Interferometric Photomechanical Spectroscopy and Imaging of Biological and Turbid Media

by

Barry P. Payne

B.S., University of Denver (1994)
S.M., Massachusetts Institute of Technology (1997)

Submitted to the Department of Mechanical Engineering in Partial fulfillment of the requirements for the degree of

Doctor of Philosophy

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

February 2001

© Barry Payne. All rights reserved.

The author hereby grants to MIT permission to reproduce and to distribute publicly paper and electronic copies of this thesis document in whole or part.

Certified by

B.B. Mikic
Professor, Department of Mechanical Engineering
Thesis Supervisor

Certified by

N.S. Nishioka
Associate Professor of Medicine, Harvard Medical School
Thesis Supervisor

Accepted by

A.A. Sonin
Chairman, Departmental Committee on Graduate Students
Interferometric Photomechanical Spectroscopy and Imaging of Biological and Turbid Media

by

Barry Payne

Submitted to the Department of Mechanical Engineering on December 5, 2000 in Partial fulfillment of the requirements for the degree of Doctor of Philosophy

Abstract

The medical field is currently experiencing rapid growth in the area of optical diagnostics. Minimally-invasive spectroscopic and imaging modalities enable physicians to make increasingly accurate diagnoses in real time, without the cost and delay associated with traditional reliance on histopathology. We have developed an ultra-high resolution interferometric system which is well suited for clinical diagnostic applications. The interferometric system has a spatial resolution of 0.1 nm and a temporal resolution of 3 ns.

We have utilized this high resolution interferometric system in two novel minimally invasive techniques. Both techniques measure surface deformation of a target after absorption of a short laser pulse. The time dependent surface deformation is a function of the target’s spatially resolved optical, thermal and mechanical properties. Therefore, accurate measurement of surface displacement can be used to extract significant diagnostic information.

The first technique is termed Interferometric Photomechanical Spectroscopy (IPMS), and is used to measure effective optical absorption depth of a sample. We used IPMS to measure effective absorption depth of both diffuse and specularly reflecting targets including well characterized colored glass samples and gelatin based tissue phantoms. The second technique is termed Interferometric Photomechanical Tomography (IPMT), and is used to image sub-surface absorbers such as tumors or blood vessels. IPMT combines high optical contrast with low attenuation of sound propagation to localize sub-surface absorbers in highly scattering media.

We have used IPMT to image sub-surface blood vessels in a phantom model and in vivo.

Interferometric techniques compare favorably against diagnostic techniques which measure surface stress instead of surface displacement. This is primarily because interferometry is a non-contact epitaxial method capable of high resolution point measurements. Further, an interferometric system is easily implemented into an optical fiber setup for use in minimally-invasive catheter based procedures.

Thesis Committee
B.B. Mikic, Professor of Mechanical Engineering, MIT
N.S. Nishioka, Associate Professor of Medicine, Harvard Medical School
R. Abeyaratne, Professor and Associate Head of Mechanical Engineering, MIT
D. Rowell, Professor of Mechanical Engineering, MIT
Barry-

Here's a notebook to journal your exciting adventures in the laser lab.

-Vasa-

- 1994
Acknowledgments

Now is the time to thank all the people who made life and research not only possible, but fun and exciting. Dr Nishioka, Dr. Mikic and Dr. Venugopalan were responsible for starting my graduate career, and for this I am extremely grateful. They have all been great mentors and friends over the last six years, and played unique roles in my development as a researcher.

Dr. Nishioka has always given me unlimited freedom and support during all my different research endeavors. His easy going nature combined with his excellent practical and clinical understanding makes him an ideal adviser for biomedical research.

Dr. Mikic has taught me so many different things regarding research and life. His insights are always amazing, and his support has never wavered. I owe much of my success at MIT to him. Thanks also to Liba for many wonderful evenings.

Dr. Venugopalan has always been available for detailed discussions on all aspects of my research. His broad knowledge of the field and his ability to question key issues has forced me pursue a deeper understanding of my own research. He was also responsible for providing me with my first lab notebook (see opposite page).

Andrew Yablon developed the first interferometric system at Wellman Laboratories and has always been readily available for long discussions regarding my research ideas. His active interest and very useful insights have helped guide my research.

I was fortunate to have both Prof. Abeyaratne and Prof. Rowell on my thesis committee. I am very grateful for their time, and pertinent suggestions regarding my research.

Brett Bouma and Gary Tearney have been invaluable regarding all aspects of my interferometric setup. Their deep understanding of optics and instrumentation has not only benefited me, but also Wellman Laboratories as a whole. In addition, they were always willing to meet and discuss my ideas, even though they were never officially involved in my research.

Hans Ludemann, the jua kali inspector has been a friend and colleague throughout my PhD work. He has helped me with many aspects of experimentation and was always willing to listen and discuss my ideas, as well as keep me thoroughly entertained. The in vivo work was done on his forearm.
Stephan Brand, John Poneros and William Puricelli have been good friends throughout my time at Wellman Laboratories. They have provided me with constant support and entertainment.

Everyone at the Wellman Laboratories has contributed to my time here in some way. Thank you all very much. In particular, Kevin Schomacker, Tom Deutsch, Rob Webb, and Apostolos Doukas were always willing to discuss various aspects of my research; Robert Redmond for the use of the laser system; Bill Farinelli for equipment help and Susan Weeks for all her help in organizing my research assistantship.

Finally, a very special thanks to my wife and family. My family got me where I am today, and my wife is taking me to where I am going. I owe my wife and family an infinite amount of thanks for everything they have done for me – Asante Sana. My wife Kelly is an amazing person, who has supported me throughout my doctoral research. She is by far the best thing I earned as a graduate student. I am so thankful for all the sacrifices she has made on my behalf, and I look forward to all the many adventures in our future. This thesis is dedicated to her.
# Table of Contents

Abstract .......................................................................................................................... 3  
Acknowledgments ........................................................................................................ 5  
List of Figures ............................................................................................................. 9  
List of Tables ............................................................................................................. 13  
Nomenclature ........................................................................................................... 15  

## Chapter 1 Introduction ............................................................................................ 17  
1.1 Background ............................................................................................................. 17  
1.2 Detection Type ........................................................................................................ 18  
  1.2.1 Detection of Input light ................................................................................ 18  
  1.2.2 Detection of Emitted Light ....................................................................... 20  
  1.2.3 Detection of Heat ...................................................................................... 21  
  1.2.4 Detection of Sound ..................................................................................... 21  
1.3 Spectroscopy and Imaging .................................................................................... 22  
1.4 Interferometry ....................................................................................................... 23  
1.5 Research Objectives ............................................................................................ 24  

## Chapter 2 Interferometric Methods for Diagnostic Applications ......................... 27  
2.1 Introduction ............................................................................................................. 27  
2.2 Interferometer Design .......................................................................................... 29  
2.3 Interferometer Capabilities .................................................................................... 33  
2.4 Future Directions in Instrumentation .................................................................. 36  

## Chapter 3 Interferometric Photomechanical Spectroscopy (IPMS) ...................... 39  
3.1 Introduction ............................................................................................................. 39  
3.2 Theoretical Modeling ............................................................................................ 42  
3.3 Verification of Interferometric Photomechanical Spectroscopy ....................... 50  
  3.3.1 Overview .................................................................................................... 50  
  3.3.2 Experimental Setup ................................................................................. 51  
  3.3.3 Results ....................................................................................................... 55  
3.4 IPMS on Tissue Phantoms ................................................................................. 61  
  3.4.1 Overview .................................................................................................... 61  
  3.4.2 Experimental Setup ................................................................................. 62
Chapter 4 Interferometric Photomechanical Tomography (IPTS) ........................................... 71

4.1 Introduction ............................................................................................................. 71
4.2 Verification of Interferometric Photomechanical Tomography ......................... 75
  4.2.1 Overview ........................................................................................................ 75
  4.2.2 Experimental Setup ...................................................................................... 77
  4.2.3 Results ........................................................................................................... 81
4.3 Discussion ............................................................................................................. 100

Chapter 5 Conclusions and Future Work ................................................................. 103

5.1 Achievements of This Thesis .............................................................................. 103
  5.1.1 Instrumentation .............................................................................................. 104
  5.1.2 Development and Verification of Interferometric Photomechanical Spectroscopy ........................................................................................................ 104
  5.1.3 Development and Verification of Interferometric Photomechanical Tomography ........................................................................................................ 105
5.2 Future Research Directions .................................................................................. 106
  5.2.1 Comprehensive Comparison with Pressure Transducers ................................ 106
  5.2.2 Clinical Instrumentation Development .......................................................... 106
  5.2.3 Image Reconstruction Development ............................................................. 107
  5.2.4 Simple Clinical Applications ........................................................................ 107

References ............................................................................................................... 109
List of Figures

Figure 2-1: Dual balanced Interferometric system used to measure surface displacement................................................................. 30

Figure 2-2: A comparison of base line traces of our current interferometric system and a previous version by Yablon and coworkers [108]. ........... 34

Figure 2-3: A comparison of surface expansion traces of colored glass during Q-switched Nd:YAG laser irradiation at 355 nm.............................. 35

Figure 3-1: Simulated analytical and FEM surface displacement traces for typical IPMS parameters............................................................ 50

Figure 3-2: Frequency doubled Q-switched Nd:YAG temporal laser profile with a FWHM pulse duration of about 7 ns........................................ 52

Figure 3-3: Q-switched Nd:YAG spatial laser profile at 532 nm with a FWHM diameter of 6 mm................................................................. 52

Figure 3-4: Schematic of experimental setup for IPMS verification.......................... 53

Figure 3-5: Absorption depth of Schott neutral density glass filters .................. 54

Figure 3-6: Sample displacement trace for Schott glass type NG1.......................... 56

Figure 3-7: Sample displacement trace and best fit solution for Schott glass type NG1................................................................. 57

Figure 3-8: Sample displacement traces and best fit solutions for Schott glass type NG1. Laser fluence ranged from 12 - 440 mJ/cm². ..................... 58

Figure 3-9: Sample displacement traces with best fit solutions for all the Schott glass filters......................................................................................................................................... 59

Figure 3-10: Calculated absorption depth for 350 traces obtained under various laser operating and alignment conditions. The axis limits correspond to the absorption depth range given by Schott Glass Technologies............................................................. 61

Figure 3-11: Sample displacement trace and best fit solution for a 0.0169M potassium chromate solution from batch A........................................ 64
Figure 3-12: Sample displacement trace and best fit solution for a gelatin doped tissue phantom. The tissue phantom was prepared with the 0.0169M potassium chromate solution from batch A. 66

Figure 3-13: Calculated absorption depth for 0.0169M potassium chromate solution (batch A) and calculated absorption depth for a gelatin tissue phantom prepared with the same solution. 67

Figure 4-1: IPMT scanning setup. Surface displacement traces across the sample were obtained by scanning the tissue phantom on a digital micrometer. 76

Figure 4-2: Spatial profile of a focused Q-switched Nd:YAG laser beam at 1064 nm. The FWHM diameter of the spot was about 0.6 mm. 78

Figure 4-3: Schematic of experimental setup for IPMT. 79

Figure 4-4: Sample surface displacement of a vessel phantom model consisting of a 1000 μm polyimide tube surrounded by pure water. 83

Figure 4-5: Sample surface displacement traces of a 1000 μm phantom vessel at different depths. The surrounding medium was pure water. 84

Figure 4-6: Sample tomographic image of a 495 μm diameter sub-surface phantom vessel in pure water. Vessel depth was 1.53 mm. 85

Figure 4-7: Sample contour image of a 495 μm diameter sub-surface phantom vessel in pure water. Vessel depth was 1.53 mm. 86

Figure 4-8: Sample contour images of three different sized phantom vessels in pure water. The depths of the phantom vessel are all about 1.5 mm. 87

Figure 4-9: Normalized maximum displacement profiles for three different phantom vessels in water. The diameters were of the vessels were 1000 μm, 495 μm and 198 μm. Gaussian functions have been fitted to the profiles. 88

Figure 4-10: Sample tomographic images for a 1000 μm phantom vessel in pure water, 1% and 7% Intralipid solution. The depth in each media was about 1.5 mm. 89

Figure 4-11: Normalized maximum displacement profiles for a 1000 μm diameter vessel in pure water, 1% and 7% Intralipid solution. Gaussian functions have been fitted to the profiles. 90

