OPTO-PHOTO-THERMO-ELASTIC DISPLACEMENT DETECTION, USING COHERENT CONFOCAL MICROSCOPE

A Thesis Presented
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to
The Department of Electrical and Computer Engineering
in partial fulfillment of the requirements for the degree of
Master of Science
in Electrical Engineering
in the field of
Electromagnetics

Northeastern University
Boston, Massachusetts

July 2008
ABSTRACT

Photothermal spectroscopy is a powerful tool to investigate the optical absorption and thermal characteristics of a sample. The photo-thermal effect, that is the basis for photothermal spectroscopy, is the conversion of optical energy into heat. Photothermal spectroscopy is implemented in a variety of methods. Biomedical imaging applications commonly implement the Photo-Thermo-Acoustic (PTA) method, that is based on measuring the acoustic pressure wave that propagates due to the photothermal effect, caused by absorption of energy from a heating laser.

This research demonstrates photothermal spectroscopy using a coherent confocal microscope. The high accuracy of the interferometer, that is the heart of the coherent confocal microscope, in detecting small changes in position makes it intrinsically adequate to measure the thermoelastic expansion of the sample that results from the photothermal process. In this research Polyvinyl-Chloride Plastisol (PVCP) samples, constructed with different absorption coefficients, were tested using different heating light fluences. The results are compared against an approximate theoretical model and are found to be in good agreement.

The Opto-Photo-Thermo-Elastic (Optical detection of elastic displacement changes due to the photothermal process) technique is demonstrated in this research as a first step toward extending the capability of confocal microscopes to image deeper into tissues than is presently possible, and to detect new modes of contrast.
ACKNOWLEDGMENTS

I would like to express my gratitudes to my advisor Prof. Charles A. DiMarzio, who inspired and guided me, with great patience and encouragement.

I would like to thank Prof. Gregory Kowalsky for the professional help and support on the thermodynamics aspects of this work, and also for serving as a committee member.

Also I would like to thank Prof. Carey M. Rappaport for serving as a member of the committee.

I would like to express my thanks for the people of the Optical Science Laboratory (OSL) in Northeastern University: Bill Warger, Yogesh G. Patel, Joe Kerimo, Heidy Sierra, Blair Simon and Matt Bouchard, for their help in the laboratory and moral support.

I would like to take this opportunity to express my gratitudes and love to my family, my lovely wife Ayelet, and my daughters Noa, Roni and Dana, which without their love, patience, support and understanding this work would not be possible.

Finally I would like to thank my parents Bat-Sheva and Eliyahu, and all my siblings for their moral support and encouragement.
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ABBREVIATIONS

2D – Two Dimensional

3D – Three Dimensional

BPC - Black Plastic Color

BPLO – Back Propagating Local Oscillator (Interferometer reference arm beam)

BS – Beam Splitter

CCD – Charge Coupling Device

CW – Continues Wave

FWHM – Full Width Half Maximum

He-Ne – Helium-Neon (Laser)

NA – Numerical Aperture

ND – Natural Density

Nd:Yag – Neodymium-doped Yttrium aluminum garnet (Laser)

OCT – Optical Coherence Tomography

OQM – Optical Quadrature Microscope

PA – Photo-Acoustic

PAT – Photo-Acoustic Tomography
PBS – Polarizing Beam Splitter

PMT – Photomultiplier Tube

PTA – Photo-Thermo-Acoustic

PTD – Photothermal Displacement

PVCP – Polyvinyl Chloride Plastisol

QWP – Quarter Wave Plate

SLD – Super-Luminescent Diode
1. INTRODUCTION

Biomedical imaging techniques are evaluated by their ability to deliver real time, non-invasive, high resolution images of a tissue, with high contrast and physiologically relevant parameters. The research in this field is driven by the need for imaging techniques that can penetrate deeper into the tissue while providing high speed, high quality and high resolution images.

Several methods are currently used: Confocal Microscopy, Ultrasonography (commonly called Ultrasound), Optical Coherence Tomography (OCT) and Photo-Thermo-Acoustic (PTA) are all used for tissue imaging. Each technique has its advantages and drawbacks. Ultrasonography, while able to penetrate deep into the body (tens of centimeters) suffers from low resolution (800μm-80μm) and lack of contrast in soft tissue. On the other hand OCT offers excellent resolution (<15μm) and image contrast, but has small depth of penetration (1mm-2mm). Between these two techniques PTA offers penetration of up to 10mm with resolution as high as 80μm. Confocal microscopy is a powerful imaging method in terms of resolution, but suffers from high scattering in turbid samples such as tissue. Although it can image deeper than standard bright field microscopy its depth of penetration is only on the order of a few tenths of a millimeter.

Figure 1.1 describes some of the imaging techniques mentioned above in terms of their penetration and resolution abilities.
In this work a novel technique is introduced utilizing coherent confocal microscopy to detect the thermoelastic displacement to determine the absorption coefficient, the same property that is detected by PTA using acoustic pressure waves. The purpose of the research is to demonstrate the potential of this method to deliver penetration that is similar to that of OCT, with the resolution capabilities that approach confocal microscopy, as shown in red dotted line in Figure 1.1.

The following sections will summarize the methods that are of interest for the work presented in this thesis.

Figure 1.1.: Resolution vs. Penetration of some biomedical imaging techniques

In this work a novel technique is introduced utilizing coherent confocal microscopy to detect the thermoelastic displacement to determine the absorption coefficient, the same property that is detected by PTA using acoustic pressure waves. The purpose of the research is to demonstrate the potential of this method to deliver penetration that is similar to that of OCT, with the resolution capabilities that approach confocal microscopy, as shown in red dotted line in Figure 1.1.

The following sections will summarize the methods that are of interest for the work presented in this thesis.
1.1. Optical Imaging

1.1.1. Confocal Scanning Optical Microscope

The confocal microscopy principal was first introduced by Minsky in 1957 [1], and was demonstrated ten years latter by Eggar and Petran. However, the major breakthrough in confocal microscopy was achieved only in the late seventies with the advance in the fields of lasers, electronic computers and digital image processing.

The fundamental principal of confocal microscopy is that a pinhole is placed in front of the detector, in a plane conjugate to the focus plane. Figure 1.2 describes a basic confocal microscope layout [2].

The point light source, usually a laser beam, is diverged, either using a pinhole or a lens, and then focused down on the specimen by the microscope's objective to a small diffraction-limited spot. Light that is scattered from the focused spot in the specimen propagates back through the objective and focuses back at the pinhole plane (solid black lines in Figure 1.2). Light that is scattered back from the out-of-focus planes (colored dotted lines in Figure 1.2) focuses back either in-front of the pinhole plane or behind it, causing the light to spread over a large area at the pinhole plane. The result is that the majority of the signal that makes it through the pinhole is scattered light from the focus plane, while most of the signal that arrives from the out-of-focus planes is being rejected by the pinhole. Since the detector receives a signal from a single spot of light in the specimen it can be a single pixel light power detector, such as: PIN photodiode, photomultiplier tube (PMT), avalanche photodiode etc. [2].
Composing a 2D image requires that the sample be scanned by the focused light beam, and at the same time the detector must capture the scattered signal from every pixel on the scanned target. The procedure of scanning the target with the spot of light is performed using one of two methods:

1. Motorized stage which moves the target while the spot of light is stationary, or

2. Actuated optical elements to scan the target with the source beam.

*Figure 1.2: A basic confocal microscope layout*

Composing a 2D image requires that the sample be scanned by the focused light beam, and at the same time the detector must capture the scattered signal from every pixel on the scanned target. The procedure of scanning the target with the spot of light is performed using one of two methods:

1. Motorized stage which moves the target while the spot of light is stationary, or

2. Actuated optical elements to scan the target with the source beam.
Since the signal that reaches the detector is mainly the signal that was scattered from the focal spot, and since the image is composed of a raster of such spots by scanning the target at the focal plane, the image that is collected by the detectors is a 2D image of the objects that are at or near the focus plane. All objects that lay in the out-of-focus planes will be very dim or invisible, depending on their distance from the focal plane. This is a key feature of the confocal microscope allowing it to section the sample, by taking multiple scans of the target and moving the focal plane between scans. Sectional images of the target can then be “stacked” to produce a 3D image of the target. Changing the focal plane can be accomplished by either moving the target on the z-axis (i.e. away or toward the objective), or by moving the objective itself.

The theoretical lateral resolution of a standard bright field microscope, $\Delta x, \Delta y$, is given by [3]: $\Delta x = \Delta y \approx 0.61 \frac{\lambda_0}{NA}$, where $NA = n \cdot \sin(\phi)$, $n$ is the index of refraction and $\phi$ is half the angle of the cone of light formed by the objective as seen in Figure 1.3, and $\lambda_0$ is the mean wavelength. However in confocal microscopes better resolution can be achieved by using a very small pinhole. Multiple constants are used to describe the lateral and axial resolution because of different approximations for the Rayleigh criterion. Those of [4] have been used here:

$$\Delta x = \Delta y = 0.4 \cdot \frac{\lambda_0}{NA}. \tag{1.1}$$
The longitudinal resolution is then defined as

$$\Delta z = 1.5 \cdot \frac{\lambda n}{NA^2}$$

(1.2)

Equations 1.1-1.2 clearly suggest that in order to improve the resolution, high NA objective should be used.

1.1.2. **Optical Coherence Tomography (OCT)**

In low-coherence reflectometry, the coherence property of light reflected from a sample provides information on the time of flight delay from the boundaries and backscattering sites in the sample [6]. The OCT provides a 2D map of the reflection at points in the sample by taking multiple longitudinal scans in a series of lateral locations. The optical lateral resolving power of the OCT, like that of the confocal microscope, is proportional to $\lambda/NA$, as shown in Equation 1.1. However while the longitudinal resolving power of the confo-
cal microscope is proportional to $\frac{\lambda n}{NA^2}$ (Equation 1.2), the OCT longitudinal resolution is limited by the coherence length of the light source.

In low-coherence reflectometry OCT, a low-coherence light, usually from a Super-Luminescent Diode (SLD) or short-coherence-laser, is split into reference and signal beams in a modified Michelson interferometer. The reference beam is reflected back from a mirror that can move along the longitudinal axis, to provide longitudinal scan of the tissue. The signal beam is reflected back from the sample, as shown in Figure 1.4.

The two beams are then combined in the BS and the power of the signal is detected using a photo detector.
The movement of the reference mirror will produce interference modulation with Doppler frequency [7]:

\[ f_D = 2 \cdot v_r \cdot \sqrt{\bar{\lambda}} \]  

where \( \bar{\lambda} \) denotes the mean wavelength and \( v_r \) is the reference mirror velocity.