Figure 4-12: Sample surface displacement of a vessel phantom model consisting of a 300 μm sub-surface polyimide tube surrounded by 7% Intralipid solution. The vessel depth was 1.04 mm. 91
Figure 4-13: Sample surface displacement traces of vessel phantoms at different depths in a 7% Intralipid solution. The phantom vessel size was 300 μm in diameter. ................................................................. 92

Figure 4-14: Sample tomographic image of a 1000 μm diameter sub-surface phantom vessel in a 7% Intralipid solution. Vessel depth was 1.36 mm. ..................................................................................... 93

Figure 4-15: Sample contour image of a 1000 μm diameter sub-surface phantom vessel in a 7% Intralipid solution. Vessel depth was 1.36 mm. ............... 94

Figure 4-16: Sample contour images of three different sized phantom vessels in a 7% Intralipid solution. The depths of the phantom vessel are all about 1.4 mm. ................................................................. 95

Figure 4-17: Normalized maximum displacement profiles for three different phantom vessels in a 7% Intralipid solution. The diameters were of the vessels were 1000 μm, 495 μm and 198 μm. Gaussian functions have been fitted to the profiles. ................................................................. 96

Figure 4-18: Sample surface displacement traces of three different vessel at a depth of 2.2 mm in a 7% Intralipid solution. The diameters of the vessels were 1000 μm, 300 μm and 198 μm. ............................................. 97

Figure 4-19: Two sample surface displacement traces obtained from the forearm of a human volunteer. The top trace was obtained directly above the vessel, and the bottom trace was obtained to the side of the vessel. The vessel depth is estimated to be 0.9 mm below the skin surface. ....... 99

Figure 4-20: Sample OCT image taken directly on top of a vessel in the forearm of a human volunteer. There is no clear indication that there is a sub-surface blood vessel. ................................................................. 100
List of Tables

Table 3-1: Material properties and calculated speed of sound for several Schott neutral density filters ........................................................................ 55

Table 3-2: Measured absorption depths for Schott neutral density filters compared with values obtained from Schott Glass Technologies ........ 60

Table 3-3: Measured absorption depth for three potassium chromate solutions from two different batches (A and B) compared with estimated values. ........................................................................ 64
Nomenclature

\( b_i \) - body force vector, [N/m²]
\( c_l \) - longitudinal speed of sound, [m/s]
\( c_v \) - heat capacity at constant volume, [J/kgK]
\( D \) - characteristic penetration depth of radiation, [m]
\( E \) - Young's modulus, [N/m²]
\( I \) - intensity of detected light, [W]
\( I_s \) - intensity of interferometric sample arm, [W]
\( I_r \) - intensity of interferometric reference arm, [W]
\( k \) - wave propagation vector [radians/m]
\( K \) - bulk modulus, [N/m²]
\( p \) - Laplace variable, [-]
\( S(t) \) - Surface displacement, [m]
\( S_0 \) - Equilibrium surface displacement, [m]
\( t \) - time, [s]
\( t_p \) - laser pulse duration, [s]
\( u_i \) - displacement vector, [m]
\( \dot{V}_i \) - acceleration vector, [m/s²]
\( z \) - depth coordinate in irradiated target, [m]
Greek

\( \alpha \)  - thermal diffusivity, [m\(^2\)/s]
\( \beta \)  - coefficient of thermal expansion, [K\(^{-1}\)]
\( \delta \) - Kronecker delta, [-]
\( \Delta \rho \) - path length difference of the interferometric system, [m]
\( \Delta w \) - AOM modulation frequency, [Hz]
\( \varepsilon_{ij} \) - strain tensor, [-]
\( \eta_{ij} \) - Lagrangian strain tensor, [-]
\( \phi_{\text{AOM}} \) - phase of AOM signal, [radians]
\( \phi_{m} \) - phase of interference signal, [radians]
\( \phi_{o} \) - incident laser fluence, [J/m\(^2\)]
\( \lambda \) - wavelength, [m]
\( \mu_{a} \) - radiation absorption coefficient, [1/m]
\( \nu \) - Poisson’s ratio, [-]
\( \pi \) - 3.14159…
\( \rho \) - density, [kg/m\(^3\)]
\( \sigma_{ij} \) - stress tensor, [N/m\(^2\)]
\( \theta \) - temperature relative to surrounding temperature, [K]
\( \theta_{0} \) - surface temperature relative to surrounding temperature, [K]
\( \tau_{m} \) - surface displacement time constant, [s\(^{-1}\)]
Chapter 1

Introduction

1.1 Background

The medical field is currently experiencing rapid growth in the area of minimally-invasive optical diagnostic techniques. This growth is primarily due to technological advances in digital signal processing and optical technologies. Non-invasive spectroscopic and imaging modalities allow physicians to make increasingly accurate diagnoses in real time, without the cost and delay associated with traditional reliance on histopathology. Optical techniques are attractive because they are generally safe and are capable of extracting information on the morphological, biochemical and molecular level. There are many different optical diagnostic techniques, each with its own advantages, limitations, targets and resolution. We have listed several recent review articles that cover a broad range of current optical diagnostic techniques [31, 78, 81, 93, 99].

All optical diagnostic techniques use light with wavelengths ranging from the ultraviolet to the infrared to obtain tissue information. These techniques differ in how this
information is extracted, and the type of information extracted. How the information is extracted is a function of what is specifically measured. There are four distinct types of detection. These are the detection of input light, detection of emitted light, detection of heat and detection of sound. These four detection types are reviewed in section 1.2. The type of information extracted is either spectroscopic based, imaged based, or a combination of both. Spectroscopy is primarily concerned with wavelength dependent properties of tissue. Imaging is concerned with spatially mapping tissue structure or function. Section 1.3 reviews two important sub-sections of spectroscopy and imaging; tissue optical properties, and imaging in highly scattering media. Understanding both how information is extracted, and what information is extracted is key in developing new optical diagnostic applications and improving existing ones.

1.2 Detection Type

1.2.1 Detection of Input light

The detection of input light can be further subdivided into the detection of coherent (ballistic) light, and the detection of diffuse light. The detection of coherent light is normally associated with diffraction limited imaging applications. The main challenge for techniques based on the detection of coherent light is to reject non-coherent or scattered light. The most common methods to reject scattered light are spatial filtering, polarization gating and time gating.

Spatial filtering is one of the simplest and oldest methods for suppressing scattered light. The concept is to use a spatial filter to block scattered light, while allowing the majority of coherent light to be detected. The most common example of a
spatial filter is a simple pin hole which is most often used to image point objects. The confocal microscope is the most widely used instrument that uses spatial filtering for high resolution imaging (e.g. [72]).

Polarization gating utilizes polarization differences between scattered light and unscattered light to reject the former. Coherent light retains its polarization state, whereas scattered light is randomly polarized. Polarization gating is generally performed by resolving the polarization state of light emerging from a sample, and using this information to subtract the diffuse background (e.g. [43]).

Time gating uses the flight time of photons to obtain path length information. In the simplest configuration, a time resolved measurement of light emerging from a sample that was illuminated by a short input pulse provides a temporal distribution of path lengths. Coherent light has the shortest flight time and is temporally separated from multiply scattered light. Simple time gating can be achieved by a streak camera [22, 37]. Improved time gating can be achieved with correlation techniques where the sample light is mixed with a short reference pulse of proper time delay. Nonlinear correlation techniques include SHG [25, 56, 110, 111], upconversion [27], Kerr gating [104], Raman amplification [6] and Photorefractive holography [47]. Linear correlation techniques include time-gated holography [61] and optical coherence tomography [13, 31, 83]. Optical coherence tomography is the most clinically successful linear correlation technique and combines heterodyne detection and low coherence interferometry. OCT was first demonstrated in the eye [44] and has since been used in many clinical applications [7, 10, 12, 14, 29, 30, 49, 50, 54, 75, 84, 85, 88, 91, 96].
The detection of diffuse light is normally used to measure tissue optical properties or to perform low resolution imaging. The optical properties or internal structure of tissue is reconstructed from diffuse transmittance or reflectance measurements by using a theoretical model of wave propagation. In general, the diffusion approximation to the radiative transport equation provides sufficient accuracy, but needs to be supplemented with complex boundary conditions [5]. Diffuse light detection is performed in either the spatial, time or frequency domain. In the spatial domain or spatially resolved method, light of constant intensity is launched into tissue and detected at different known spatial locations [20, 28, 45]. In the time domain or time resolved method, a pulse of light is launched into tissue. Changes in the pulse shape due to attenuation and scattering are used to extract optical absorption and scattering properties. One source-detector separation is sufficient to characterize the optical properties [51, 64, 70]. The third approach uses high frequency (MHz) intensity modulated light to create diffuse photon density waves (PDW). The PDW propagate with a wave vector that is a function of optical properties [15, 32, 71, 74, 86, 100].

1.2.2 Detection of Emitted Light

The detection of emitted light covers all fluorescence based techniques. The principles of fluorescence spectroscopy and fluorescence imaging are well understood (e.g. [59, 105]). The advantage of fluorescence based techniques is the potential to link biochemical and morphological properties of tissue to individual patient care. There are numerous fluorescence techniques and configurations which are based on one, two and three photon excitation, confocal microscopy, fluorescence life times as well as a wide
range of both endogenous and exogenous fluorophores [24, 39, 40, 72, 78, 79, 81, 90, 92, 103, 107].

1.2.3 Detection of Heat

The detection of heat (temperature) during and after laser irradiation can be used to extract tissue optical properties and to perform low resolution imaging. For example, pulsed photothermal radiometry measures the temporal evolution of radiative emission from the surface of a tissue sample after the absorption of a sub-ablative laser pulse. This measurement is used to compute the original thermal field, thereby extracting optical or structural information [53, 62, 63, 65, 66, 102]. This is an inverse diffusion problem and it is therefore very difficult to accurately reconstruct the original thermal profile.

1.2.4 Detection of Sound

The detection of sound that is generated from the absorption of light is a potentially powerful technique for extracting the optical properties of tissue, or imaging sub-surface structures like blood vessels or tumors within tissue. The generation of sound by light absorption is often termed the photoacoustic or optoacoustic effect. Detailed theoretical and experimental aspects of photoacoustic processes are listed in three excellent references, including a review paper, book chapter and book [36, 46, 87]. Although the field of photoacoustics is well established, there has been a recent trend towards applying photoacoustic techniques for medical diagnostic applications. These techniques include photoacoustic spectroscopy and photoacoustic imaging [26, 41, 55, 58, 67, 68]. Although photoacoustic spectroscopy and imaging are inverse problems,
where the original stress distribution is reconstructed from time dependent stress measurements, it is not prone to the same instabilities and inaccuracies of diffusion based inverse problems such as pulsed photothermal radiometry. This is simply because wave propagation is not significantly affected by turbid media, as is the case for diffusion based light propagation. This is a major advantage for imaging in highly scattering media such as tissue.

1.3 Spectroscopy and Imaging

The optical properties of tissue is an important sub-section of spectroscopy, and play a central role in many diagnostic applications. Tissue optical properties are responsible for the propagation of light in tissue, and can also yield useful diagnostic information regarding metabolic, physiologic and structural status of the tissue [18, 106]. For example, changes in optical properties have been used to study the hemodynamics of brain, [35, 89, 98]. The fundamental optical properties of tissue are the absorption coefficient, scattering coefficient, and scattering phase function or scattering anisotropy. Often the scattering coefficient and phase function are combined to form a reduced scattering coefficient. For one dimensional geometry, the penetration of light decreases exponentially, and an effective penetration depth is used which is a function of the absorption and reduced scattering coefficient.

Imaging modalities capable of imaging deep sub-surface absorbers in highly scattering media would be very useful in many diagnostic applications such as breast cancer screening. Coherent imaging techniques such as optical coherence tomography and confocal microscopy cannot be used to detect deep absorbers since tissue scattering
is too great. Currently, non-optical imaging techniques such as X-ray radiography, 
magnetic resonance imaging and ultrasound imaging are used to image deep sub-surface 
absorbers. Although these techniques are valuable, the major limitation is usually 
associated with low contrast, leading to insufficient sensitivity for detection of small 
tumors.

1.4 Interferometry

Interferometric methods have shown great promise in a variety of diagnostic 
applications. The success of interferometric techniques is primarily due to the high spatial 
and temporal resolution that can be achieved. In addition, an interferometric system is 
easily implemented into an optical fiber setup for use in minimally-invasive catheter 
based procedures. For example, optical coherence tomography has been successfully 
during endoscopic procedures. (e.g. [12, 85, 95]).

Interferometry has also been successfully used in pump-probe techniques where 
the thermo-mechanical response of biological tissue was investigated by measuring the 
time dependent surface displacement of biological tissue after the absorption of a short 
laser pulse [4, 21, 73, 82, 108]. The time dependent surface displacement is a function of 
the sample’s optical, thermal and mechanical properties. Interferometric monitoring of 
surface deformation after laser irradiation is therefore a potentially powerful diagnostic 
tool. Stress transducers could also be employed as a diagnostic tool to measure material 
properties [55, 58, 67, 68]. However, stress transducers are not ideal in many medical 
applications since they are contact based methods and are not easily used in conjunction 
with minimally invasive catheter type procedures.
1.5 Research Objectives

Given the importance of tissue optical properties, and the need for sub-surfacing imaging in scattering media, the goal of this thesis was to develop a minimally-invasive spectroscopic technique, and a minimally-invasive imaging technique. Interferometry is a non-contact minimally invasive high resolution technique and is well suited for both these diagnostic applications. The specific objectives of this work are listed below:

I. Develop a high resolution interferometric system capable of accurately measuring surface displacement on both diffuse and specular targets

II. Develop an accurate spectroscopic technique to measure the effective absorption depth of well characterized targets and tissue phantoms

III. Develop an interferometric technique to image sub-surface absorbers such as blood vessels in highly scattering media.

Given these objectives, the rest of this thesis is organized into four sections. Chapter 2 describes the interferometric system developed and illustrates its capabilities as compared to previous interferometric systems. Chapter 3 describes a novel minimally invasive spectroscopic technique termed Interferometric Photomechanical Spectroscopy (IPMS). IPMS was used to accurately measure the optical properties of colored glass as well as tissue phantoms. Chapter 4 describes a novel minimally invasive imaging technique
termed Interferometric Photomechanical Tomography (IPMT). IPMT was used to image sub-surface blood vessels in a phantom model and in vivo. Finally, Chapter 5 summarizes this thesis and discusses future directions of this work.
Chapter 2

Interferometric Methods for Diagnostic Applications

2.1 Introduction

Interferometric methods are attractive for non-invasive diagnostic imaging applications. In particular, heterodyne interferometry, a linear correlation technique capable of shot noise limited detection has shown great promise in a variety of medical imaging applications [81]. The success of interferometric techniques is primarily due to the high spatial and temporal resolution, and the ability to be implemented within an optical fiber system. High resolution imaging through optical fibers is valuable for minimally invasive catheter based diagnostic applications. For example, Optical Coherence Tomography (OCT) is a very promising minimally invasive interferometric technique used for high resolution imaging of biological tissue [31, 83]. The basic principle of OCT is analogous to ultrasound, where echoes from sub-surface structures are used to create tomographic images. OCT uses echoes of light rather than echoes of sound, and therefore has superior resolution to ultrasound. OCT was first demonstrated in
vitro in the human retina and atherosclerotic plaques in 1991 [44]. The first human studies with OCT were performed in 1993 in ophthalmology [29, 91], and have expanded to include dermatology [84], dentistry [7], cardiology [14, 30], urology [54, 96], gynecology [10] and gastroenterology [12, 49, 50, 75, 85, 88].

Interferometry has also been successfully used in time resolved pump-probe techniques to study the thermo-mechanical response of biological tissue [4, 21, 73, 82, 108]. These techniques measured the time dependent surface displacement of biological tissue after the absorption of a short laser pulse. The time dependent surface displacement is a function of the sample’s thermo-mechanical properties as well as the specific laser-tissue geometry such as spot size. The thermo-mechanical response of biological tissue can be used to extract useful diagnostic information about the tissue’s optical, thermal and mechanical properties. Photoacoustics or optoacoustics is an analogous technique which uses stress instead of displacement to extract the optical properties of tissue [55, 58, 67, 68]. Stress detection via a pressure transducer is not ideal in many medical applications since it is a contact method and is not easily used in conjunction with minimally invasive catheter based procedures.

The success of interferometric systems in medical applications is highly dependent on the temporal and spatial resolution of the instrument. Previous interferometric systems employed to measure surface displacement had temporal resolutions of 3-4 ns, and spatial resolutions of 1-4 nm [4, 21, 73, 82, 108]. Although the temporal resolution is adequate for most applications, the spatial resolution is not sufficient for diagnostic applications which must differentiate subtle changes in tissue properties, or imaging applications attempting to image deep sub-surface absorbers.
We have developed an ultra-high resolution heterodyne interferometric system capable of angstrom spatial resolution, and sub nanosecond temporal resolution. The high spatial and temporal resolution is ideal for measuring surface displacements with extremely high accuracy. In Chapter 3, we have used this interferometric system to measure optical absorption depth of well characterized glass samples and tissue phantoms. In Chapter 4, we have used this interferometric system to image sub-surface absorbers within tissue phantoms and in vivo.

2.2 Interferometer Design

The interferometric system developed and employed in this thesis is shown in figure 2-1. This configuration is based on a previous version by Yablon and coworkers [108], with two important differences. First, the focusing lens was replaced with a gradium index lens with a focal length of 80 mm. The gradium index lens maintained better spatial coherence in the sample beam, and therefore enabled a higher interference depth of modulation and lower noise. The interference depth of modulation is the ratio of interfered light to the total available light. A depth of modulation of 100% means that all the available light is being used for interference, implying perfect spatial and temporal coherence. The shorter focal length of the gradium index lens reduced the probe spot diameter to about 10 μm, which enabled more coherent light to be reflected back off diffuse surfaces. Although a smaller focal length lens could be used to decrease the spot size further, the sample working distance would be reduced which is not practical for most applications.
Second, the avalanche photodiode was replaced with dual balanced Si PIN photodiodes. The Si PIN photodiodes have very high temporal resolution (0.2 ns) and very low noise owing to the small dark current (2 pA). The dual balanced detection system reduces the effect of local oscillator noise by subtracting their photocurrents to cancel unwanted DC components [1]. Ideally, a transimpedance amplifier should be used to amplify the subtracted photocurrents, although a broadband low noise amplifier is sufficient.

Figure 2-1: Dual balanced Interferometric system used to measure surface displacement.
A polarized 3mw helium-neon (HeNe) laser beam (Melles Griot, Irvine, CA) with a wavelength of 632.8 nm is used as the interferometric probe beam. An acoustic optical modulator (IntraAction Corp., Bellwood, IL) is used to deflect and impart a frequency shift of 110 MHz to the reference beam. The unshifted beam, also called the sample beam, passes through a polarized beamsplitter and quarter wave plate and is directed towards the target by a dielectric mirror. The quarter wave plate rotates the polarization state by 45°. When necessary, a 10X beam expander (Melles Griot) and 80 mm focal length gradium index lens is used to increase the numerical aperture, yielding a spot size of about 10 µm. This is useful for imaging diffuse surfaces such as gelatin phantoms and tissue targets. The HeNe laser reflects off the sample surface due to a refractive index mismatch between the air and target, and passes back through the quarter wave plate, rotating the polarization state by an additional 45°. The polarization state of the sample beam is therefore rotated by a total of 90°, causing the beam to be reflected by the polarized beamsplitter, rather than transmitted. The reflected sample beam passes through a half wave plate to restore the original state of polarization and is recombined with the reference beam by a 50/50 non-polarizing beamsplitter. The recombined beams have a 90° phase difference and are imaged by 25.4 mm focal length lenses on two Si PIN photodiodes (Hamamatsu, Bridgewater, NJ). The small active area of each PIN diode (0.126 mm²) enables confocal imaging of the target. The photocurrents generated by the Si PIN photodiodes are subtracted, canceling any inherent laser noise. The subtracted photodiode current is amplified by a commercial 500 MHz broadband low noise amplifier (Mini-Circuits, Branson, MO) and is digitized by a 500 MHz bandwidth oscilloscope (Tektronix, Wilsonville, OR). The output of the commercial low noise
broadband amplifier was compared to a lab built transimpedance amplifier. The signal to noise ratio for the transimpedance amplifier was slightly better than the signal to noise ratio of the broadband amplifier, but the gain curve was not as flat. A commercial broadband transimpedance amplifier would probably yield better performance than a commercial low noise broadband amplifier.

When the target is stationary, the photodiodes detect interference fringes that oscillate at the AOM drive frequency of 110 MHz. When the sample surface moves, the path length difference between the two arms changes, and the phase of the modulation frequency is altered. The polarity of the phase shift depends on the direction the sample moves. The intensity reaching the photodiodes is a function of time ($t$), path length difference ($\Delta p$), and intensities of the sample and reference arms and is given by:

$$I(\Delta p, t) = I_1 + I_2 + 2\sqrt{I_1 I_2} \cos(\phi_m).$$  \hspace{1cm} (2.1)

$I_1$ and $I_2$ are the intensities of the sample arm and reference arm, and $\phi_m$ is the phase of the modulated signal which is given by:

$$\phi_m = k\Delta p + \Delta wt.$$  \hspace{1cm} (2.2)

$k$ is the propagation vector ($2\pi/\lambda$) and $\Delta w$ is the AOM modulation frequency. The surface displacement $S(t)$ is equal to half the path length difference and given by:

$$S(t) = \frac{\Delta p}{2} = \frac{\phi_m - \Delta wt}{2k} = \frac{\phi_m - \phi_{AOM}}{2k}.$$  \hspace{1cm} (2.3)
The surface displacement is therefore computed by subtracting the phase of the AOM drive signal from the phase of the detected signal. The phase of both signals are obtained with the aid of the Hilbert Transform. In general, the AOM signal is sufficiently pure that subtraction of a simulated AOM phase is equivalent to subtracting the phase from the real AOM signal.

2.3 Interferometer Capabilities

The improvements of our interferometric system over a previous version developed by Yablon and coworkers [108] is best illustrated by comparing sample baseline traces from both systems as shown in figure 2-2. Both baseline traces were obtained under similar conditions and filtered with a 80MHz band pass filter resulting in a temporal resolution of 4 ns. The spatial resolution defined by the standard deviation (STD) of the displacement signal has been improved by an order of magnitude.
Figure 2-2: A comparison of base line traces of our current interferometric system and a previous version by Yablon and coworkers [108].

Two surface expansion traces of colored glass after Q-switched Nd:YAG laser irradiation at 355 nm are shown in figure 2-3. One trace was obtained with our new interferometric system, and one trace was obtained with the previous interferometric system. The maximum displacement in both cases was about 30 nm, although they have been normalized for display purposes. The significant improvement in spatial resolution is highly desirable for many diagnostic applications. For example, subtle changes in the optical absorption depth may allow contrast between pathological and non-pathological structures.
In general, there is a trade off between the temporal and spatial resolution of the interferometric system. For the experiments in this thesis, a 80 MHz digital band pass filter was used resulting in a 4 ns rise time. The best STD achieved on a highly reflecting target without using the 10X beam expander or focusing lens was 0.08 nm. This spatial resolution represents the limit of the 8 bit digitizing oscilloscope. For samples that reflect only about 4% of the incident light such as glass, the STD increases slightly to 0.09 nm.

When the beam expander and focusing lens were used, the interference depth of modulation decreased from 98% to about 75%. This meant there was some spatial incoherence between the reference and the sample beams caused by imperfections of the

Figure 2-3: A comparison of surface expansion traces of colored glass during Q-switched Nd:YAG laser irradiation at 355 nm.
beam expander optics. Spatially incoherent light incident on detectors contributes to noise. Consequently the best STD achieved on a high reflecting surface was 0.09 nm, and the best STD achieved on a low reflecting sample such as glass was to 0.12 nm. The small increase in STD due to spatial incoherence highlights the benefit of using low noise detectors such as Si PIN photodiodes.

For diffuse surfaces such as gelatin tissue phantoms, the amount of coherent light reflected off the surface decreases and the STD increases. The best STD achieved on a gelatin tissue phantom was 0.16 nm.

The temporal resolution of the interferometric system is limited only by the 500 MHz bandwidth of the digitizing oscilloscope and amplifier. Therefore, sub-nanosecond rise times are achievable with the current system, although the spatial resolution will degrade. For example, the STD for a temporal resolution of 0.9 ns on a highly reflecting surface was 0.28 nm. Since the Si PIN photodetectors have a bandwidth of 1.5 GHz, one could in theory measure rise times as low as 230 ps.

2.4 Future Directions in Instrumentation

There are two distinct tasks for improving the interferometric system described in this chapter. The first task involves improving existing components in the current system, and the second task involves transforming the current system into a fiber based clinically useable tool.