Interference in this low-coherence illuminated interferometer will occur only when the path difference between the two waves is less than the coherence length of the light.

Assuming \( L_s \) and \( L_r \) represents the round trip optical path length of the Signal beam and reference beam respectively. Then \( \Delta L \) that is the optical path difference is:

\[ \Delta L = L_s - L_r = 2 n_0 (l_s - l_r) \]  

where \( l_s \) and \( l_r \) are the geometric length of the signal and reference arms and \( n_0 = 1 \) is the refractive index for air, assuming only air in the interferometers arms. While in confocal microscopy both the lateral and longitudinal resolution are determined by the NA of the objective, the higher the NA the better the resolution since the focused beam will be smaller. In low-coherence OCT only the lateral resolution is determined by the objective NA. The longitudinal resolution is related to the coherence length \( L_c \) of the light source which in turn is related to Full-Width-Half-Maximum (FWHM) of the light source \( \Delta \lambda \), by:

\[ L_c = \left( \frac{4 \sqrt{\ln 2}}{\pi} \right) \left( \frac{\bar{\lambda}^2}{\Delta \lambda} \right) \]  

(1.5)
Since the OCT scans the longitudinal axis fast by moving the reference mirror, it needs a long depth of field, i.e. low NA. This causes poor resolution on both the lateral axis and the longitudinal sectioning power, comparing to this of confocal microscopy, since small NA results in larger $\lambda / NA$ and $\lambda n/NA^2$.

A low NA objective will result in a lower resolution in OCT compared to that of a confocal microscope, but the OCT offers greater depth of penetration, and since the information of the image is held not in the intensity of the signal but in the interfering signal, the dependency of the image contrast on the depth in the sample is lower then in confocal microscopy, i.e. OCT offers greater dynamic range the confocal microscopy.

1.1.3. Optical Quadrature Microscope

One of the problems in optical imaging is obtaining phase information out of an image. The advantage of phase information is significant when imaging transparent samples, or using low illumination levels, as are often needed in biological imaging, such as monitoring the viability of embryos [8]. Conventional methods for obtaining phase require a reference beam that is offset in frequency form the signal beam [9][10], which usually requires additional complex apparatus to be added to the system. On the other hand implementing the quadrature detection technique [11] will result in phase detection of the image using a direct and simple approach[12].

Optical Quadrature Microscope (OQM) combines conventional bright field microscopy with an interferometric quadrature detection technique to capture both the amplitude and the phase of an image [13].
The OQM is based on a modified Mach-Zehnder Interferometer, as shown on Figure 1.5. A laser beam polarized at 45° to the vertical, is sent into the interferometer, split into the reference and signal arms. The reference arm contains a QWP placed at 45° to the vertical that changes the linear beam polarization into circular. The sample is placed between the condenser and the objective in the signal arm of the interferometer. The reference arm of the interferometer contains an identical pair of condenser and objective lenses to match the reference field curvature, with that of the signal beam, at the CCD cameras. The two beams are combined in a second BS to produce a pair of output beams that mix the field from the signal and reference arms:

*Figure 1.5.: OQM layout, courtesy of the Center of Subsurface and Imaging System (CenSSIS) [14]*
\[ \vec{E}_1 = \vec{E}_{\text{sig}} + \vec{E}_{\text{ref}} \]
\[ \vec{E}_2 = \vec{E}_{\text{sig}} - \vec{E}_{\text{ref}} \]

The minus sign is because of the sign difference for internal and external reflections, and is originated from energy conservation. Each one of these beams is then sent through a PBS to split the \(x\) and \(y\) components of the fields. Each one of the components is then imaged using a CCD camera. The image that is detected by each camera is then given by the following equations:

\[
\begin{align*}
\text{CCD } 0 : \quad & |E_0|^2 = |E_{\text{sig}}|^2 + |E_{\text{ref}}|^2 + 2 \Re (E_{\text{sig}}^* E_{\text{ref}}) \\
\text{CCD } 1 : \quad & |E_1|^2 = |E_{\text{sig}}|^2 + |E_{\text{ref}}|^2 + 2 \Im (E_{\text{sig}}^* E_{\text{ref}}) \\
\text{CCD } 2 : \quad & |E_2|^2 = |E_{\text{sig}}|^2 + |E_{\text{ref}}|^2 - 2 \Re (E_{\text{sig}}^* E_{\text{ref}}) \\
\text{CCD } 3 : \quad & |E_3|^2 = |E_{\text{sig}}|^2 + |E_{\text{ref}}|^2 - 2 \Im (E_{\text{sig}}^* E_{\text{ref}})
\end{align*}
\] (1.7)

These images are recorded in a computer. Subtracting CCD 2 from CCD 0, and CCD 3 from CCD 1, and defining \(E'_{\text{sig}}\) and \(\phi\) so that:

\[
\Re (E'_{\text{sig}}) = 4 \Re (E_{\text{sig}}^* E_{\text{ref}}) = |E_{\text{sig}}^* E_{\text{ref}}| \cos \phi \\
\Im (E'_{\text{sig}}) = 4 \Im (E_{\text{sig}}^* E_{\text{ref}}) = |E_{\text{sig}}^* E_{\text{ref}}| \sin \phi
\] (1.8)

and \(\phi\) is the phase difference between the signal and reference arms. Eventually by dividing the amplitudes with the measured reference beam field and combining the real and imaginary parts to a complex field:

\[ E(x, y) = E_{\text{sig}}(x, y) \cdot e^{i \phi(x, y)} \] (1.9)
1.1.4. Coherent Confocal Microscopy

Coherent confocal microscopy is similar to OCT, in that it provides an interferometric image of the tissue. The difference between the low-coherence OCT and the coherent confocal microscopy is that the light source in the coherent confocal microscope has a long coherence length, that is larger than the optical path difference between the interferometer arms, Referring to Equation 1.4 and Equation 1.5: $L_c > \Delta L$.

This property results in an interference signal throughout the sample in the longitudinal axis. Figure 1.7 depicts a coherent confocal microscope in a Michelson interferometer configuration. The light beam in the signal arm is focused to a diffracted limited spot on

Figure 1.6. An amplitude and phase images of a mouse oocyte taken using a OQM, courtesy of the Center of Subsurface and Imaging System (CenSSIS) [14]

Figure 1.6 Shows an image of a mouse oocyte using OQM, courtesy the Center for Subsurface Sensing and Imaging Systems (CenSSIS).
the sample. Light scattered back, by small scatterers in the sample, will propagate back through the same path to the BS and then to the photo detector.

*Figure 1.7: A basic layout of a coherent confocal microscope*

In the BS the signal arm is mixed with the reference arm that is polarized 45° to the vertical in the interferometer reference arm. The signal is sent through a QWP that changes the linear polarization of the signal beam to circular polarization, a PBS is then used to split the field to its $x$ and $y$ component to implement the quadrature detection technique, as will be discussed in greater detail in section 3.1, in which a description of the coherent confocal microscope used in this research is given. The back propagating wavefront of
the reference beam (BPLO), that matches the wavefront of the signal beam at the photo
detector location, coincides with the diffracted limited spot of the interferometer signal
beam in the sample, allowing interfering signal only from this specific location. There-
fore it replaces the pinhole of the confocal microscope, interchanging the alignment of
the pinhole in a confocal microscope system with the proper alignment of the reference
and signal beams to achieve an interference signal at the detector. Since interference will
occur only when the wavefronts of the reference and signal beams are well matched, and
since the BPLO diffracted limited spot is placed at the same location as that of the trans-
mitted beam, if the wavefronts are well matched, the interference signal detected is high-
ly sensitive to focus; hence the sectioning power of the coherent confocal microscope.
The signal that is detected by the detector is proportional to the combined irradiance of
the signal and back propagating local oscillator beams. The resulting signal will depend
not only on the irradiance of the two beams but also on the phase difference between
them. The phase difference between these two beams is related to the difference between
the local oscillator arm and the signal arm optical paths, $L_r$ and $L_s$:

$$\Theta = \frac{2 \pi}{\lambda} (L_s - L_r) ,$$  \hspace{1cm} (1.10)

and the difference between the optical paths in Equation 1.10 can be related to the geo-
metrical length of the interferometer using Equation 1.4.

As the signal beam scans the target, the difference in phase between the local oscillator
arm of the interferometer and the signal arm of the interferometer, resulting from changes
in the position of the scatterers in the sample will be detected as changes in the total sig-
nal that is detected because of destructive or enhancing interference effects. In a classic OCT system the direction of the interference zone along the longitudinal axis is controlled by moving the reference mirror. Therefore the direction of movement, although it can not be determined from the Doppler signal, providing no special techniques are implemented to the apparatus, can be deduced from the direction the mirror was moved. In the presented coherent confocal microscope, the direction of the Doppler signal is detected using the quadrature detection technique.

1.2. Photo-Thermal (PT)

Photo-Thermal (PT) techniques are implemented in a variety of research fields to determine the optical properties of materials, namely the optical absorption. The fundamental principal of PT is that optical energy that is absorbed in the material is converted into heat by exciting electrons in the atoms or molecules, which give up their energy through non-radiative transitions [15]. The photothermal effect is used to reveal the optical absorption properties of the sample by means of probing the resultant heat, or its effect on the material.

In this work PVCP samples were used incorporating low scattering coefficient [16], and thus defined to be absorption-dominated Media [17], in which \( \mu_s' \geq \mu_a \), where:

\[
\mu_s' = \mu_s (1 - g) \tag{1.11}
\]

In these equations \( \mu_a \) is the absorption coefficient, \( \mu_s' \) is the optical reduced scattering coefficient, \( \mu_s \) is the scattering coefficient and \( g \) is the anisotropy factor [16], which is usually of the order of 0.8-0.9 for different types of tissues and changes negligibly over the op-
tical spectrum and from one tissue type to another [18]. The penetration of the laser radiation as a function of the depth $z$, in non scattering medium is [17]:

$$\Psi(z) = \Psi_0 e^{-\mu_a z} \quad (1.12)$$

and $\Psi_0$ is the incident irradiance of the beam on the surface of the sample in [W/cm²]. The Optical Zone $d_0$, is then defined as the depth at which the irradiance rate drops to $1/e$ of the incident irradiance, or:

$$d_0 = \frac{1}{\mu_a} \quad (1.13)$$

For a broad beam with a diameter of $2\omega_0 > 1/\mu_a$ the optical zone is defined to be:

$$d_0 \approx \frac{1}{\mu_a} ; \quad 2\omega_0 > \frac{1}{\mu_a} \quad (1.14)$$

Since the laser beam used in this research is focused on the sample, it is considered to be a narrow beam with a waist diameter in the focal plane $2\omega_0$, where $\omega_0$ is the waist radius.