The major limiting component in the current system is the HeNe probe laser which has insufficient power to enable shot noise limited detection on low reflecting surfaces. Ideally, a dual balance detection system should be operated just under the
threshold of the photodetectors. This was not possible with our current system for low reflecting targets. For highly reflecting targets, noise calculations indicated that detection was limited by shot noise. These calculations were based on a transimedance amplifier configuration, but should be comparable to a broadband low noise amplifier. A higher power HeNe laser would allow shot noise limited detection even on diffuse surfaces. The specific laser choice must be made carefully. Generally, higher power lasers have a longer cavity length, and therefore shorter beat frequency [38]. If the beat frequency is too close to the carrier frequency, the interference phase is corrupted and spatial resolution is significantly reduced. Although filter techniques will reduce this effect, we feel shorter cavity higher power lasers are best suited.

The next major improvement would be to implement this system into a optical fiber based system. This would not only ease alignment, increase the depth of modulation, but also enable the current system to be used in real clinical applications, including minimally invasive catheter based procedures. The authors feel that implementing the current system into an optical fiber setup should not be difficult. This is because interferometric fiber based technologies already exist for other techniques like optical coherence tomography.

Finally, the clinical value of the interferometric system would be greatly increased by enabling the probe beam and pump beam to be scanned. This would allow multiple spatial displacement traces to be obtained quickly. This is particularly useful for imaging purposes. Alternatively, one could envision a probe array for collecting multiple points simultaneously. This would avoid scanning difficulties and allow 3-dimensional reconstruction of sub-surfaces absorbed in a single shot.
Chapter 3

Interferometric Photomechanical Spectroscopy (IPMS)

3.1 Introduction

The optical properties of tissue play a central role in many therapeutic and diagnostic medical applications. For example, therapeutic applications such as laser surgery and photodynamic therapy rely on predicting the spatial distribution of laser energy within the tissue, and hence ensuring correct laser dosimetry. The spatial distribution is a function of the absorption and scattering properties of tissue. Tissue optical properties also yield useful diagnostic information regarding metabolic, physiologic and structural status of the tissue state. In many applications, it is often convenient to characterize the spatial energy distribution for a large diameter beam by an effective absorption depth. The effective absorption is a function of absorption coefficient, scattering coefficient and an anisotropy parameter which governs the angular dependence of scattering [18, 106].
There are a number of methods used to measure the effective absorption depth of a sample. These methods either directly measure optical properties via simple attenuation measurements, or indirectly extract optical properties via a theoretical model. In general indirect methods are based on the diffusion approximation to the radiative transport equation and measure the diffuse optical transmission and reflectance of a sample at different wavelengths. These techniques are performed in either the spatial, time or frequency domain. In the spatial domain or spatially resolved method, light of constant intensity is launched into tissue and detected at different known spatial locations [20, 28, 45]. In the time domain or time resolved method, a pulse of light is launched into tissue. Changes in the pulse shape due to attenuation and scattering are used to extract optical absorption and scattering properties. One source-detector separation is sufficient to characterize the optical properties [51, 64, 70]. The third approach uses high frequency (MHz) intensity modulated light to create diffuse photon density waves (PDW). The PDW propagate with a wave vector that is a function of optical properties [15, 32, 71, 74, 86, 100].

Recently, photoacoustic or optoacoustic techniques have been used to extract optical properties of turbid media [55, 58, 67]. Photoacoustic spectroscopy extracts the optical properties of a sample by measuring thermoelastic stresses caused by the absorption of a short laser pulse. Thermoelastic stresses are a function of the initial energy distribution within the sample, and therefore also a function of the sample’s optical properties. Although photoacoustic spectroscopy is an inverse problem, where optical properties are extracted by reconstructing the original stress distribution, it is not prone to the instabilities and inaccuracies of diffusion based inverse problems. This is
simply because wave propagation through turbid media is not altered significantly compared with diffusion based propagation. Consequently, it is possible to measure optical properties of structures deep inside a sample, where photon diffusion based techniques fail.

Current photoacoustic spectroscopy techniques employ pressure transducers to measure laser induced stresses. In most cases, the measurement must be performed in an epitaxial or so called backward mode since access to both sides of a tissue target is not possible. Standard front surface pressure transducers cannot be used as they would physically block the incident laser light. Investigators have therefore used a variety of creative techniques to overcome this problem, including piezoelectric transducers with separated light and sound fields, transparent transducers measuring optical reflectance, annular piezoelectric elements, and acoustic conductors where sound generated by an obliquely incident laser pulse propagates predominantly normal to the tissue surface [8, 9, 17, 55, 57, 69]. Despite these technologies, stress detection via a pressure transducer is not ideal in many medical applications since it is a contact method and is not easily used in conjunction with minimally invasive catheter based procedures.

In this Chapter, we have developed a novel minimally-invasive non-contact interferometric technique used to measure the effective absorption depth of a sample with extremely high accuracy. We call this technique Interferometric Photomechanical Spectroscopy (IPMS). We use the word photomechanical instead of photoacoustic or optoacoustic since we measure displacement and not stress. IPMS uses an interferometric system to measure surface expansion during and after the absorption of a short laser pulse. The expansion of the sample is caused by thermoelastic stress relaxation and the
time constant of this relaxation is proportional to absorption depth. The magnitude of expansion is a function of incident laser energy and thermo-physical properties of the sample. This technique is complementary to a similar technique called Interferometric Photothermal Spectroscopy (IPTS) [108, 109]. IPTS calculates effective absorption depth by measuring surface cooling due to thermal diffusion.

The concept of using an interferometric based system to calculate absorption depth by monitoring surface expansion has been noted previously, although it was not termed IPMS nor was it employed to measure an unknown absorption coefficient [2, 4, 48] [3]. Recently, the same group made some preliminary absorption depth measurements on meniscus samples [21]. However, IPMS has yet to be fully verified, or its accuracy accessed. In addition, the spatial resolution of the interferometric system used in these experiments is 50 times worse than the spatial resolution of our interferometric system.

3.2 Theoretical Modeling

In this section, we derive the one dimensional relationship between surface displacement and absorption depth using the theory of elasticity. A non-linear fitting algorithm is used to extract the absorption depth from the displacement traces acquired by the interferometric system. We begin by deriving Green’s solution which assumes the incident laser energy is absorbed instantaneously. The following derivation was based on two references [60, 97].

The stress and strain relationship in any material are governed by three equations; the stress tensor, the equation of motion and the constitutive relation for that material. We
neglect effects of thermal diffusion since the time scale of stress relaxation is much smaller than the time scale for thermal diffusion. The strain tensor \( \varepsilon_{ij} \) is given by eq. \((3.1)\) and is the small strain approximation of the Lagrangian strain tensor \( \eta_{ij} \). The subscripts represent indicial standard notation.

\[
\eta_{ij} \approx \varepsilon_{ij} = \frac{1}{2} (u_{i,j} + u_{j,i}),
\]

(3.1)

where \( u_i \) is the displacement vector. The equation of motion is derived from the conservation of momentum and is given by:

\[
\sigma_{i,j} + b_i = \rho \ddot{v}_i,
\]

(3.2)

where \( \sigma_{ij} \) is the stress tensor, \( b_i \) is the body force vector, \( \rho \) is the density and \( \ddot{v}_i \) is the acceleration vector. The constitutive relation for a linear isotropic material is given by:

\[
\varepsilon_{ij} = \frac{1 + \nu}{E} \sigma_{ij} - \frac{\nu}{E} \sigma_{kk} \delta_{ij} + \beta \theta \delta_{ij},
\]

(3.3)

where \( \nu \) is Poisson’s ratio, \( E \) is elastic modulus, \( \beta \) is the coefficient of thermal expansion, \( \theta \) is the relative temperature and \( \delta \) is the Kronecker delta. Assuming the laser energy is absorbed exponentially, the temperature distribution will be:
\[ \theta = \theta_0 e^{-\frac{z}{D}}, \]  

(3.4)

where \( \theta_0 \) is the surface relative temperature, and \( D \) is the effective absorption depth which is equal to the inverse of the absorption coefficient. Substituting eq. (3.4) and (3.3) into eq. (3.2), we obtain the partial differential equation for displacement as a function of depth (\( z \)) and time (\( t \)).

\[ \frac{\partial^2 u}{\partial t^2} = c_i^2 \frac{\partial^2 u}{\partial z^2} + \frac{\beta K \theta}{D \rho} e^{-\frac{z}{D}}, \]  

(3.5)

where \( c_i \) is the longitudinal speed of sound and is defined in eq. (3.6) and \( K \) is the bulk modulus and is defined in eq. (3.7).

\[ c_i^2 = \frac{E(1-\nu)}{\rho(1+\nu)(1-2\nu)} \]  

(3.6)

and

\[ K = \frac{E}{3(1-2\nu)}. \]  

(3.7)

The initial conditions and boundary conditions are as follows:
\[ u = 0 \quad t = 0, \]
\[ \frac{\partial u}{\partial t} = 0 \quad t = 0, \quad (3.8) \]
\[ u = 0 \quad z \to \infty \]

and

\[ \sigma = 0 \quad z = 0. \]

The solution is conveniently obtained by using the method of Laplace transforms. The Laplace transform is obtained by the following integral transform:

\[ L\{f(t)\} = \hat{f}(p) = \int_0^\infty e^{-pt} f(t) dt. \quad (3.9) \]

Transforming eq. (3.5) into the Laplace domain yields

\[ q^2 \bar{u} = \frac{\partial^2 \bar{u}}{\partial z^2} + \frac{Q}{p} e^{\frac{-z}{p}}, \quad (3.10) \]

where

\[ Q = \frac{\beta K \theta_o}{Dpc_i^2} \quad \text{and} \quad q = \frac{p}{c_i}. \quad (3.11) \]

The general solution is given by:

\[ \bar{u} = c_1 e^{-qz} + c_2 e^{+qz} + \frac{Q}{p} \left[ \frac{D^2}{q^2 D^2 - 1} \right] e^{\frac{-z}{p}}. \quad (3.12) \]
Solving for the constants of integration $c_1$ and $c_2$ by applying the boundary conditions in (3.8) yields the final solution in the Laplace domain:

\[ \bar{u} = \frac{QDc_1}{p^2 - \gamma^2} e^{-\gamma p} + \frac{QD^2c_2}{p(p^2 - \gamma^2)} e^{-\frac{z}{c_2}}, \]  

(3.13)

where

\[ \gamma = \frac{c_1}{D}. \]  

(3.14)

The final solution is transformed back into the time domain using the following transforms:

\[ L\left\{ \begin{array}{ll}
0, & t < \alpha \\
 f(t-\alpha), & t \geq \alpha
\end{array} \right\} = e^{-\alpha p} \tilde{f}(p), \quad \frac{1}{P} = 1 \quad \text{and} \quad \frac{1}{P + \alpha} = e^{-\alpha p}. \]  

(3.15)

The one dimensional surface displacement is finally given by:

\[ u = \frac{\beta K \theta D}{2 \rho c_i^2} \left[ e^{\frac{t}{\eta \left( \frac{z}{\alpha} \right)}} + e^{\frac{t}{\sigma \left( \frac{z}{\alpha} \right)}} - 2e^{\frac{z}{c_i}} \right] \quad t \geq \frac{z}{c_i} \]  

(3.16)

and

\[ u = \frac{\beta K \theta D}{2 \rho c_i^2} \left[ e^{\frac{t}{\eta \left( \frac{z}{\alpha} \right)}} + e^{\frac{t}{\sigma \left( \frac{z}{\alpha} \right)}} - 2e^{\frac{z}{c_i}} \right] \quad t < \frac{z}{c_i}. \]  

(3.17)
At the surface (z=0), the solution simplifies to:

\[ S(t) = -u_z = S_0 \left[ 1 - e^{-\frac{z_0}{D}} \right], \]  

(3.18)

where

\[ S_0 = \frac{\beta K \Theta_o D}{\rho c_i^2} = \frac{\beta \Theta_o D}{3} \left( \frac{1 + \nu}{1 - \nu} \right) = \frac{\beta \phi_o}{3 \rho c_v} \left( \frac{1 + \nu}{1 - \nu} \right). \]  

(3.19)

\( \phi_o \) is the incident laser fluence, and \( c_v \) is the heat capacity at constant volume. The time constant of the surface displacement is a function of absorption depth \( D \) and longitudinal speed of sound \( c_i \) only: \( \tau_m = D/c_i \).