For a narrow beam with $2\omega_0 < 1/\mu_a$ the optical zone $d_0$ is defined to be of the order of the waist diameter $2\omega_0$:

$$d_0 \approx 2\omega_0 ; \quad 2\omega_0 < \frac{1}{\mu_a} \quad (1.15)$$

Once the laser energy is deposited and turns into heat, the thermal energy can diffuse out of the optical zone and into the tissue. If the laser-pulse duration, $t_p$, is sufficiently short thermal diffusion is too slow to allow the laser energy dissipate into the tissue during the laser pulse. Therefore all the energy deposited is confined in the optical zone, allowing
maximum thermal densities in the tissue. This situation is called *thermal confinement*, and the criterion that enables thermal confinement is:

\[ t_p \leq \frac{d^2}{D_t} \tag{1.16} \]

where \( D_t \) is the thermal diffusivity, given by:

\[ D_t = \frac{k}{\rho \cdot C_p} \left[ \frac{m^2}{s} \right] \tag{1.17} \]

where \( k \) [W·m\(^{-1}\)·K\(^{-1}\)] is the thermal conductivity, \( \rho \) [kg·m\(^{-3}\)] is the equilibrium density, and \( C_p \) [J·kg\(^{-1}\)·K\(^{-1}\)] is the specific heat capacity. The thermal diffusivity is often notated by the Greek letter \( \alpha \), but in this work \( D_t \) was chosen to denote thermal diffusivity, to avoid confusion with the linear expansion coefficient, that is also denoted \( \alpha \).

The laser-induced temperature rise in the optical zone causes a thermoelastic expansion of the tissue, which in turn yields mechanical stress. The stress will dissipate in the medium at the velocity of sound \( v_s \), in the specific medium. Similar to the thermal confinement, a very short laser pulse will deposit energy and generate stress before the stress can propagate out of the optical zone. This situation is called *stress confinement*, and the criterion for the pulse time to enable it is:

\[ t_p \leq \frac{d}{v_s} \tag{1.18} \]

where \( v_s \) is the velocity of sound in the medium.
The localized heating of the material will cause a thermal gradient in the medium that will diffuse the heat in the medium according to the conducting heat differential Equation [15]:

$$\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2} = \nabla^2 T(x, y, z; t) = \frac{1}{D_t} \frac{\partial T(x, y, z; t)}{\partial t},$$  \tag{1.19}

Equation 1.19 assumes no internal heat generation. The heat equation can be generalized to include internal sources of heat generation:

$$\nabla^2 T(x, y, z; t) + \frac{\dot{g}}{k} = \frac{1}{D_t} \frac{\partial T(x, y, z; t)}{\partial t},$$  \tag{1.20}

where $\dot{g}$ [J·m$^{-3}$] is the generation rate of energy per unit volume, that is the rate at which optical energy turns into heat energy.

1.2.1. **Photo-Thermo-Acoustic (PTA)**

Photo-thermo-acoustic (photoacoustic) spectroscopy is a technique that probes the photo-thermal effect using an indirect acoustic method [15]. It was Alexander Graham Bell who first discovered the photo-acoustic effect, while working on an innovative “photophone” [19]. Bell was attempting to modulate a light beam with voice, when he noticed that selenium that was exposed to a modulated beam of light emitted an audible signal.

A material that is exposed to optical energy absorbs part of the optical energy, that transforms into heat. The heating of the material will in turn generate an acoustic pressure wave in the material. This process is called the thermo-optical mechanism of stress generation [20].
The thermal expansion of the instantaneously heated medium causes a pressure rise that is proportional to the thermal coefficient of volume expansion, $\beta$ [1/K°], of the given medium by [18]:

$$P_0 = \frac{1}{\gamma} \frac{\Delta V}{V} = \frac{1}{\gamma} \beta \Delta T = \frac{1}{\gamma} \frac{\beta E_{abs}(z)}{\rho C_v} = \frac{BC_s^2}{C_p} \Phi(0) \mu_a ,$$

(1.21)

where $\gamma$ [1·bar⁻¹] is the thermodynamic coefficient of isothermal compressibility

$$\gamma = \frac{C_p}{C_v} \frac{1}{\rho C_s^2} ,$$

(1.22)

$\Delta V$ [m³] is volume increase caused by thermoelastic expansion, $V$ [m³] is the volume irradiated, $\rho$ [kg·cm⁻³] is the medium density, $C_p$ [J·Kg⁻¹·K°⁻¹] is the heat capacity at constant pressure, $C_v$ is the heat capacity at constant volume, $E_{abs}$ [J·cm⁻³] is the absorbed energy density and $\Phi_0$ [J·cm⁻²] is the laser irradiance at the medium surface.

The acoustic pressure wave generated during the photothermal effect depends not only on the sample properties, but also on the properties of the heating laser optical source. The spatial and temporal profiles of the laser pulse determine the profile and magnitude of the pressure wave. Intensive investigation of these profiles for different types of laser pulses and sample properties was made by A. A. Karabutov et al. [21].

Photo-Acoustic Tomography (PAT) was demonstrated by Wang et al. to produce in vivo imaging of the brain of a rat [22]. The PAT setup, and imaging results are described in Figure 1.8. A heating laser source, such as an Nd:Yag (1064nm), is used to deposit optical energy into the sample, causing it to locally heat. The localized heating of the sample
forms an acoustic pressure wave that propagates in the tissue. Pulsed lasers are often used because a source that will enable thermal confinement, and stress confinement will maximize the acoustic pressure wave that will propagate in the sample. The acoustic pressure wave is then detected by an ultrasound transducer. The heating laser beam is used to trigger the data acquisition system, and together with the ultrasound transducer data is stored in a computer for further signal processing, and image formation.

Scanning the sample with the heating laser while acquiring the acoustic data at the same time, will allow the user to construct a 2D image of the sample. Since the acoustic pressure wave propagates at the speed of sound it is possible to determine the axial and lateral position of the acoustic pressure wave using time resolved techniques and array of transducers, and therefore to construct a 3D image of the target.

Figure 1.8.: Left – PAT setup, Right – PAT imaging of a rat brain in-vivo, (a)- non invasive PAT, (b) Open skull photograph. Taken from Wang et al. [22]
The advantages of PAT over other imaging techniques are:

- Image is based on absorbed light, rather than scattered, and

- PAT can penetrate deeply into the tissue, using wavelengths with low absorbing and scattering coefficients, such as 1064nm

Since the PAT is based on the photothermal effect, and the acoustic pressure wave that is generated due to the photothermal effect depends only on the absorbed light, scattered light, which presents such a big problem in conventional medical imaging, does not play a role in the image formation. Consequently, one can obtain images from systems that are optically opaque. However, scattering combined with absorption, will determine the penetration depth of the heating laser pulse, and therefore will be a limiting criteria for the depth of penetration. Also attenuation of the propagating pressure wave will result in a limiting criteria on the ultra-sound detection system.

### 1.2.2. Opto-photo-thermo-elastic Displacement Detection

In this research a different approach is suggested to measure the photothermal effect. Instead of measuring the acoustic pressure wave, the displacement that occurs due to the temperature rise is probed directly. One of the methods to probe the Photothermal Displacement (PTD), is to use an interferometer [15]. The accuracy of the interferometer in measuring small changes in position makes it suitable for the PTD measurements. Methods that involve interferometers usually implemented to detect the acoustic pressure wave on the surface of the sample [23][24]. This technique was also used to demonstrate
Angstrom-level displacement resolution and nanosecond temporal resolution to detect subsurface blood vessels within model tissue phantoms and a human forearm \textit{in vivo} [25].

The motivation to use the coherent confocal microscope is to maintain the resolution of the confocal microscope, while increasing the depth of penetration. The problem that confocal microscopy experiences in turbid tissue is that light scattered from locations in front of the focal plane cause clutter signals that are reflected back to the detectors. Using the coherent confocal microscope combined with the photothermal process in the focal plane will cause the scatterers in the focal spot to move. This displacement is then measured by the coherent confocal microscope. The clutter signals still exist, but since the coherent confocal microscope measures the displacement using an interferometer that implements coherent detection, the signal that varies in phase can be distinguished from the stationary one.

The Optophotothermoelastic technique, suggested in this work, is based on measuring the expansions and contractions of the tissue due to PTD process directly, using a coherent confocal microscope. In order to maximize the PTD, it is ideal to use laser pulses that will provide thermal confinement, but not stress confinement.
2. SIMULATION

In this chapter a simple model is developed, based on a solution for the heat diffusion equation using integration of the Green's function, in order to predict the displacement expected in the sample due to the photothermal process. The model is used to simulate the displacement in the Polyvinyl-chloride Plastisol (PVCP) samples used in the experiment, with the same heating laser powers used in the experiment.

2.1. Simulation Method

A computational model is used in this research to compare qualitatively and quantitatively the experiment results against theoretical approximated results. The model is based on an analytical solution for the heat diffusion equation given in Equation 1.19, repeated here [26]:

\[
\nabla^2 T(\bar{r}, t) - \frac{1}{D_t} \frac{\partial T(\bar{r}, t)}{\partial t} = \frac{Q(\bar{r}, t)}{\rho C_p}. \tag{2.1}
\]

\(D_t\) is the thermal diffusivity as in Equation 1.17:

\[
D_t = \frac{k}{\rho \cdot C_p} \left[ \frac{m^2}{s} \right], \tag{2.2}
\]

\(\rho \) [Kg·m\(^{-3}\)] is density of the medium, \(C_p\) [J·kg\(^{-1}\)·°K\(^{-1}\)] is the specific heat, and \(k\) [W·m\(^{-1}\)·°K\(^{-1}\)] is the thermal conductivity. In this work Polyvinyl Chloride Plastisol (PVCP) is used as the phantom. The properties of PVCP are given in Table 2.1.
<table>
<thead>
<tr>
<th>Property</th>
<th>Symbol</th>
<th>value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>$\rho$</td>
<td>1380</td>
<td>[Kg·m$^{-3}$]</td>
</tr>
<tr>
<td>Specific heat</td>
<td>$C_p$</td>
<td>900</td>
<td>[J·kg$^{-1}$·°K$^{-1}$]</td>
</tr>
<tr>
<td>Thermal conductivity</td>
<td>$k$</td>
<td>0.16</td>
<td>[W·m$^{-1}$·°K$^{-1}$]</td>
</tr>
<tr>
<td>Thermal diffusivity</td>
<td>$D_t$</td>
<td>$1.2882 \cdot 10^7$</td>
<td>[m$^2$·s$^{-1}$]</td>
</tr>
<tr>
<td>Linear expansion coefficient</td>
<td>$\alpha$</td>
<td>$8 \cdot 10^5$</td>
<td>[°K$^{-1}$]</td>
</tr>
</tbody>
</table>

*Table 2.1.: PVCP properties*

The model described here assumes a homogeneous infinite medium, having no boundaries with other materials, for simplicity. The samples that are investigated in this research have low absorption coefficients, described in Table 3.3. The thicknesses of the

*Figure 2.1.: Nd:Yag source laser beam focused into the sample*
samples are small compared to the inverse of the absorption coefficient, indicating low loss due to absorption as the beam focuses in the phantom. The laser beam irradiance decays due to absorption in the sample according to:

\[ I(z) = I_0 e^{-\mu_a z}, \quad (2.3) \]

where \( I_0 \) [W·m\(^{-2}\)] is the irradiance of the light at the phantoms surface \((z = 0)\). The absorbed power per unit volume can be written:

\[ \frac{dP_{abs}}{dv} = I(z) \cdot \mu_a \quad (2.4) \]

The beam area is large at the surface of the phantom and is getting smaller as it approaches the focal spot. Using a high NA will cause the cone of light to converge into the focal spot with a large head angle \( \theta \), as seen in Figure 2.1, combined with a thin, low absorption coefficient sample allows to neglect absorption of the light before it reaches the focal spot can. Equation 2.4 can then be written to describe the power absorbed:

\[ P_{abs} = I_0 \cdot \text{Spot volume} \cdot \mu_a \quad (2.5) \]

The optical zone for the samples can be found using Equations 1.14 and 1.15. Since the beam of the Nd:Yag (1064nm wavelength) laser source is focused in the sample, the beam diameter, \( 2\omega \), will be the diffraction limited spot in the focal point. Since the beam diameter \( 2\omega \) is smaller then \( 1/\mu_a \), a narrow beam is assumed, and the optical zone, given by Equation 1.15, is determined to be in the focal spot of the laser beam. Since a high NA objective is used, it will result in a strongly converging cone of light, as described in Figure 2.1, it can be then assumed that most of the power absorbed in the medium is con-
fined within a small region that is the diffracted limit spot of the Nd:Yag laser [3] with diameter given by:

\[
d = 1.22 \frac{\lambda}{NA},
\]

For simplicity this region is considered to be a sphere. Using a high NA of the order of 1-1.3 for water, will result in a diffraction limited spot that is approximately 1\( \mu \)m in diameter.

The velocity of sound \( v_s \) in PVCP is 1.4\( \cdot 10^3 \) [m/s] [16]. Stress confinement will require a laser pulse length, \( \tau \), of:

\[
\tau < \frac{d}{v_s} = \frac{5 \cdot 10^{-6} m}{1.4 \cdot 10^3 m/s} = 3.57 \cdot 10^{-9} s = 3.57 \text{ns}
\]

and thermal confinement will require a laser pulse of:

\[
\tau_t < \frac{d^2}{4 D_t} = \frac{(5 \cdot 10^{-6} m)^2}{4 \cdot 1.2882 \cdot 10^{-7} m^2/s} = 4.8517 \cdot 10^{-5} m = 48.517 \mu s
\]

Since the Nd:Yag laser that is used in this research has a pulse length of \( \tau = 250 \mu s \), the experiment is neither in the stress confinement, nor thermal confinement zones, allowing the use of the diffusion equation in this model.

Using the expression for the laser beam irradiance [27]:

\[
I = I_0 f(t) H(x, y) e^{-\mu z}
\]

where \( I_0 \) is the irradiance on the surface, \( f(t) \) is the temporal profile of the pulse, and \( H(x, y) \) is the lateral power distribution of the beam, which for lasers has a Gaussian profile. Assuming that the power of the beam is uniformly distributed over time, \( f(t) = \text{con-} \)
stant, neglecting absorption of the laser beam before the focal spot, and writing the equation in terms of energy instead of irradiance, we get the energy delivered to the optical zone (focal spot) per unit time per unit volume [26]:

\[
q(t) = \frac{P_{\text{laser}}}{\pi \omega^2} e^{\frac{x'^2 + y'^2}{\omega^2}} e^{-\mu_z \cdot \mu_a} ; \quad 0 \leq t \leq \tau
\]  

(2.10)

and the \( \omega \) is given by:

\[
\omega = \frac{d_0}{2} \sqrt{1 + \left(\frac{4 \lambda z}{\pi d_0^2}\right)^2}
\]  

(2.11)

\( d_0 = 2\omega \) is the Gaussian beam waist diameter [28], \( \lambda \) is the optical wavelength and \( z \) is the axial distance.

For an isotropic, homogeneous, infinite medium the 3D Green's function is given by [26]:

\[
g(\vec{r}, t) = \frac{1}{4\pi D_t t^{3/2}} e^{-\frac{|\vec{r}^2|}{4D_t t}}
\]  

(2.12)

A heat source \( Q \) will cause the temperature to be:

\[
T(\vec{r}, t) = \int \int \int \frac{1}{4\pi D_t t^{3/2}} e^{-\frac{|\vec{r}^2 - \vec{r}'^2|}{4D_t (t-t')}} \frac{Q(\vec{r}', t) dt'}{\rho C_p} dx' dy' dz'
\]  

(2.13)

\[
T(\vec{r}, t) = \int \int F(\vec{r}, \vec{r}', t) \frac{1}{\rho C_p} dx' dy' dz' ,
\]  

(2.14)

where \( F \) is:

\[
F(\vec{r}, \vec{r}', t) = \int_{0}^{t} \frac{1}{4\pi (t-t')}^{3/2} e^{\frac{|\vec{r}^2 - \vec{r}'^2|}{4D_t (t-t')}} Q(\vec{r}', t') dt'.
\]  

(2.15)
Assuming uniform distribution of the source power over time:

\[ F(\vec{r}, \vec{r'}, t) = \int_0^\tau \frac{1}{4\pi(t-t')} \left[ \frac{|r - r'|}{4D(t-t')} \right] Q(\vec{r}) dt'. \]  

(2.16)

Letting \( r = |\vec{r} - \vec{r'}| \) as shown in Figure 2.2, and substituting

\[ \sqrt{\frac{r^2}{4D(t-t')}} = u, \]  

(2.17)

Figure 2.2.: The geometry of the analytical solution, the small ball is the source, \( r \) is the observation point \( r' \) is a point in the source

taking the positive square root, and noting that time is always positive in the simulation, so that \( (t - t') > 0 \),

\[ dt' = -\frac{r^2}{2D'u^3} du. \]  

(2.18)

28
Also we substitute:

\[
[4 \pi D_t(t, t')]^{3/2} = \pi^{3/2} \frac{t^3}{u^3}.
\]  

(2.19)

The integral becomes:

\[
F = \frac{Q(\tilde{r})}{4\pi D_r r} \int \frac{1}{\pi^{3/2} r^3} e^{-u^2} \frac{r^2}{2 D_r u^3} du = \frac{1}{4\pi D_r r} \frac{2}{\sqrt{\pi}} \int \frac{r^2}{\sqrt{\frac{r^2}{4 D_r (t-\tau)}}} e^{-u^2} du,
\]

(2.20)

Using the error function [29]:

\[
\text{erf}(x) = \frac{2}{\sqrt{\pi}} \int_0^x e^{-u^2} du
\]

(2.21)

\[
F = \frac{Q(\tilde{r})}{4\pi D_r r} \left[ \text{erf}\left(\sqrt{\frac{r^2}{4 D_r (t-\tau)}}\right) - \text{erf}\left(\sqrt{\frac{r^2}{4 D_r t}}\right) \right].
\]

(2.22)

In order to simplify the analysis, instead of the source described in Equation 2.10, the source is assumed to be a sphere, with a 3D Gaussian distribution of the energy in it:

\[
Q(\tilde{r}) = Q_0 e^{-\frac{x^2 + y^2 + z^2}{\alpha^2}},
\]

(2.23)

with a diameter of \(d_0 = 2\omega = 5\mu m\), according to Equation 4.

Using Equation 2.23 in Equation 2.22, and into Equation 2.14, the change in temperature for every position in space at any given time can be calculated, by integrating over the source using \(r = |\tilde{r} - \tilde{r}'|\) as shown in Figure 2.2 for every point in the source. Since spherical symmetry is assumed, calculation are made for one spatial dimension and time.

The result is an array, of the temperature change, \(\Delta T\), where the horizontal dimension de-
scribes the radial distance from the center of the focal spot, and the vertical dimension describes time. This can be seen in the top left plot in Figure 2.3. Multiplying the temperature change from the initial condition, $\Delta T$, by the linear expansion coefficient $\alpha$,

$$\nabla \vec{x} = \alpha \cdot \Delta T(r, t)$$ (2.24)

will result in unitless, absolute divergence that each point experiences, as shown in the top right plot in Figure 2.3.

Figure 2.3.: Simulation results. PVCP sample properties similar to sample ID 050-100-000, laser power 0.438W, beam pulse 250µs, beam diameter 1µm. (a) – Temperature change as a function of position (x-axis) and time(y-axis), (b) – unit less absolute divergence as a function of position and time, (c) – Integration of the actual displacement of each point in the array, (d) – Displacement vs. time curves for some locations away from the center of the focal spot.
Finally to obtain the spatial displacement of a given position \( n \), the divergence is summed over the space from the center of the focal spot to the given position:

\[
\Delta x = \int_0^x \nabla x(x') \, dx'.
\]  

(2.25)

As seen in the bottom left plot. Profile curves of the displacement vs. time, for various positions along the x-axis from the center of the focal spot outward, are shown in the bottom right plot in the figure. These curves are basically vertical slices of bottom left plot. The lowest curve refers to position that is closest to the center of the focal spot and moving outwards in radius results in higher displacements. The location that experience

\[dx'\]

\[dx' + \nabla x(x') \, dx'\]

Figure 2.4.: Displacement by integration
maximum displacement is seen shifted to the right in the bottom left plot, from the source center. This is because every positions experiences the displacement that results from the temperature rise, as well as the cumulative divergence of all the position that are closer to the center of the source. This is described in Figure 2.4.

2.2. Simulation Results

The simulation method described in Section 2.1, was implemented using Matlab (“Mathworks”, Cambridge USA). Simulations were performed to predict the displacement in samples with different absorption coefficients, using various laser pulse energy levels. The absorption coefficients simulated were chosen to match the absorption coefficients that were implemented in the PVCP phantoms, described in Table 3.3. The laser power was selected according to the power levels that were used in the experiment, according to Section 4.1.