In general, the input laser pulse has a finite duration and the impulse solution must be convoluted with the laser pulse temporal profile. The surface displacement can be calculated from the following equation:

\[ S(t) = \int_0^\infty (1 - e^{-dx}) g(x) dx, \]  

(3.20)

where \( d = \tau_m^{-1} \) and \( g(x) \) is the laser temporal profile. The temporal profile of the laser system used in this thesis resembled a Gaussian function given by:

\[ g(x) = I e^{-ax^2}, \]  

(3.21)
where

$$a = \frac{2.8}{\text{FWHM}^2}. \quad (3.22)$$

FWHM is the full width half max of the laser temporal profile and I is the peak intensity.

Solving eq. (3.20) yields the final one dimensional surface displacement caused by the absorption of a Gaussian laser pulse:

$$S(t) = \frac{I\beta\phi_0}{3\rho c_v} \left(\frac{1+\nu}{1-\nu}\right) \sqrt{\frac{\pi}{4a}} \left[ 1 + \text{erf}\left(\frac{1}{a^2 t}\right) - e^{-d^2/4a} e^{-d^2} \left\{ 1 + \text{erf}\left(\frac{2at-d}{4a}\right) \right\} \right]. \quad (3.23)$$

For short laser pulses or long absorption depths ($t_p << \tau_m$) eq. (3.23) will simplify to the impulse solution given in eq. (3.18). For long laser pulses or short absorption depths ($t_p >> \tau_m$) the surface displacement will simply replicate the integral of the incident laser pulse profile. In the case of a Gaussian profile, the surface displacement will be given by eq. (3.24). Note, the onset of surface expansion in both eq. (3.23) and eq. (3.24) occurs before $t = 0$.

$$S(t) = \sqrt{\frac{\pi}{4a}} \left[ 1 + \text{erf}\left(\frac{1}{a^2 t}\right) \right]. \quad (3.24)$$
It is interesting to note that eq. (3.24) could be used to study photochemical processes such as fluorescence lifetimes, where there is a delay thermalizing absorbed laser energy.

For all of our experiments, the effect of the finite laser pulse was small since the laser pulse duration $t_p$ was much shorter than the time constant $\tau_m$ of the surface displacement. This condition is termed stress confinement and is defined as $t_p \ll \tau_m$.

The governing equations were also implemented numerically using a finite element method (FEM) partial differential equation solver (Matlab, The Mathworks, Natick, MA). The FEM solution and analytical solution were identical and are both shown in figure 3-1. The properties used for the figure correspond to typical parameters used in the IPMS verification experiments.
3.3 Verification of Interferometric Photomechanical Spectroscopy

3.3.1 Overview

To test the validity and access the accuracy of IPMS, a set of well characterized glass samples were investigated. The glass samples chosen were absorbing neutral density filters obtained from Schott Glass Technologies Inc. (Duryea, PA). Neutral density filters were chosen because the absorption depth does not vary significantly with
wavelength. In particular, the absorption depth between 450 nm and 600 nm remains essentially constant.

3.3.2 Experimental Setup

The interferometric system was previously described in Chapter 2 and illustrated in figure 2-1. The beam expander and 80 mm gradium focusing lens were not necessary since the glass targets have smooth surfaces. The larger interferometric spot size compensated for minor spatial inhomogeneities within the incident pump laser beam and avoided the need for complex beam homogenizing techniques.

Laser System

The pump laser used was a Q-switched Nd:YAG (yttrium aluminum garnet with neodymium ions) laser, frequency doubled to operate at 532 nm. This wavelength was chosen since it is centered in the flat portion of the absorption spectrum of the neutral density filters. The pulse duration of the laser beam was about 7 ns, and had a Gaussian temporal shape. figure 3-3 illustrates a typical temporal profile. The spatial profile was analyzed using a CCD Camera (Cohu 4800, Cohu Inc), and found to resemble a flat top. The full width half max (FWHM) diameter of the raw beam was estimated to be 6 mm. Figure 3-4 illustrates the spatial profile.
Figure 3-2: Frequency doubled Q-switched Nd:YAG temporal laser profile with a FWHM pulse duration of about 7 ns.

Figure 3-3: Q-switched Nd:YAG spatial laser profile at 532 nm with a FWHM diameter of 6 mm.
The frequency doubled Nd:YAG laser irradiation was directed toward the sample by dielectric mirrors. The angle of incidence at the sample was 15°. The angle of laser radiation propagation within the target is a function of the angle of incidence and the refractive index of the target. The refractive index for glass is about 1.5, and so the angle of laser radiation propagation within the glass targets was about 10°. The measured absorption depth was corrected for this angle by dividing by the cosine of 10°. A diagram of the setup is shown in figure 3-4. The laser fluence (energy per unit area) used for experiments ranged from 10 - 550 mJ/cm². It should be noted though, that IPMS does not rely on incident laser fluence to calculate absorption depth.

Figure 3-4: Schematic of experimental setup for IPMS verification
Glass Targets

The absorption depth for several neutral density filters is shown in Figure 3-5.
The absorption depth was calculated from transmission curves supplied by the manufacturer, and under the assumption that the laser energy is absorbed exponentially with depth (z).

![Figure 3-5: Absorption depth of Schott neutral density glass filters](image)

The only property necessary to calculate absorption coefficient using IPMS is the speed of sound ($c_1$). Although the speed of sound is usually known, it can be calculated from Young’s modulus, Poisson Ratio and density as illustrated in eq. (3.6). Table 3-1
summarizes these properties and the resulting speed of sound. The speed of sound of glass is about 3.5 times larger than the speed of sound in water, the main constituent of biological tissue.

<table>
<thead>
<tr>
<th>Glass Type</th>
<th>Young's Modulus $X 10^3$ Kgm/(s$^2$mm$^2$)</th>
<th>Poisson Ratio</th>
<th>Density g/cm$^3$</th>
<th>Speed of Sound (μm/ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG1</td>
<td>62</td>
<td>0.229</td>
<td>2.49</td>
<td>5.37</td>
</tr>
<tr>
<td>NG10</td>
<td>62</td>
<td>0.229</td>
<td>2.47</td>
<td>5.39</td>
</tr>
<tr>
<td>NG9</td>
<td>62</td>
<td>0.229</td>
<td>2.44</td>
<td>5.42</td>
</tr>
<tr>
<td>NG3</td>
<td>62</td>
<td>0.229</td>
<td>2.44</td>
<td>5.42</td>
</tr>
<tr>
<td>NG4</td>
<td>62</td>
<td>0.227</td>
<td>2.43</td>
<td>5.43</td>
</tr>
<tr>
<td>NG5</td>
<td>62</td>
<td>0.229</td>
<td>2.43</td>
<td>5.43</td>
</tr>
</tbody>
</table>

Table 3-1: Material properties and calculated speed of sound for several Schott neutral density filters

3.3.3 Results

A sample displacement trace for glass filter NG1 is shown in figure 3-6. A single pulse of Nd:YAG pump laser is absorbed by the glass sample at 0 ns, generating a thermoelastic stress within the glass sample. The thermoelastic stress propagates through the glass, reflecting off the free surface and causing a non-linear surface displacement that is a function of the sample’s absorption depth $D$ and speed of sound $c_r$. Before the laser pulse is absorbed, the glass surface is stationary, and there is no surface displacement. For times longer than 300 ns, shear waves influence the surface profile,
and the laser-target geometry is no longer one dimensional. The surface displacement will eventually decay back to the zero baseline via thermal diffusion. The time scale for diffusion is $\tau \sim D^2/\alpha$ where $\alpha$ is the thermal diffusivity of the target. For the absorption depths used in these experiments, the thermal diffusion time ranges from 0.02 – 6 s. The maximum surface displacement is 45 nm and is a function of material properties and incident laser energy only. This information could be used as an additional diagnostic metric, although the need for an accurate local fluence may limit its potential.

Figure 3-6: Sample displacement trace for Schott glass type NG1.
A Gauss-Newton non-linear least squares fitting algorithm was used to compute the absorption depth and maximum displacement of each trace. Figure 3-7 depicts the surface displacement with the best fit solution.

![Graph showing displacement over time](image)

Figure 3-7: Sample displacement trace and best fit solution for Schott glass type NG1

The best fit surface displacement solution had an absorption depth of 101 μm and clearly matched the measured surface displacement. We repeated these measurements for laser energies ranging from 12 - 440 mJ/cm². The displacement traces and best fit curves are shown in figure 3-8. The maximum displacement at the lowest fluence of 12 mJ/cm² displacement trace was 0.8 nm
Figure 3-8: Sample displacement traces and best fit solutions for Schott glass type NG1. Laser fluence ranged from 12 - 440 mJ/cm².

Figure 3-9 shows surface displacement traces with best fit solutions for all the Schott glass filters used. The laser fluence was 550 mJ/cm². For convenience, the maximum displacements have been normalized.
Figure 3-9: Sample displacement traces with best fit solutions for all the Schott glass filters

The best fit solutions perfectly match the measured surface displacement traces for all glass samples. Table 3.2 summarizes the measured absorption depths for all glass samples, compared to the values computed from transmission curves given by Schott Glass Technologies. The measured absorption depth is given as the mean ± one standard deviation.
The IPMS technique yields absorption depths well within the range given by Schott Technologies. The relatively large ranges given by Schott Glass Technologies, indicate the inherent inaccuracies of extracting absorption depth of highly absorbing materials using standard transmission spectroscopy. Since IPMS is not performed in transmission mode, it may be a more accurate technique for measuring absorption depth in highly absorbing materials.

To evaluate the consistency of IPMS, we calculated the absorption depth of NG 9 from 350 separate surface displacement traces. The traces were obtained over a period of two months under different operating and alignment conditions. Figure 3-10 plots all 350 traces and clearly illustrates the robustness of IPMS. The y-axis limits correspond to the absorption depth range given by Schott Glass Technologies.

Table 3-2: Measured absorption depths for Schott neutral density filters compared with values obtained from Schott Glass Technologies.

<table>
<thead>
<tr>
<th>Glass Type</th>
<th>Measured Absorption Depth (60 points)</th>
<th>Schott Given Absorption Depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG1</td>
<td>101 ± 0.8 μm</td>
<td>106 ± 50 μm</td>
</tr>
<tr>
<td>NG10</td>
<td>187 ± 1.5 μm</td>
<td>188 ± 11 μm</td>
</tr>
<tr>
<td>NG9</td>
<td>305 ± 2.2 μm</td>
<td>306 ± 50 μm</td>
</tr>
<tr>
<td>NG3</td>
<td>435 ± 3.5 μm</td>
<td>434 ± 40 μm</td>
</tr>
<tr>
<td>NG4</td>
<td>858 ± 10 μm</td>
<td>856 ± 70 μm</td>
</tr>
<tr>
<td>NG5</td>
<td>1780 ± 80 μm</td>
<td>1790 ± 170 μm</td>
</tr>
</tbody>
</table>
3.4 IPMS on Tissue Phantoms

3.4.1 Overview

IPMS is not limited to smooth specularly reflecting targets, and can be applied to diffuse targets such as tissue. When measuring surface displacements on diffuse samples, the interferometric system must include the beam expander and target focusing lens. This ensures sufficient coherent light is reflected from the diffuse surface. To demonstrate IPMS on a diffuse surface, the surface displacement of gelatin phantoms doped with an
absorbing dye were investigated. The absorbing dye was used to increase the absorption of the tissue phantom to ensure one dimensional laser-tissue geometry. Potassium Chromate ($K_2CrO_4$, Aldrich Chemical Company, Milwaukee, WI) was chosen as the absorbing dye, since the absorption properties have been measured and it is reported to be photochemically stable [55, 67]. These investigators estimated the absorption depth of a 35g/L $K_2CrO_4$ solution to be 10μm. The extinction coefficient ($\varepsilon$), a more common descriptor for absorbing dyes is given by the following expression:

$$\varepsilon = \frac{1}{\ln(10)C_m D}$$  \hspace{1cm} (3.25)

where $C_m$ is the molar concentration, and $D$ is the absorption depth. The extinction coefficient was calculated to be 2410 Lmol$^{-1}$cm$^{-1}$.

3.4.2 Experimental Setup

The experimental setup was similar to the setup used in the IPMS verification experiments described in section 3.2, except the beam expander and target focusing lens was used. The Q-switched Nd:YAG pump laser was operated at its third harmonic with a wavelength of 354.7 nm. The spatial profile resembled a top hat with a FWHM diameter of 6 mm. The temporal profile was Gaussian in shape with a FWHM pulse duration of about 8 ns. The spatial and temporal profiles were similar to the profiles when the laser was operated at 532 nm in the previous section. The laser fluences used in these experiments were 70 mJ/cm$^2$. 