Results of the simulations are shown in Figure 2.5 for 10Hz Nd:Yag laser repetition rate, and in Figure 2.6 for 5Hz Nd:Yag laser repetition rate. The plots on top shows the displacement vs. energy in a single laser pulse on the sample surface, for different absorption coefficients. The bottom plots describe the displacement as a function of absorption coefficient, for some laser pulse power levels. The simulation results indicates that over the range of absorption coefficients, and energy levels that were simulated, a linear response is expected in terms of the displacement.
Figure 2.5: Simulation results for 10Hz repetition rate. Top - Displacement vs. pulse energy, for various absorption coefficients. Bottom – Displacement vs. absorption coefficient, for various pulse power levels.
Figure 2.6: Simulation results for 5Hz repetition rate. Top - Displacement vs. pulse energy, for various absorption coefficients. Bottom – Displacement vs. absorption coefficient, for various pulse power levels.
3. EXPERIMENT

This chapter describes the apparatus and the samples that are used in this experiment. Some properties of the apparatus are discussed and the theoretical background of the technique that is implemented is developed.

3.1. Quadrature Coherent Confocal Microscope

The coherent confocal microscope used in the experiment is based on a modified Michelson Interferometer incorporating quadrature detection [11], as described in Figure 3.1.

Figure 3.1.: The coherent confocal microscope setup
A vertically, linearly polarized, He-Ne (632.8nm wavelength) with TEM$_{00}$ mode, continuous wave (CW) (Research Electro Optics, INC. LHRP-1701 Cylindrical Helium-Neon Laser Head), laser beam is expanded and then collimated using a lens. The vertical will be denoted the x-axis while the direction of propagation will be the z-axis. The beam is passed through an iris to block the high transverse spatial modes of the beam and allow only the center of the beam to propagate, providing more uniform illumination. The beam is then split to the reference beam and the signal beam using a non-polarizing 50%-reflecting beam splitter (BS). The reference beam is passed through a polarizer at 45º to the vertical, and reflected back to the non-polarizing BS from a mirror. The signal beam is focused using a microscope objective (Newport M-10X 0.25) on the sample. The scattered light from the sample propagates back through the objective and into the same beam splitter where it is mixed with the reference beam. The two beams are then passed through a QWP at 45º to the vertical (the same as the polarizer in the reference beam path), that changes the polarization of the signal beam to circular polarization. After the two signals are mixed, a polarized beam splitter (PBS) is used to separate the vertical (x-axis) and horizontal (y-axis) components of the fields. These fields are then measured by amplified PIN Diodes (Thorlabs PDA-150). The data from the amplified PIN diodes is displayed and stored in an oscilloscope (LeCroy Waverunner LT344). The oscilloscope is connected to a computer, data is recorded as files in the computer for further processing using computer software.
Jones Calculus [28] will be used to describe the polarization changes in the system, and to demonstrate the quadrature detection process mathematically. Equation 3.1 describes the time harmonic electrical field of an electromagnetic wave that propagates on the positive z direction and has \( x \) and \( y \) fields component [30].

\[
E = \hat{a}_x e_x^+ e^{i(\omega t - \beta z)} + \hat{a}_y e_y^+ e^{i(\omega t - \beta z)} \tag{3.1}
\]

\( e_x^+ \) and \( e_y^+ \) in Equation 3.1 are complex. The state of polarization can then be described using a two element vector, one for the \( x \) component and one for the \( y \) component, as shown in Equation 3.2 [28]. Assuming \( e_x^+ \) and \( e_y^+ \) have the same magnitude \( E_0 \), then the vector can be normalized to hold only the phase in the terms \( e_x \) and \( e_y \).

\[
E = \begin{bmatrix} e_x^+ e^{i(\omega t - \beta z)} \\ e_y^+ e^{i(\omega t - \beta z)} \end{bmatrix} = E_0 e^{i(\omega t - \beta z)} \begin{bmatrix} e_x \\ e_y \end{bmatrix} \tag{3.2}
\]

This vector is called the *Jones vector*, and it describes the state of polarization for light sources that holds a given state of polarization, it can not describe light that is randomly polarized or holds several polarization states. Some examples of polarization states described using Jones vectors are shown in Table 3.1. The upper element is chosen arbitrary to represent \( x \) component of the polarization and the lower element to represent the \( y \) component, the beam is linearly polarized to the \( x \) axis.
### State of polarization

<table>
<thead>
<tr>
<th>State of polarization</th>
<th>Jones vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear polarization in the x-axis</td>
<td>$\begin{bmatrix} 1 \ 0 \end{bmatrix}$</td>
</tr>
<tr>
<td>Linear polarization in the y-axis</td>
<td>$\begin{bmatrix} 0 \ 1 \end{bmatrix}$</td>
</tr>
<tr>
<td>Linear polarized at 45° from the x-axis</td>
<td>$\frac{1}{\sqrt{2}}\begin{bmatrix} 1 \ 1 \end{bmatrix}$</td>
</tr>
<tr>
<td>Right circular polarized</td>
<td>$\frac{1}{\sqrt{2}}\begin{bmatrix} 1 \ -i \end{bmatrix}$</td>
</tr>
<tr>
<td>Left circular polarized</td>
<td>$\frac{1}{\sqrt{2}}\begin{bmatrix} 1 \ i \end{bmatrix}$</td>
</tr>
</tbody>
</table>

**Table 3.1.: Polarization states and their corresponding Jones vector**

### Component/Operator

<table>
<thead>
<tr>
<th>Component/Operator</th>
<th>Notation</th>
<th>Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mirror</td>
<td>$M_m$</td>
<td>$M_m = \begin{bmatrix} 1 &amp; 0 \ 0 &amp; 1 \end{bmatrix}$</td>
</tr>
<tr>
<td>Quarter Wave Plate (QWP)</td>
<td>$M_{QWP}$</td>
<td>$M_{QWP} = \begin{bmatrix} e^{i\frac{\pi}{4}} &amp; 0 \ 0 &amp; e^{-i\frac{\pi}{4}} \end{bmatrix}$</td>
</tr>
<tr>
<td>X-axis polarizer</td>
<td>$M_{px}$</td>
<td>$M_{px} = \begin{bmatrix} 1 &amp; 0 \ 0 &amp; 0 \end{bmatrix}$</td>
</tr>
<tr>
<td>Y-axis polarizer</td>
<td>$M_{py}$</td>
<td>$M_{py} = \begin{bmatrix} 0 &amp; 0 \ 0 &amp; 1 \end{bmatrix}$</td>
</tr>
<tr>
<td>Rotation $\theta$ [rad]</td>
<td>$M_{rot}(\theta)$</td>
<td>$M_{rot}(\theta) = \begin{bmatrix} \cos(\theta) &amp; \sin(\theta) \ -\sin(\theta) &amp; \cos(\theta) \end{bmatrix}$</td>
</tr>
</tbody>
</table>

**Table 3.2.: Jones Matrices for some optical components and for rotation operator**
Using Jones vectors we describe the He-Ne main beam using the Jones vector in Equation 3.3.

\[ J_{mb} = \begin{bmatrix} 1 \\ 0 \end{bmatrix}. \tag{3.3} \]

The polarization state of the beam can be tracked during the propagation process, through different optical components, using the Jones calculus \(^{[28]}\). Table 3.2 describes some optical components and their Jones Matrices. The rotation operator allows to change the angle of which the optical component is set.

Using the above matrices the polarization vectors for the Local Oscillator (LO- reference Beam) beam is given by (before passing through the PBS):

\[ J_{LO} = M_{\text{rot}}(\text{–}\pi/4) M_{\text{qwp}} M_{\text{rot}}(\pi/4) \frac{1}{\sqrt{2}} M_{\text{rot}}(\text{–}\pi/4) M_{\text{px}} M_{\text{rot}}(\pi/4) M_m M_m M_{\text{rot}}(\text{–}\pi/4) M_{\text{px}} M_{\text{rot}}(\pi/4) \frac{1}{\sqrt{2}} M_m J_{mb} = \begin{bmatrix} 0.1768 + 0.1768i \\ -0.1768 - 0.1768i \end{bmatrix} \times 0.1768 \begin{bmatrix} 1 + i \\ -1 - i \end{bmatrix}. \tag{3.4} \]

All rotations are at \( \theta = \pi/4 \). Both the Polarizer and the QWP are at 45° to the vertical (x-axis). The two \( 1/\sqrt{2} \) factors arise from the beam going through the BS twice, the first time when it splits to the reference and signal arms, and second time when it returns and mixes with the signal beam in the BS.

For the signal beam:

\[ J_{sig} = M_{\text{rot}}(\text{–}\pi/4) M_{\text{qwp}} M_{\text{rot}}(\pi/4) \frac{1}{\sqrt{2}} M_m \frac{1}{\sqrt{2}} J_{mb} = \begin{bmatrix} 0.3536 + 0i \\ 0 - 0.3536i \end{bmatrix} = 0.3536 \begin{bmatrix} 1 \\ -i \end{bmatrix}. \tag{3.5} \]
The signal beam undergoes additional attenuation when it scattered back from the sample, that is proportional to the absorption and scattering coefficients of the sample. In the case of the samples studied in this work only the absorption coefficient cause attenuation.

The PBS will add an additional mirroring to the y-axis components, and will split each of the fields into their x and y components. The x component will be denoted $I$ (In-Phase) and the y component will be denoted $Q$ (Quadrature). Each component of the field is detected using an amplified PIN photodiode. The PBS will also multiply each of the fields component in additional $1/\sqrt{2}$ factor. The four component of the fields: I and Q for the LO beam and I and Q for the signal beam are described in Equation 3.6,

$$
\begin{align*}
J_{ILO} &= Mpx \frac{1}{\sqrt{2}} J_{LO} = 0.125 \begin{bmatrix} 1+1 \\ 0 \end{bmatrix} \\
J_{QLO} &= Mpy \frac{1}{\sqrt{2}} J_{LO} = 0.125 \begin{bmatrix} 0 \\ -1-i \end{bmatrix} \\
J_{Isig} &= Mpx \frac{1}{\sqrt{2}} J_{sig} = 0.25 \begin{bmatrix} 1 \\ 0 \end{bmatrix} \\
J_{Qsig} &= Mpy \frac{1}{\sqrt{2}} J_{sig} = 0.25 \begin{bmatrix} 0 \\ -i \end{bmatrix}
\end{align*}
$$

Combining the $I$ terms and the $Q$ terms and using Equation 3.2, one can obtain:

$$
\begin{align*}
E_I &= E_{ILO} + E_{Isig} = 0.125 E_0 e^{i(\omega t - \beta z)} \begin{bmatrix} 1+i \\ 0 \end{bmatrix} + 0.25 E_0 e^{i(\omega t - \beta z + \phi)} \begin{bmatrix} 1 \\ 0 \end{bmatrix} \\
E_Q &= E_{QLO} + E_{Qsig} = 0.125 E_0 e^{i(\omega t - \beta z)} \begin{bmatrix} 0 \\ -1-i \end{bmatrix} + 0.25 E_0 e^{i(\omega t - \beta z + \phi)} \begin{bmatrix} 0 \\ -i \end{bmatrix},
\end{align*}
$$

and $\phi$ is the the phase difference between the reference and signal beam when recombined. Taking out $(1+i) = e^{i\pi/4}$:
Since the detectors output signal that is proportional to the power of the received signal:

\[
E_I = E_{ILO} + E_{Isig} = 0.125 E_0 e^{i(\omega t - \beta z)} \begin{bmatrix} 0 \\ e^{\frac{i\pi}{4}} \end{bmatrix} + 0.25 E_0 e^{i(\omega t - \beta z + \phi)} \begin{bmatrix} 1 \\ 0 \end{bmatrix}
\]

\[
E_Q = E_{QLO} + E_{Qsig} = 0.125 E_0 e^{i(\omega t - \beta z)} \begin{bmatrix} 0 \\ e^{-i\frac{\pi}{4}} \end{bmatrix} + 0.25 E_0 e^{i(\omega t - \beta z + \phi)} \begin{bmatrix} 0 \\ -i \end{bmatrix}
\]

(3.8)

The asterisks in Equation 3.9 denote complex conjugate, and \(E_{0LO}, E_{0sig}\) are the magnitudes of the LO and signal beams respectively. Ignoring for now the squared power parts of the signals, the conjugate terms are:

\[
\tilde{P}_I = |E_{0LO}|^2 + |E_{0sig}|^2 + E_{ILO} E^{*}_{Isig} + E^{*}_{ILO} E_{Isig}
\]

\[
\tilde{P}_Q = |E_{0LO}|^2 + |E_{0sig}|^2 + E_{QLO} E^{*}_{Qsig} + E^{*}_{QLO} E_{Qsig}
\]

(3.9)

The locus in the complex plane for this expression will be a closed curve, as shown in Figure 3.2. The DC component, that defines the center of the circular curve, arise from the combined powers of the interferometer signal and reference arms. The tail of the vector that describes the interference signal starts at this point, and its magnitude is related to the powers of the reference and signal beams as described in Equation 3.11.
The relative displacement is found by determining half the maximum phase change divided by $2\pi$, multiplied by the laser wavelength:

$$
\Delta z = \frac{\Delta \phi \cdot \lambda}{2 \pi} \frac{1}{2} = \frac{\Delta \phi \cdot \lambda}{4 \pi},
$$

provided that the phase is first wrapped if it exceeds $2\pi$. The factor of 2 arises from the doubling of the Doppler shift by reflection from the moving target.

*Figure 3.2.: Quadrature detection, display of the complex power signal $I+i\cdot Q$*
Figure 3.3 describes 0.25mm motions that were made using a micrometer and highly reflective target. The graphs on the left describe an upward movement (toward the objective).

**Figure 3.3:** Example of motion detection, using the coherent confocal microscope, (a) – 0.25mm upward motion (toward the objective), (b) – 0.25mm downward (away from the objective. The motion was measured using a micrometer. Top graphs are the quadrature detected signal, bottom graphs are the unwrapped phase angle vs. time.
The graphs to the right are result of a downward motion (away from the objective. The top two graphs are the complex I+iQ signal, as described in Figure 3.2. The bottom two graphs shows the unwrapped phase as a function of time, in radians. Using Equation 3.12 the upward motion can be determined in high accuracy:

\[ \Delta z = \frac{+5224}{4\pi} 632.8 \cdot 10^{-9} = +2.6306e^{-4} m = +0.26306 \text{mm} \] (3.13)

Similarly the downward motion can be found:

\[ \Delta z = \frac{-5274}{4\pi} 632.8 \cdot 10^{-9} = -2.6558e^{-4} m = -0.26558 \text{mm} \] (3.14)

3.1.1. Resolution

3.1.1.1. Lateral Resolutions

The Rayleigh criterion of resolution for incoherent light states that two light sources can be resolved when the distance between these points, \( d \), is such, that the peak of the second point is situated just over the first dark ring in the first Airy disk [3], see Figure 3.4.

Using the Rayleigh criterion for imaging resolution the distance \( d \) is determined:

\[ d = 0.61 \frac{\lambda}{NA} \] (3.15)

As was described earlier the confocal microscope can achieve better resolution according to Equation 1.1,
$d = 0.4 \cdot \frac{\lambda_0}{NA}$,

and $\lambda_0$ is the mean wavelength for the incoherent confocal microscope.
3.1.1.2. **Longitudinal Resolution, \( R_z \)**

The interferometer theoretical resolving power in the longitudinal dimension can be split to two types of resolutions. The resolution of the diffraction limited spot of the interferometer beam, depends on the wavelength and NA of the objective and the index of refraction, and will determine the spot size in the longitudinal axis.

![Diagram showing longitudinal resolution, \( R_z \), of the microscope](image)

*Figure 3.5.: Longitudinal resolution, \( R_z \), of the microscope*

Figure 3.5 shows the longitudinal resolution \( R_z \) for the coherent confocal microscope. The solid curved lines describes the He-Ne laser beam of the interferometer treated as a Gaussian beam [28] that converges in a short distance due to a high NA microscope objective. The small gray dots represents scatterers in the sample that reflects the light back to the...
interferometer detectors. The ellipse between the curved lines, is the diffracted limited
spot of the laser beam, and it defines the microscope depth of field, or the axial resolution
$R_z$. Light that scattered back from this spot is detected as an interference signal.

In the longitudinal axis the size of the diffracted limited spot defines the longitudinal
resolution $R_z$ of the microscope, and is given by the expression [4]:

$$R_z = 1.5 \frac{\lambda n}{NA^2}$$  \hspace{1cm} (3.17)

Hence the sectioning power of the microscope when operating as a coherent confocal mi-
croscope will be similar to that of a standard confocal microscope.

3.1.1.3. **Displacement Measurement Resolution, $\Delta z$**

When the microscope is detecting optophotoelastic displacement, it will achieve better
resolution in the axial dimension then using it as a coherent confocal microscope. The
reason for this is that when the microscope heats the diffracted limited area (the optical
zone) using the Nd:Yag laser, the signal that is detected is related to the displacement of
any scatterers in the optical zone due to the photothermal process, as shown in Figure 3.6.

The red curved lines shows the interferometer Gaussian beam, the second set of curved
lines describe the Nd:Yag laser Gaussian beam, heating the optical zone. This will cause
the optical zone to expand causing the scatterers inside this area to change their position,
as shown for one of the scatterers in the optical zone, by $\Delta z$. This change in position,
since it occurs within the coherent confocal microscope longitudinal resolution $R_z$, is then
detected by the interferometer.
When the displacement of the scatterers $\Delta z$ is moving out of the coherent confocal microscope resolution $R_z$ area, the signal that is detected by the interferometer degrades rapidly, this can be seen in Figure 3.7. In this figure the quadrature signal is shown for an experimental results of sample ID: 050-250-000, 5Hz repetition rate and 600V charging voltage ($2.16 \cdot 10^{-1}$ W). As the sample is heated by the Nd:Yag laser pulse, the quadrature signal radius decreases. This change in the signal is due to the decrease in the signal arm beam power. The signal arm is sensitive to focus and moving away from the focal plane will result in reduction in the power that is reflected back to the detectors. The reduction of the radius of the circle and the shift of the center of the circle toward the lower left cor-

*Figure 3.6.* The interferometer displacement resolving power $\Delta z$
ner of the original signal is expected according to Figure 3.2.

Figure 3.7.: Sample ID 050-250-000, 5Hz repetition rate, 600V charging voltage
3.2. **Coaxial Optophotothermoelastic Microscope**

The laser that was used in this research to initiate the photothermal effect is a pulsed, Nd:YAG 1064nm wavelength, with maximum output power of 10W, and random polarization (Schwartz Electro-Optics, Inc. LASER 1-2-3). The power that this laser outputs is much higher than what is required for our experiment. Therefore the power that was injected to the sample was limited by using a glass plate as a combining optical element for the heating laser and interferometer beams, instead of a more efficient element such as a dichoric.

*Figure 3.8.: Photothermoelastic displacement detection microscope*
The integration of the heating laser into the microscope is described in Figure 3.8. The pinhole is placed to allow for uniform illumination, and is blocking part of the power of the powerful laser. The alignment mirrors allow alignment of the heating laser with the interferometer He-Ne laser.

Figure 3.9 describes the transmission and reflection coefficients for the power of an electromagnetic field that is incident on a glass plate with \( n=1.5 \) (\( \varepsilon=2.25 \)) vs. the angle of incidence. The transmitted power is described by the dotted red line and the reflected power is by the solid blue line. In Figure 3.9 the two polarization states are shown, perpendicular polarization and parallel polarization. The equations that describe the reflection coefficient, of the electric field, for the perpendicular polarization is [30]

\[
\Gamma_\perp = \frac{\cos \phi_i - \sqrt{\varepsilon_1 / \varepsilon_2} \cdot \sqrt{1 - (\varepsilon_1 / \varepsilon_2) \cdot \sin^2 \phi_i}}{\cos \phi_i + \sqrt{\varepsilon_2 / \varepsilon_1} \cdot \sqrt{1 - (\varepsilon_1 / \varepsilon_2) \cdot \sin^2 \phi_i}} \tag{3.18}
\]

where \( \phi_i \) is the incident angle, \( \varepsilon_1 \) is the permittivity of the material that the incident field propagates at and \( \varepsilon_2 \) is the permittivity of the material that the incident electric field is transmitted into. The equation that describes the reflection coefficient, of the electric field, for the parallel polarization case is

\[
\Gamma_\parallel = \frac{-\cos \phi_i + \sqrt{\varepsilon_1 / \varepsilon_2} \cdot \sqrt{1 - (\varepsilon_1 / \varepsilon_2) \cdot \sin^2 \phi_i}}{\cos \phi_i + \sqrt{\varepsilon_2 / \varepsilon_1} \cdot \sqrt{1 - (\varepsilon_1 / \varepsilon_2) \cdot \sin^2 \phi_i}}, \tag{3.19}
\]

and the corresponding transmission coefficients, of the electric fields, are:

\[
T_\perp = 1 - \Gamma_\perp \quad T_\parallel = 1 - \Gamma_\parallel. \tag{3.20}
\]
Figure 3.9.: Transmission (red-dashed line) and Reflection (blue solid line) of light from a glass plate with $n=1.5$, for both perpendicular and parallel polarization.
Figure 3.9 describes the power of the reflected and transmitted fields, found by squaring the the coefficients in Equations 3.18 - 3.20. The data in Figure 3.9 suggests a reflection coefficient of 9.2% for the power in the perpendicular polarization, and for the power in the parallel polarization a reflection coefficient of 0.8%. According to the specifications of the Nd:Yag laser the polarization is random, therefore assuming equal distribution of the power at this two polarization, then the total power that is reflected is

$$\Gamma = \frac{(0.092 + 0.008)}{2} = 0.05 \ .$$

Thus the total power that is reflected from the glass plate that is tilted at 45°, is approximately 5% of the power of the incident beam.