62
Two separate batches (A and B) of potassium chromate solutions were prepared with molar concentrations of 0.0188M, 0.0169M and 0.0154M, with estimated absorption depths of 95 µm, 106 µm and 117µm respectively. These solutions were prepared so we could verify the extinction coefficient of potassium chromate, and confirm the accuracy of IPMS by differentiating subtle changes in absorption coefficient. To minimize preparation errors, all potassium chromate solutions were filtered dilutions of two 0.169M stock solutions. The filters used were 0.2 µm sterile Acrodiscs (Gelman Sciences, Ann Arbor, MI). All solutions were prepared with distilled water. Tissue phantoms were prepared by mixing 10% of gelatin (G-1890 Type A gelatin, Sigma, St. Louis, MO) by weight with a 0.0169M potassium chromate solution from batch A. This enabled the influence of gelatin to be directly observed.

3.4.3 Results

Potassium Chromate Solutions

A sample displacement trace and best fit solution for a 0.0169M potassium chromate solution from batch A is shown in figure 3-11. The best fit solution clearly matched the measured displacement trace and had an absorption depth of 86 µm, which was lower than the estimated value of 106 µm.
Figure 3-11: Sample displacement trace and best fit solution for a 0.0169M potassium chromate solution from batch A.

Table 3.3 summarizes the results for the three potassium chromate solutions from batch A and B.

<table>
<thead>
<tr>
<th>Molar Concentration (λ = 354.7 nm)</th>
<th>(A) Measured Absorption Depth (30 points)</th>
<th>(B) Measured Absorption Depth (30 points)</th>
<th>Estimated Absorption Depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0188M</td>
<td>75 ± 1.9 μm</td>
<td>86 ± 2.0 μm</td>
<td>95 ± ~ 50 μm</td>
</tr>
<tr>
<td>0.0169M</td>
<td>86 ± 2.5 μm</td>
<td>96.3 ± 1.8 μm</td>
<td>106 ± ~ 50 μm</td>
</tr>
<tr>
<td>0.0154M</td>
<td>93.2 ± 3.0 μm</td>
<td>106 ± 2.4 μm</td>
<td>117 ± ~ 50 μm</td>
</tr>
</tbody>
</table>

Table 3-3: Measured absorption depth for three potassium chromate solutions from two different batches (A and B) compared with estimated values.
These results show excellent agreement within each batch, clearly differentiating subtle changes in absorption depth. However, there is some discrepancy between each batch and the estimated absorption depth. These discrepancies are most likely due to differences in preparing the potassium chromate solutions. If we calculate the extinction coefficient based on the measured absorption depths, we get 2972 Lmol\(^{-1}\)cm\(^{-1}\) and 2623 Lmol\(^{-1}\)cm\(^{-1}\) for batch A and B respectively. Both these values are reasonably close to the value estimated from the literature (2410 Lmol\(^{-1}\)cm\(^{-1}\)), and the value we obtained via standard transmission experiments (2800 Lmol\(^{-1}\)cm\(^{-1}\)).

Our results highlight the uncertainty in obtaining accurate absorption depth information from highly absorbing samples. In such cases, IPMS appears to be a very suitable technique capable of detecting small absorption depth differences.

**Gelatin Phantoms**

A sample displacement trace and best fit solution for a gelatin doped tissue phantom is shown in figure 3-12. The best fit solution clearly matched the measured displacement trace and had an absorption depth of 136 µm. This absorption depth was higher than the absorption depth of the 0.0169M potassium chromate solution alone (A - 86 µm).
Figure 3-12: Sample displacement trace and best fit solution for a gelatin doped tissue phantom. The tissue phantom was prepared with the 0.0169M potassium chromate solution from batch A.

The absorption depth of gelatin doped tissue phantoms appeared to decrease with repeated laser pulses. Although this decrease was small, it was clearly detectable. Figure 3-13 compares the calculated absorption depth for the 0.0169M potassium chromate solution with the gelatin phantom prepared with the same solution.
Figure 3-13: Calculated absorption depth for 0.0169M potassium chromate solution (batch A) and calculated absorption depth for a gelatin tissue phantom prepared with the same solution.

It was unclear why the absorption depth decreased with each additional laser pulse. The experiments were also performed on other gelatin types, including type A (G-2625, Sigma, St. Louis, MO), type B (G-9391, Sigma, St. Louis, MO) and Knox unflavored Gelatine (Nabisco, Hanover, NJ). The calculated absorption depth of these phantoms exhibited dependence on gelatin type, concentration of potassium chromate solution and laser fluence. Potassium chromate solutions alone were stable, although they did exhibit slight photobleaching effects (< 8%) at high fluences. The absorption of all gelatin type phantoms prepared without the dye was small (D ~ 3 mm – 8 mm). The specific role of gelatin type, potassium chromate concentration and laser fluence on
absorption depth was not investigated. Explanations for these effects may include photochemical processes, or laser induced changes in spatial absorption properties of the phantom. For example, the concentration of dye within the gel may be slightly altered after each laser pulse, thereby creating inhomogeneous absorption. We have observed these phenomena in gelatin phantoms prepared with India ink, and pumped with infrared wavelengths where no photochemical effects play a role.

Gelatin type, absorbing dye and laser energy do influence optical properties of tissue phantoms. Although some of these effects may be small, they were clearly detectable with IPMS. We feel it is important to keep these effects in mind when preparing tissue phantoms to be used for testing new techniques or applications.

3.5 Discussion

Interferometric Photomechanical Spectroscopy (IPMS) is a powerful non-contact technique which accurately extracts the absorption depth of a sample. The absorption depth is calculated from the sample’s surface expansion after absorption of a short laser pulse. The time dependent surface expansion is a function of optical penetration depth.

We have verified the accuracy of IPMS by calculating the absorption depth of well characterized absorbing glass samples. The range of computed absorption depths were more than an order of magnitude lower than the values given by the glass manufacturer. In addition, IPMS was very robust, producing consistent results when performed under different alignment and laser operating conditions. The consistent accurate results of IPMS are primarily due to the high spatial and temporal resolution of the interferometric system. IPMS relies on non-linear fitting to the time dependent
surface displacement of a sample. If the surface displacement is acquired with negligible noise, or very high spatial resolution, the range of possible fitting parameters is narrow. This is particular beneficial when fitting to only a portion of a sample’s surface displacement, as is the case for samples from long absorption depths. This is an attractive feature which avoids the need for using the full three dimensional numerical wave solution to extract absorption depths for weaker absorbing samples. The maximum surface displacement could also be used to extract additional diagnostic information, since it is a function of thermal expansion coefficient, Poisson ratio, density and specific heat.

The challenge of diagnostic spectroscopy is to measure changes in optical properties such as effective absorption depth, and equate these changes with specific changes in tissue state. Often, changes in the optical properties are subtle, and must therefore be measured with high resolution techniques. IPMS is a high resolution technique capable of differentiating small changes in absorption depth. Further, IPMS could be implemented into an optical fiber system and be used as a clinically valuable minimally invasive diagnostic tool.
Chapter 4

Interferometric Photomechanical Tomography (IPMT)

4.1 Introduction

There is great demand for non-invasive techniques to image deep sub-surface absorbers such as tumors and blood vessels in highly scattering tissue. Such techniques would be very useful for breast cancer screening, laparoscopic and endoscopic procedures, and a variety of laser-tissue interactions including treatment of port wine stains. The major challenge in imaging absorbers deep in tissue light is to maintain high resolution despite significant tissue scattering. Coherent imaging techniques such as optical coherence tomography and confocal microscopy cannot be used to detect deep absorbers in tissue. Standard confocal microscopy has a penetration depth of only 100-200 μm, and research based confocal microscopy have obtained depths up to 400 μm [13, 19, 33, 77, 84]. Optical coherence tomography tends to have a slightly larger penetration of about 1-2 mm for highly scattering tissue [13, 84], although in our experience, a high resolution clinical OCT instrument had a penetration depth of under less than 1 mm in
skin. The same instrument used in the gastrointestinal (GI) tract had penetration depths of up to 2 mm [12].

There are other optical techniques that use diffuse light instead of coherent light for imaging or characterization of sub-surface absorbers such as photon migration [99]. In addition, there are non-optical imaging techniques that are used for detecting deep tumors. These techniques include X-ray radiography, magnetic resonance imaging and ultrasound imaging. Although these techniques do not suffer from optical scattering, the major limitation is usually associated with low contrast leading to insufficient sensitivity for the detection for small tumors or blood vessels. Photoacoustic tomography (optoacoustic imaging) is a new technique which utilizes high contrast optical absorption to generate mechanical transients which propagate relatively unaffected through large tissue sections (e.g. [26, 41, 55, 68, 101]). Photoacoustic imaging uses a pump probe technique, where a short pulse of laser light is preferentially absorbed by a sub-surface target such as a tumor or blood vessel. The sub-surface target expands and creates an acoustic or stress wave that propagates to the tissue surface where it is measured by one or a series of pressure transducers. Photoacoustic tomography essentially captures the most beneficial attributes of optical and ultrasound imaging techniques by combining high optical contrast with deep penetration properties of sound transients.

In general, non-invasive photoacoustic medical imaging techniques must be performed in an epitaxial or so called backward mode. Consequently, standard front surface pressure transducers cannot be used as they would physically block the incident laser light. To solve this problem, investigators have used a variety of creative techniques, including piezoelectric transducers with separated light and sound fields,
transparent transducers measuring optical reflectance, annular piezoelectric elements, and acoustic conductors where sound generated by an obliquely incident laser pulse propagates predominantly normal to the tissue surface [8, 9, 17, 55, 57, 69]. Alternatively, an interferometric technique that is inherently epitaxial could be used to image sub-surfaces absorbers. The ultra high spatial and temporal resolution interferometer discussed in Chapter 2 is ideal for imaging sub-surface absorbers by measuring surface displacements caused by acoustic transients. We call this technique Interferometric Photomechanical Tomography. We use the term photomechanical rather than photoacoustic or optoacoustic, because we measure surface deformation and not acoustic transients.

In addition to being epitaxial, there are many significant advantages for using an interferometric surface monitoring technique. First, interferometric monitoring of surface displacement is a non-contact method, which is highly beneficial in medical applications. Second, IPMT is a point measurement, limited only by the numerical aperture of the imaging optics. It has been shown that the lateral resolution for optoacoustic imaging is limited by transducer size [41]. Third, the interferometer has a very high bandwidth limited only by the digitizing system (500 MHz) and does not suffer from calibration uncertainty. Fourth, the interferometric system could easily be implemented into an optical fiber setup, enabling minimally invasive sub-surface imaging during catheter based procedures such as endoscopy. Fiber optic components and related technologies for minimally invasive interferometric techniques such as optical coherence tomography already exist and could be readily adapted to our interferometric system. Lastly, the interferometric system could be modified to produce an array of probe beams to allow
simultaneous point measurements. This would enable full three-dimensional imaging in a single shot.

Although interferometric surface monitoring of photomechanical transients has many significant advantages over measurements made by pressure transducers, it has not been pursued in the medical community. In fact, we only found one article that used an interferometric system for medical imaging of acoustic transients [52]. This is surprising because the pioneering studies by Albagli and coworkers showed great potential [2, 3, 48]. The reason why interferometric techniques were not pursued for medical imaging may be due to the relatively low spatial resolution (± 4 nm) reported in these earlier studies. Photomechanical transients from deep absorbers are attenuated inversely to distance traveled, and consequently sensitive surface displacement or stress detection is necessary. The interferometric system described in Chapter 2 is capable of a spatial resolution which is 50 times more sensitive than the interferometric system in these earlier studies. We feel that this significant increase in spatial resolution, combined with the advantages listed above, should enable interferometric surface monitoring techniques such as IPMT to become a powerful tool for imaging in vivo deep sub-surface absorbers such as tumors and blood vessels. In addition, current work in photoacoustic image reconstruction for stress based techniques can be applied to interferometric photomechanical techniques.

In this chapter, we have successfully applied Interferometric Photomechanical Tomography (IPMT) to image sub-surface blood vessels in a phantom model and in vivo. We used a simple scanning technique to extract vessel size and depth information.
4.2 Verification of Interferometric Photomechanical Tomography

4.2.1 Overview

IPMT was performed in several well characterized tissue-blood vessel phantom models, and in the forearm of a volunteer human subject. The tissue phantoms consisted of either pure water, 1% Intralipid solution or 7% Intralipid solution. Although the majority of investigators use 1% Intralipid solutions as tissue phantoms, we feel a 7% Intralipid solution more accurately mimics highly scattering biological tissue. The blood vessel phantoms consisted of different diameter polyimide tubes. The polyimide walls were acoustically thin (19 μm – 50 μm), so stress reflections due to impedance mismatch between the vessel wall and surrounding medium were not observed. Stress reflections were observed in thicker walled phantom vessels. Although stress reflections could be used to extract size information, such phantom vessels are not realistic.