*Figure 3.10: Interferometer beam (gray, dashed line) and heating laser beam (red solid line) interaction with glass plate*
The rest will be transmitted into the glass plate and will be incident with the back surface. At the back surface of the glass plate the beam will be reflected and transmitted, as shown in Figure 3.10. The majority of the light that is incident on the second surface will be transmitted out to the air, but a small part of it will be reflected back into the glass and will be incident on the first surface, as shown in Figure 3.10. Part of the light that is incident on the first surface from within the glass will be reflected back while most of it will be refracted and will travel parallel to the light that was reflected from the first surface on the first incident. It is important to find a relation between the beams radius $r$ and the glass plate thickness $d$, to insure distance between the center axes of the beams $h$, that will satisfy:

$$h > 2r.$$  \hspace{1cm} (3.22)

This is required to avoid interference between this two beams that will result in reduction in the power of the heating laser beam that propagates to the objective and into the sample. Since the incident angle of the main beam of the heating laser is $\theta_i = 45^\circ$ and the refraction coefficient of the glass is $n_i = 1.5$, then using Snell Law [28]:

$$n_i \sin \theta_i = n_t \sin \theta_t$$ \hspace{1cm} (3.23)

the angle of which light will be transmitted into the glass plate $\theta_t$ will be

$$\sin \theta_t = \frac{n_i \sin \theta_i}{n_t} = \frac{1}{1.5} \sin \frac{\pi}{4} = 0.4714.$$ \hspace{1cm} (3.24)

In our case $\pi/2 - \theta_i = \theta_t$, and therefore
\[ h = (2 \cdot d \tan \theta_i) \sin \theta_i = (2 \cdot d \cdot 0.5097) \cdot 0.7071 = 0.7208d \]  \hspace{1cm} (3.25)

and this will constrain the beam radius according to inequality 3.22:

\[ r < 0.7208d \]  \hspace{1cm} (3.26)

The laser beam from the interferometer goes through a similar path as the heating laser beam. Figure 3.10 describes the propagation of the interferometer main beam through the glass plate. It is clear from Figure 3.10 that the restriction on the beam radius, described in inequality 3.26 applies to the interferometer beam as well, to avoid interfering between the main beam that is transmitted through the glass plate, and the secondary beam that propagates in parallel after two reflections in the glass plate, and then refraction out of the plate glass, and parallel to the main beam.

### 3.3. Phantom Samples

The testing of new methodologies in biomedical research on phantoms allows a controlled study of the method, and optimization of it, before testing on animals and humans are performed. A number of phantoms were developed and suggested to mimic the optical and acoustical properties of tissue. Intralipid [31], polyacrylamide gels [32], albumin [33] and agar [34] are materials that are used to make tissue phantoms in biomedical research. Polyacrylamide gels, agar and albumin phantoms absorb water, which makes them not suitable for applications that require contact with water. Also the optical properties of these materials might change with time due to humidity. Agar and albumin phantoms are fragile.
and rapidly degrade over time due to fungal growth, which shorten their life time and requires constantly preparing phantoms for the study.

In this research a Polyvinyl-Chloride Plastisol (PVCP) [16] samples are used to study the photothermo elastic expansion. These samples present optical properties that resemble those of tissue. Also PVCP is based on oil and therefore has a longer life time, when stored properly.

The use of PVCP sample with Black Plastic Color (BPC) allows good control of the sample absorption coefficient $\mu_a$. In this research only BPC was added to the samples to control the absorption coefficient. Titanium Dioxide (TiO2) can be added to the samples as well, to introduce scattering [16]. In this work scattering was not implemented and its effects on the photothermo elastic expansion were not tested. The phantoms that are study in this research are similar to transparent tissues, such as the cornea. The procedure that was developed to prepare the phantoms is detailed in Appendix B.

The absorption coefficient depends on the concentration of the BPC in the PVCP according to [16]:

$$\mu_a = 12.818 A_{BPC} [cm^{-1}]$$

In Equation 3.27 $A_{BPC}$ is the percentage of BPC concentration in the PVCP.

Four phantoms are tested in this work. A list of the phantoms used and their properties is detailed in Table 3.3. The nomenclature used for the sample ID is as follows:
1. The first three digits describe the amount of PVCP in [ml], that was used to prepare the sample

2. The second series of three digits show the amount of BPC in [µl], that was added to the solution of PVCP, to introduce absorption

3. The third series of three digits is reserved to indicate the amount of TiO₂ in [mg/ml] added to the solution, to introduce scattering. This will be used in future work.

<table>
<thead>
<tr>
<th>Sample Id.</th>
<th>PVCP Vol.</th>
<th>BPC Vol. (A)</th>
<th>BPC %</th>
<th>Absorption Coefficients $\mu_a$ [cm⁻¹]</th>
<th>Sample Width [µm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>050-025-000</td>
<td>50ml</td>
<td>25µl</td>
<td>0.05%</td>
<td>0.6409</td>
<td>229</td>
</tr>
<tr>
<td>050-050-000</td>
<td>50ml</td>
<td>50µl</td>
<td>0.1%</td>
<td>1.2818</td>
<td>274</td>
</tr>
<tr>
<td>050-100-000</td>
<td>50ml</td>
<td>100µl</td>
<td>0.2%</td>
<td>2.563</td>
<td>230</td>
</tr>
<tr>
<td>050-250-000</td>
<td>50ml</td>
<td>250µl</td>
<td>0.5%</td>
<td>6.409</td>
<td>160</td>
</tr>
</tbody>
</table>

*Table 3.3: PVCP Samples properties*

In this work no samples incorporating TiO₂ are used.
A picture that presents the PVCP samples that are used in this study is shown in Figure 3.11. The lower right sample is the PVCP sample that incorporate the lowest concentration of BPC, the lower left sample has a higher concentration of BPC, the sample at top right is the third and the sample at top left is the sample that incorporate the highest level of BPC. Each sample is molded in several shapes, a thin sheet that is a few tenth of a millimeter thick, a bulk disk that is 1-2 inches thick, and small disks that are few a millimeters in thick.

![PVCP samples used in the research. The top left sample contains the highest level of BPC, concentrations is lower moving to the top right sample and moving to the bottom samples the lower right sample contains the lowest level of BPC](image-url)
The mechanical structure of every sample is described in Figure 3.12. A thin film of ~0.2mm is placed on top of an aluminum foil, on a microscope slide. A cover slip is then placed on top of the phantom, and pressed using a small 50g weight. Two drops of glue are placed on the side of the cover glass, to hold the structure, and are not in contact with either the sample, nor the the aluminum foil. The samples can be seen in Figure 3.11 at the middle of the different kind of molds for each sample, on a microscope slide.

*Figure 3.12.: PVCP Sample mechanical structure*

The aluminum foil on the bottom of the sample is the reflecting material for the interferometer signal. This approach was taken to avoid Signal-to-Noise (SNR) issues that may arise because of the use of PIN Photodiodes as detectors, which is not optimal for detecting small signals that are expected to reflect back from the low scattering samples used in this work. Each one of the samples is measured using a confocal microscope to determine
the exact thickness of the phantom. Figure 3.13 shows a confocal images of sample ID: 050-250-000. The thickness measurements are made on a single position for each sample, but because the process used to make the thin film of PVCP can not promise a uniform thickness, variations on the thickness across the phantom slice are certain. The image and measurements are courtesy of the W.M. Keck 3D Fusion Microscope, at Northeastern University.

Figure 3.13.: Confocal image of sample 050-250-000, (a) - under the cover glass, (b) - a section image inside the sample, (c) - aluminum foil at the bottom, the white dots are the particles of BPC in the sample (courtesy of the W.M. Keck 3D Fusion Microscope)
4. MEASUREMENTS AND RESULTS
This chapter describes measurements of different characteristics of the apparatus. The experimental results are shown, and the experimental results are compared with the theoretical expected results that were obtained in Chapter 2.

4.1. He-Ne Laser Power Measurements
The power in the interferometers laser beams was measured using a laser power and energy meter (Scientech, Vector H410).

First the laser output power was measured and was found to be 12\(\text{mW}\). The beam is then passed through a pinhole that blocks a significant amount of the power, and an iris is used to reduce the beam diameter, and is blocking part of the beam. Measurements of the interferometer arms after split in the beam splitter indicates that the reference arm power after the polarizer is 0.57\(\text{mW}\). The measured power in the signal arm of the interferometer, after the glass plate and before the microscope objective, is 0.45-0.55\(\text{mW}\). This measurements suggests that the power of the laser beam after the pinhole and iris is in the order of \(~1-1.2\text{mW}\).
4.2. Nd:Yag Output Power Measurements

The Nd:Yag laser allows the user to control the charging voltage to the laser igniting mechanism, and the power of the output beam is related to the charging voltage. The Nd:Yag laser offers charging voltages from 0V to 1500V. The Output power of the Nd:Yag heating laser was measured to define a linear range of operation in various repetition rates. The measurements were made using an amplified silicon photodiode (THORLABS FDS100 Si Photodiode). Measurements were made after the plate glass, shown in Figure 3.8 that combines the interferometer and the refracted Nd:Yag laser beams coaxially, and in front of the microscope objective. The typical circuit that is shown in Figure 4.2 for the FDS100 Si photodiode was used to measure the beam power. Neutral Density (ND) filters were used to prevent the Photodiode from saturating or been damaged during the power measurements as laser power is increased between measurements.