Currently, photoacoustic imaging is performed by measuring acoustic transients generated from a sub-surface absorber at multiple surface locations. Each spatially resolved stress history is combined in a reconstruction algorithm to create an image of the sub-surface absorber. An excellent example of this technique was recently illustrated by Hoelen and co-workers who imaged phantom blood vessels using a transducer array in the forward mode [23, 41, 42]. IPMT could also be performed in a similar fashion, where an array of interferometer probes measuring surface displacements are used to reconstruct sub-surface tomography. However, we used an alternate technique which has not been
previously used in photoacoustic imaging. It is a simple scanning technique which avoids
the need for complex image reconstruction techniques.

The scanning technique is illustrated in figure 4-1. The interferometer probe beam
is centered within the incident pump beam on the target surface. Single shot
measurements were obtained across the sample’s surface by moving the sample via a
digital micrometer stage. Each spatially resolved time trace was stored and combined
later to form a tomographic image.

Figure 4-1: IPMT scanning setup. Surface displacement traces across the sample were obtained by
scanning the tissue phantom on a digital micrometer.
4.2.2 Experimental Setup

Laser System

The pump laser used was a Q-switched Nd:YAG laser, operating at its fundamental wavelength of 1064 nm. This wavelength was chosen since it is absorbed more strongly by blood than the surrounding tissue and therefore could also be used for the in vivo experiments described later. The pulse duration of the laser beam was about 8 ns, and had a Gaussian temporal shape. The spatial profile was analyzed using a CCD Camera (Cohu), and found to resemble a flat top. The full width half max (FWHM) diameter of the raw beam was measured to be about 6.6 mm. For the scanning experiments, the beam was focused to a spot with a diameter of 0.6 mm. The lens had a long focal length of 1000 mm to ensure the beam diameter did not vary significantly at the target. Figure 4-2 illustrates the spatial energy distribution of the focused spot at the target.

The Q-switched Nd:YAG was directed towards the sample by gold mirrors. The angle of incidence at the sample was about 15°. A diagram of the setup is shown in figure 4-3. The laser fluence used for the experiments ranged from 0.2 J/cm² to 1.5 J/cm² depending on the amount of scattering of the tissue phantom being probed.
Figure 4-2: Spatial profile of a focused Q-switched Nd:YAG laser beam at 1064 nm. The FWHM diameter of the spot was about 0.6 mm.
Figure 4-3: Schematic of experimental setup for IPMT

Phantom Model

The tissue phantoms consisted of either pure water, 1% Intralipid, or 7% Intralipid solution. The Intralipid solutions were made by diluting stock 10% Intralipid solution (Pharmacia Inc., Clayton, NC). The optical properties of Intralipid at 1064 nm were obtained from Royston and coworkers [80]. Note, the authors quote the optical properties of Intralipid as a percentage of 10% stock solution, not the total percent. The reduced optical scattering coefficient at 1064 nm were is 0.65 mm$^{-1}$ and 4.55 mm$^{-1}$ for 1% and 7% Intralipid solution respectively. A 7% Intralipid solution more accurately mimics highly scattering tissue such as skin, which has a reduced scattering coefficient of 3.55 mm$^{-1}$ [18].
The blood vessel phantoms consisted of thin walled polyimide tubes of diameters ranging from 200 – 1000 μm (Cole-Parmer, Vernon Hills, IL). The wall thickness was between 19 μm – 50 μm and were considered acoustically thin. The polyimide tubes did not absorb significant laser energy. A dilute solution of black India ink (Higgins, Bellwood IL) was used to simulate the absorption properties of blood. The absorption coefficient of whole blood at 1064 nm is about 0.5 mm⁻¹ [76, 94]. The optical properties of India ink at 1064 nm were obtained from Royston and coworkers and verified using IPMS [80]. A 0.125% India ink solution (by volume) has an absorption coefficient of 0.45 mm⁻¹ or absorption depth of 2.2 mm and was used in all experiments. A simple syringe setup was used to flow the dilute India ink solution through the polyimide tubes after each measurement to prevent any ink particle separation.

In Vivo Human forearm

A vessel in the forearm of a human volunteer was also imaged using IPMT. It was not feasible to accurately scan the forearm through the pump and laser beam. Instead, single measurements were made on spatial locations either on or off the vessel. The vessel location was determined visually, by the presence of a faint blue/green line. A thin layer of water was used on top of the skin surface. This was not to used to generate a specular surface, but used simply to aid in alignment. This would not be necessary for a fiber optic based clinical system. Since there was no scanning, the 1064 nm Q-switched Nd:YAG laser was not focused onto the surface of the skin. We also used a clinical OCT system [11] to image the same location.
4.2.3 Results

Pure Water

A sample surface displacement trace of a vessel tissue phantom is shown in figure 4-4. The surface displacement is caused by the absorption and subsequent expansion of a sub-surface 1000 μm phantom blood vessel and surrounding pure water. The incident pump laser and interferometric probe beam were centered directly above the phantom blood vessel. A single pulse of Nd:YAG pump laser is absorbed by both the sub-surface absorber and surrounding water at 0 ns. The initial surface displacement is due to the absorption of laser radiation in the surrounding Intralipid. The surface displacement caused by the submerged phantom blood vessel starts at 2.52 μs. The time delay represents the time for the acoustic wave generated by the phantom vessel to reach the surface. The vessel depth is calculated by multiplying the delay time with the speed of sound in pure water. The longitudinal speed of sound in pure water at 25°C is 1.496 mm/μs [16]. The vessel depth was therefore calculated to be 3.78 mm. The upper axis of figure 4-4 represents depth as computed from delay time. The actual vessel depth was 3.82 mm, which is 40 μm less than the measured value. The actual depth is obtained by comparing the height of the vessel surface before any water is added, to the height of the water surface after the water is added. This is achieved by using a digital micrometer to move the phantom surface (vessel or water) vertically into the focal plane of the interferometric probe. The vertical resolution of this technique is about 10 μm, and is a function of the micrometer and the depth of focus of the interferometer probe beam.

This technique can be used to measure the speed of sound in an unknown liquid by measuring transit time of an acoustic wave through a known distance. We tested the
accuracy of this technique by calculating the speed of sound of a planar wave in pure water. The planar wave was generated by irradiating a Schott glass filter (NG1) through pure water. The average speed of sound at ten different depths was measured to be 1.493 – 1.513 mm/µs, which closely matches the given value of 1.496 mm/µs for pure water. Alternatively, assuming the speed of sound is 1.496 mm/µs, the average depth error was 14 µm. We repeated this measurement for 7% Intralipid solution, and measured the speed of sound to be 1.487 – 1.529 mm/µs, which is slightly higher than for pure water. This value is slightly lower than the value reported for human milk, which is 1.54 mm/µs [34]. Although these differences are small they should be taken into account when extracting accurate depth information.
Figure 4-4: Sample surface displacement of a vessel phantom model consisting of a 1000 µm polyimide tube surrounded by pure water.

Figure 4-5 illustrates displacement traces obtained from the same 1000 µm phantom vessel at different depths. The maximum displacement decreases linearly with depth as expected. The calculated vessel depths were 3.78 mm, 5.79 mm, 7.24 mm, which were within ± 45 µm of the actual depths (measured via digital micrometer).

Although the depth errors were small, they were significantly larger than the depth errors for the planar wave experiments (14 µm). The larger depth errors occurred because wave propagation effects for non-planar waves such as diffraction were not taken into account. Wave propagation effects should be accounted for if accurate depth information is necessary. Nonetheless, the high spatial resolution of the interferometer is beneficial.
when measuring surface displacement caused by acoustic waves generated from deep absorbers.

Figure 4-5: Sample surface displacement traces of a 1000 μm phantom vessel at different depths. The surrounding medium was pure water.

Surface displacement traces obtained at different spatial locations across the phantom model can be combined to form a tomographic image of the time dependent deformation. No other processing is necessary. A sample tomographic image for a 1.53 mm deep 495 μm diameter phantom blood vessel is shown in figure 4-6. A contour plot is shown in figure 4-7. The x-axis is the scanning axis, and the y-axis is the temporal or depth axis.
Figure 4-6: Sample tomographic image of a 495 μm diameter sub-surface phantom vessel in pure water. Vessel depth was 1.53 mm.
Figure 4-7: Sample contour image of a 495 µm diameter sub-surface phantom vessel in pure water. Vessel depth was 1.53 mm.

Figure 4-8 compares tomographic images for three different phantom vessels with diameters of 1000 µm, 495 µm and 198 µm respectively. The vessel depths were all about 1.5 mm. The bar in figure 4-8, and all other figures represents 500 µm. The vessel sizes are clearly distinguishable both in horizontal scanning axis (x-axis), as well as in the temporal or depth axis (y-axis).
Figure 4-8: Sample contour images of three different sized phantom vessels in pure water. The depths of the phantom vessel are all about 1.5 mm.

One way to obtain a quantitative measure on vessel size is to plot the maximum surface displacement as a function of length or scanning distance (x-axis) as is illustrated in figure 4-9. The maximum displacement profiles have been normalized and fitted to a Gaussian function. The width of the three profiles calculated at 60% of the maximum displacement are 970 µm, 510 µm and 230 µm for phantom vessel diameters 1000 µm, 495 µm and 198 µm respectively. The maximum displacement profile is clearly a good indicator of vessel size in a non-scattering medium.
Figure 4-9: Normalized maximum displacement profiles for three different phantom vessels in water. The diameters were of the vessels were 1000 μm, 495 μm and 198 μm. Gaussian functions have been fitted to the profiles.

Comparison of Pure Water, 1% Intralipid Solution and 7% Intralipid Solution

To access the effect of scattering, we repeated these experiments in 1% Intralipid solution and 7% Intralipid solution. Figure 4-10 compares tomographic images for a 1000 μm phantom vessel in pure water, 1% and 7% Intralipid solution. The depth in all three cases was about 1.5 mm. The vessel images for the 1% and 7% Intralipid solutions have been broadened in the horizontal scanning axis (x-axis), but not the temporal or depth axis (y-axis). The temporal profile is not strongly affected by the scattering properties of the phantom medium.
Figure 4-10: Sample tomographic images for a 1000 μm phantom vessel in pure water, 1% and 7% Intralipid solution. The depth in each media was about 1.5 mm.

The normalized maximum displacement profiles are shown in Figure 4-11. The width of the three profiles calculated at 60% of the maximum displacement are 970 μm, 1250 μm and 1720 μm for a 1000 μm diameter vessel in pure water, 1% and 7% Intralipid solution respectively. The maximum displacement profiles are broadened by scattering. The 1% Intralipid image and the pure water image are relatively similar indicating that scattering in 1% Intralipid solution is not very significant.
Figure 4-11: Normalized maximum displacement profiles for a 1000 \( \mu \)m diameter vessel in pure water, 1\% and 7\% Intralipid solution. Gaussian functions have been fitted to the profiles.

\textbf{7\% Intralipid Solutions}

A sample surface displacement of a 300 \( \mu \)m vessel tissue phantom is shown figure 4-12. The incident pump laser and interferometric probe beam were centered directly above the phantom blood vessel. A single pulse of the Nd:YAG pump laser is absorbed by both the sub-surface absorber and surrounding Intralipid at 0 ns. The initial surface displacement is due to the absorption of laser irradiation in the surrounding Intralipid. Since the Intralipid absorbs more laser energy than pure water, the initial surface displacement is greater than it was for the pure water phantoms (see figure 4-4). The surface displacement caused by the submerged phantom blood vessel starts at 0.67 \( \mu \)s.
The time delay represents the time for the acoustic wave generated by the phantom vessel to reach the surface. The vessel depth is calculated by multiplying the delay time with the speed of sound in 7% Intralipid, which was measured to be 1.508 mm/μm (see the results section under Pure Water). The vessel depth was therefore calculated to be 1.01 mm. The upper axis of figure 4-12 represents depth as computed from delay time. The actual vessel depth was 1.04 mm, which is 30 μm more than the measured value. The actual depth was obtained by comparing the height of the vessel surface before any Intralipid is added, to the height of the Intralipid surface after the Intralipid is added (as mentioned before).

Figure 4-12: Sample surface displacement of a vessel phantom model consisting of a 300 μm sub-surface polyimide tube surrounded by 7% Intralipid solution. The vessel depth was 1.04 mm.
Figure 4-13 illustrates displacement traces obtained for the same 300 μm phantom vessel at different depths. The calculated vessel depths were 1.04 mm, 1.65 mm, 2.13 mm, 2.72 mm, 3.27 mm, 3.82 mm which were within ± 60 μm of the actual depths (measured via digital micrometer). The maximum displacement no longer decreases linearly with depth. This is because the amount laser energy reaching the deeper vessels is significantly reduced due to scattering. In non-scattering media, the maximum displacement was proportional to depth (see figure 4-5). For a highly scattering medium, laser light penetration may be a limiting factor when imaging subsurface absorbers.

Figure 4-13: Sample surface displacement traces of vessel phantoms at different depths in a 7% Intralipid solution. The phantom vessel size was 300 μm in diameter.
Surface displacement traces obtained at different spatial locations across the phantom model were combined to form a tomographic image of the time dependent deformation. A sample tomographic image for a 1.36 mm deep 1000 μm diameter phantom blood vessel is shown in figure 4-14. A contour plot is shown in figure 4-15. The x-axis is the scanning axis, and the y-axis is the temporal or depth axis. The initial surface displacement due to laser absorption be the Intralipid is clearly visible.

Figure 4-14: Sample tomographic image of a 1000 μm diameter sub-surface phantom vessel in a 7% Intralipid solution. Vessel depth was 1.36 mm.
Figure 4-15: Sample contour image of a 1000 μm diameter sub-surface phantom vessel in a 7% Intralipid solution. Vessel depth was 1.36 mm.

Figure 4-16 compares tomographic images for three different phantom vessels with diameters of 1000 μm, 495 μm and 198 μm respectively. The vessel images are significantly broadened in the horizontal scanning axis (x-axis). The temporal or depth axis (y-axis) has not been significantly affected by the scattering properties of the phantom medium.
Figure 4-16: Sample contour images of three different sized phantom vessels in a 7% Intralipid solution. The depths of the phantom vessel are all about 1.4 mm.

The normalized maximum displacement profiles are shown in Figure 4-17. The width of the three profiles calculated at 60% of the maximum displacement are 1720 μm, 1370 μm and 1150 μm for phantom vessel diameters 1000 μm, 495 μm and 198 μm respectively. The maximum displacement profile is no longer a good indicator of vessel size in a highly scattering medium. However, the temporal profile is still a good indicator of vessel size, since it is not significantly affected by the highly scattering medium. This clearly illustrates the advantage of using wave propagation over diffuse light propagation for obtaining high resolution images in highly scattering mediums. To underscore this point further, the temporal profile of three different phantom vessels with diameters of 1000 μm, 300 μm, and 198 μm is shown in figure 4-18. The depth of all the three phantoms was about 2.2 mm. The temporal profile and maximum displacement is significantly different for each phantom vessel and could both be used to extract size information. The exact temporal profile is a function of vessel size, and wave propagation properties including diffraction and attenuation.
Figure 4-17: Normalized maximum displacement profiles for three different phantom vessels in a 7% Intralipid solution. The diameters were of the vessels were 1000 μm, 495 μm and 198 μm. Gaussian functions have been fitted to the profiles.
Figure 4-18: Sample surface displacement traces of three different vessels at a depth of 2.2 mm in a 7% Intralipid solution. The diameters of the vessels were 1000 µm, 300 µm and 198 µm.

**In Vivo human forearm**

Since a scanning setup was not feasible, single measurements were made on spatial locations either on or off the vessel. Figure 4-19 illustrates two sample surface displacement traces caused by the absorption of 1064 nm laser radiation within the forearm. The top trace was obtained when the pump and probe beam were centered on top of the vessel, and the bottom trace was obtained when the pump and probe beam were centered to the side of the vessel. In both cases, the alignment was not optimized, and the baseline STD was 0.25 nm. A fiber optical setup would circumvent alignment
difficulties, and increase spatial resolution. Nonetheless, the presence of the blood vessel is clearly detected.

The initial surface displacement is caused by absorption of laser radiation within the water layer. At 1.6 µs, there is an increase in surface displacement owing to absorption within the forearm skin. The increase of absorption is expected, since skin contains additional chromophores such as melanin and blood. The water thickness is estimated from the transit time of 1.6 µs to be 2.4 mm. The displacement in the top trace caused by the submerged blood vessel starts at 2.2 µs. Using a speed of sound of 1.5 mm/µs for skin [34], the vessel depth was estimated to be 0.9 mm. This appears to be a realistic estimate, since the vessel was faintly visible from the surface.
Figure 4-19: Two sample surface displacement traces obtained from the forearm of a human volunteer. The top trace was obtained directly above the vessel, and the bottom trace was obtained to the side of the vessel. The vessel depth is estimated to be 0.9 mm below the skin surface.

An OCT image was also taken directly on top of the same vessel and is shown in figure 4-20. The bar indicates 500 μm. Although the image indicates some texture, there is no clear indication of a blood vessel.
Figure 4-20: Sample OCT image taken directly on top of the same vessel in the forearm of a human volunteer. There is no clear indication that there is a sub-surface blood vessel.

4.3 Discussion

In this chapter, we have developed and verified a new technique called Interferometric Photomechanical Tomography (IPMT). IPMT was used to image sub-surface phantom blood vessels as well as an \textit{in vivo} blood vessel in a human forearm. IPMT was performed in a simple scanning mode to obtain tomographic images of sub-surface phantom vessels. The scanning technique was not feasible for the \textit{in vivo} experiments. A clinical optical fiber based interferometric system would be more suited for \textit{in vivo} applications.

We demonstrated IPMT in both non-scattering and highly scattering media including real tissue. In non-scattering media, the scanning technique produced accurate tomographic images in both the horizontal or scanning axis, and the temporal or depth axis. A quantitative measure of vessel size was achieved by comparing maximum surface
displacement profiles as a function of the scanning. The horizontal or scanning axis resolution in a non-scattering medium is a function of the laser spot size.

For highly scattering media, the horizontal or scanning axis resolution is significantly degraded. The maximum displacement profiles are broadened and can no longer be used to extract vessel size. The temporal profile however, is still a good indicator of vessel size, since it is not significantly affected by a scattering medium. This result highlights the advantage of using wave propagation over diffuse light propagation for obtaining high resolution images in highly scattering mediums. Phantom models made with 1% Intralipid solution behaved more like water than the 7% Intralipid. We feel a 7% Intralipid solution more accurately mimics highly scattering tissue, and should be used in tissue phantom experiments.

The vessel depth in both non-scattering and scattering mediums were accurately measured to within ±60 μm. The depth error resulted because wave propagation effects such as diffraction were not taken into account. For planar geometry, the depth error was significantly lower (14 μm). Wave propagation effects should be accounted for if accurate depth information is necessary.

Interferometric Photomechanical tomography is a potentially powerful non-contact technique used to image sub-surface absorbers in highly scattering media. A simple scanning technique can be used to accurate extract the size and depth of a sub-surface absorber. In highly scattering media, the size information must be extracted from the temporal profile. A clinical scanning setup would enable sub-surface tomographic images similar to ultrasound. IPMT could be combined with an image processing algorithm to increase the horizontal resolution in scattering media. For example, a simple
algorithm could utilize the spatially dependent transit times to reduce spatial broadening. IPMT could also be used with more complex reconstruction algorithms, similar to ones currently used for photoacoustic (e.g.,[41]). Since IPMT could be incorporated into an optical fiber setup, it may have real clinical value.
Chapter 5

Conclusions and Future Work

5.1 Achievements of This Thesis

We have developed two novel minimally invasive diagnostic techniques. The first technique is termed Interferometric Photomechanical Spectroscopy (IPMS) and measures the effective optical absorption depth of a sample. We used IPMS to measure effective absorption depth of both diffuse and speculary reflecting targets including well characterized colored glass samples and gelatin based tissue phantoms. The second technique is termed Interferometric Photomechanical Tomography and images sub-surface absorbers in scattering media. We used IPMT to image sub-surface blood vessels in a phantom model and in vivo. Both techniques measure surface deformation of a target after absorption of a short laser pulse, and relate this surface deformation to the target's spatially resolved optical, thermal and mechanical properties. We have also developed an ultra-high resolution interferometric system to be used in both these techniques.
The specific achievements of this thesis are given in the following three sections: Instrumentation, development and verification of Interferometric Photomechanical Spectroscopy, development and verification of Interferometric Photomechanical Tomography.

5.1.1 Instrumentation

We have designed and built an ultra-high resolution interferometric capable of angstrom spatial resolution, and sub nanosecond temporal resolution. This configuration was based on a previous version by Yablon and coworkers [108]. The spatial resolution has been improved by more than an order of magnitude. The primary reason for this improvement is the implementation of a dual balance detection system, as well as a gradium index lens. The best spatial resolution achieved on a highly reflecting target was 0.08 nm. The best spatial resolution achieved on a gelatin tissue phantom was 0.16 nm.

The improvement in spatial resolution is useful for detecting weak surface deformation when imaging deep sub-surface absorbers, or when differentiating subtle changes in optical properties.

5.1.2 Development and Verification of Interferometric Photomechanical Spectroscopy

A novel minimally invasive spectroscopic technique called Interferometric Photomechanical Spectroscopy (IPMS) was developed and verified. IPMS is a powerful non-contact technique which accurately extracts the absorption depth of a sample. The absorption depth is calculated from the sample’s surface expansion after absorption of a short laser pulse. The time dependent surface expansion is a function of optical
penetration depth. IPMS produced repeatable results with standard deviations of about 1%
of the sample’s absorption depth. The mean extracted absorption depths were wellwithin the range given by the manufacturer.

5.1.3 Development and Verification of Interferometric Photomechanical Tomography
A novel minimally invasive imaging technique called Interferometric Photomechanical Tomography (IPMT) was developed. IPMT was implemented in a simple scanning setup to image sub-surface phantom blood vessels in highly scattering media. IPMT was also used to image a blood vessel in the forearm of a human volunteer.

We compared sub-surface images of non-scattering and highly scattering media. The vessel depth in both non-scattering and scattering mediums were accurately measured to within ± 60 μm, although this value can be reduced by including the effects of wave propagation. The spatial or scanning resolution was significantly degraded when scattering was present. The temporal profile was not significantly affected by the highly scattering medium, and could be used to extract vessel size. This result highlights the advantage of using wave propagation over diffuse light propagation for obtaining high resolution images in highly scattering mediums.

We were able to clearly detect the presence of a blood vessel in vivo. The same vessel was not visible using optical coherence tomography. Interferometric Photomechanical tomography is a potentially powerful non-contact technique to image sub-surface absorbers in highly scattering medium.
5.2 Future Research Directions

This thesis has illustrated two potentially useful diagnostic techniques, based on a high resolution interferometric system. Although instrumentation advancements are possible (as described in section 2.3), we feel future research should be directed toward enabling real clinical applications. Four relevant areas are discussed below.

5.2.1 Comprehensive Comparison with Pressure Transducers

Interferometric techniques compare favorably against diagnostic techniques which measure surface stress instead of surface displacement. This is primarily because interferometry is a non-contact epitaxial method capable of high resolution point measurements. However, a comprehensive comparison with state of the art pressure transducers under similar conditions should be done, and clinical practicalities explored.

5.2.2 Clinical Instrumentation Development

The current interferometric system should be implemented into a optical fiber based setup. This would enable both IPMS and IPMT to be used for in vivo applications. Implementation of the current system into an optical fiber setup should not be difficult since interferometric fiber based technologies already exist for other techniques like optical coherence tomography. A scanning system or probe array could also be developed enabling fast 3-dimensional imaging.
5.2.3 Image Reconstruction Development

Although a simple scanning technique can be used to obtain reasonable tomographic images, image processing or reconstruction will improve spatial resolution in scattering media. Reconstruction techniques can be as simple as utilizing the spatially dependent transit times to reduce spatial broadening, or as complex as current photoacoustic imaging reconstruction techniques (e.g. [41]).

5.2.4 Simple Clinical Applications

Finally, simple clinical trials are necessary to confirm the diagnostic potential of both IPMS and IPMS. For example, IPMS could be used to compare effective absorption depth between pathological and non-pathological tissue. IPMT could be used during endoscopic procedures to localize sub-surface blood vessels in the gastrointestinal (GI) tract.
References


[38] E. Hecht, "Optics," 2 ed. 1990, Reading: Addison-Wesley. 676.