The output voltage of the circuit described in Figure 4.2 can be found using

\[ V_o = P \cdot \mathcal{R}(\lambda) \cdot R_{Load} \]  

(4.1)

where \( \mathcal{R}(\lambda) \) is the responsivity of the photodiode in [A/W], \( P \) is the power of light beam and \( R_{Load} \) is the value of the resistor at the circuit output. The responsivity of the THORLAB FSD100 is shown in Figure 4.1 according to manufacturer specifications.
The vertical line points to 1064\textit{nm} wavelength, that is the Nd:Yag Laser wavelength, and

\[ \mathcal{R}(1064\text{nm}) = 0.34 \left[ \frac{A}{W} \right]. \]

The laser's output power was measured at three repetition rates: 5\text{Hz}, 10\text{Hz} and 15\text{Hz}. For each repetition rate measurements were made for different voltages charging the laser to

---

\textit{Figure 4.1.}: Responsivity vs. Wavelength for FSD100 Photodiode (THORLAB FSD100 Photodiode Datasheet, with permission)

\textit{Figure 4.2.}: Typical Circuit for THORLAB FSD100 Photodiode (THORLABS FDS100 Datasheet, with permission)
produce a plot of the output power of the laser vs. the charging voltage of the pulse. The reason for measuring over different repetition rates is to determine the maximum charging voltage per repetition rate to which the laser can fully recharge, without losing energy due to short charging time. A typical temporal profile of the beam power is shown in Figure 4.3.

The blue dark line in Figure 4.3 is the actual measured voltage at the circuit output, the cyan light line is low pass filter result of the original curve, to reduce the high frequencies noise. Figure 4.3 clearly shows the 250µs FWHM pulse of the Nd:Yag laser. To determine the power and energy of the laser pulse Equation 4.1 is used to determine the power of the laser pulse at every time stamp, including corrections for ND filters that were used to attenuate the laser pulse power. Integrating the data over time then resulted in the optical energy produced by a single pulse. For every repetition rate charging voltages of the laser was varied from 350V to 1000V in 50V steps. The results of the power measure-

Figure 4.3.: Typical Nd:Yag Beam Profile, Charging 750V, 5Hz rep. rate, 2.1ND filter
ments of the Nd:Yag laser are shown in Figure 4.4. The 15Hz repetition rate plot flattens at approximately 650V, suggesting that at this repetition rate the laser power source can not complete the charging process at charging voltages higher then 650V. The 10Hz plot starts showing the same behavior at around 750V-800V. According to the results shown on Figure 4.4, two repetition rates are chosen: 5Hz and 10Hz. The charging voltages for each one of the repetition rates were set to be: 550V, 600V, 650V and 700V, as detailed in Table 4.1.

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
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<td>5Hz</td>
<td>550V</td>
<td>1.24·10⁻¹</td>
<td>3.11·10⁻⁵</td>
</tr>
<tr>
<td></td>
<td>600V</td>
<td>2.16·10⁻¹</td>
<td>5.40·10⁻⁵</td>
</tr>
<tr>
<td></td>
<td>650V</td>
<td>3.28·10⁻¹</td>
<td>8.19·10⁻⁵</td>
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<tr>
<td></td>
<td>700V</td>
<td>3.94·10⁻¹</td>
<td>9.84·10⁻⁵</td>
</tr>
<tr>
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<td>550V</td>
<td>2.75·10⁻¹</td>
<td>6.87·10⁻⁵</td>
</tr>
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<td></td>
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<tr>
<td></td>
<td>650V</td>
<td>4.38·10⁻¹</td>
<td>1.09·10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>700V</td>
<td>4.49·10⁻¹</td>
<td>1.12·10⁻⁴</td>
</tr>
</tbody>
</table>

Table 4.1.: Selected repetition rates and charging voltages for the experiment, and the corresponding measured laser beam power and energy per pulse
Figure 4.4: Top - Energy vs. Charging Voltage (per pulse), Bottom - Power vs. Charging Voltage, for the 1064nm Nd:Yag Laser
4.3. **Noise Measurements**

The results and measurements of the experiment are affected from three major sources of noise:

1. Quantum noise – arising from the quantum fluctuations of the laser light

2. Electronic noise – arising from various components of the electronic circuits in the experiment setup, and

3. Mechanical noise – this noise affects the measurements of the interferometer, and arises from mechanical vibration of the target and optical components that the interferometer is made of. It is caused by vibrations of the optical table, acoustic pressure waves in the air, *etc*.

The Nd:Yag laser that is used in this experiment is cooled using a water cooling system. The Nd:Yag laser is placed on top of the optical table on which the interferometer is installed. The water flows to the Nd:Yag laser head from the cooling system, and back, through rubber hoses. The mechanical vibrations that are caused by the mechanical parts in the cooling system as well as by the flow of water in the hoses, cause the interferometer to measure false motions, either by causing the sample to vibrate on the stage, or by causing the optical components that the interferometer is made of, to vibrate. Figures 4.5 and 4.6, show measurements of noise that were taken between optophotothermoelastic displacement measurements with the Nd:Yag laser turned off, and the water cooling system turned on. Comparing these plots to the plots in Figures 4.7 and 4.8, that were taken
with the water cooling system off, clearly indicates that the mechanical noise that the water cooling system of the Nd:Yag laser introduce into the system dominates.

In Figures 4.5-4.8 the top left plots shows the quadrature signal that is detected by the detectors. The circle at the background is a reference signal, taken by gently vibrating the optical table, and the darker segment that overlaps it, is the quadrature signal measured in the experiment. This plot shows the output voltage of the in-phase and quadrature detectors in volts. The reference signal is used to locate the center of the quadrature signal, to allow accurate detection of the quadrature signal phase angle. The top left plot is the unwrapped angle of the quadrature signal in radians as a function of time. The bottom left

Figure 4.5.: Noise measured with cooling system turned on, sample ID: 050-025-000

In Figures 4.5-4.8 the top left plots shows the quadrature signal that is detected by the detectors. The circle at the background is a reference signal, taken by gently vibrating the optical table, and the darker segment that overlaps it, is the quadrature signal measured in the experiment. This plot shows the output voltage of the in-phase and quadrature detectors in volts. The reference signal is used to locate the center of the quadrature signal, to allow accurate detection of the quadrature signal phase angle. The top left plot is the unwrapped angle of the quadrature signal in radians as a function of time. The bottom left
The Doppler frequencies are obtained by implementing a Fast Fourier Transform (FFT) on the unwrapped angle signal. This plots shows normalized intensity of the as a function of the frequency of the signal. The bottom right plot shows the relative displacement of the sample as a function of time. The plots in Figures 4.5-4.8 are typical figures of the optophotothermoelectric detection microscope results.

Figure 4.6.: Noise measured with cooling system turned on, sample ID: 050-100-000
The plots in Figures 4.5 and 4.6 show that the noise level with the cooling system turned on is equivalent to vibrations of 100nm in the apparatus, while Figures 4.7 and 4.8 indicates that when the water cooling system is off the equivalent noise level is of the order of 40nm-15nm, indicates an improvement of more than twice in the interferometer resolving power. Other measures can be taken to improve the interferometer resolution further, but since the water cooling system is the dominate in the given setup it will not improve the resolution.

Figure 4.7.: Noise measured with the Nd:Yag and water cooling system off

The plots in Figures 4.5 and 4.6 show that the noise level with the cooling system turned on is equivalent to vibrations of 100nm in the apparatus, while Figures 4.7 and 4.8 indicates that when the water cooling system is off the equivalent noise level is of the order of 40nm-15nm, indicates an improvement of more than twice in the interferometer resolving power. Other measures can be taken to improve the interferometer resolution further, but since the water cooling system is the dominate in the given setup it will not improve the resolution.
Figure 4.8.: Noise measured with the Nd:Yag and water cooling system off
4.4. **Results**

4.4.1. **Qualitative Results**

The expected motion curves that describe the displacement of the measured point due to the photothermal process are shown in the computational simulation results, in Figure 2.3(d). During the first 250μs the laser pulse heats the sample and the sample expands due to the photothermal effect. At time 250μs the laser pulse is off and the relaxation of the sample begins, the sample contracts back to its original size exponentially, as can be seen in the cooling curve. A similar curve of displacement is shown in Figure 4.9. The absorption coefficient of the sample simulated in Figure 4.9 matches the absorption coefficient of sample ID: 050-025-000, and the laser power used in the simulation is similar to applying 650V in 5Hz repetition rate. Figure 4.10 shows experimental results for sample 050-0250-000 with 650V charging voltage to the Nd:Yag laser. The upper left plot in Figure 4.10 shows the quadrature signal that was measured during repetitive heating, the dark line in the foreground. The light line in the background describes the reference quadrature signal, as explained earlier, this signal was recorded while causing vibration to the optical table, with no heating laser power, before taking the measurement.
The upper right plot shows the unwrapped angle of the quadrature signal. Five pulses are seen in this one second frame as expected from the 5Hz repetition rate. The lower left plot is the power density spectrum of the displacement, obtained by implementing a Fast Fourier Transform (FFT) on the unwrapped angle information. The 5Hz repetition rate is seen clearly along with some higher harmonics of it at 10Hz and higher. The same pattern of noise seen in Figures 4.13 and 4.14 is also evident in this plot. The lower right plot describes the displacement measured. This is a zoom of the displacement plot on one of the pulses transition.

Figure 4.9.: Simulated displacement plot, \( \mu_a = 0.6409 \text{ cm}^{-1} \), laser power 0.328W

The upper right plot shows the unwrapped angle of the quadrature signal. Five pulses are seen in this one second frame as expected from the 5Hz repetition rate. The lower left plot is the power density spectrum of the displacement, obtained by implementing a Fast Fourier Transform (FFT) on the unwrapped angle information. The 5Hz repetition rate is seen clearly along with some higher harmonics of it at 10Hz and higher. The same pattern of noise seen in Figures 4.13 and 4.14 is also evident in this plot. The lower right plot describes the displacement measured. This is a zoom of the displacement plot on one of the pulses transition.
The plots exhibit similar behavior as the simulated displacement results, seen in Figure 4.9. The expanding behavior of the plot is not clear at the heating process, this will be discussed in section 4.3.2. During the cool down and relaxation period of the heating process both plots exhibit an exponential contractions, suggesting good agreement of the experiment with the approximated theoretical model, but the simulation plot shows higher displacement and shorter relaxation time.

Figure 4.10.: Optophotoelastic detection of sample 050-025-000, Nd:Yag laser 650V 5Hz Repetition Rate (0.328W), upper left – the quadrature signal, upper right – unwrapped angle, lower left – FFT of the unwrapped angle, lower right – displacement (zoom in)
The maximum displacement value seen in the simulation results, in figure 4.9, is 488nm. The results in the experiment shown in Figure 4.10 indicates a displacement of only 222nm. The difference between the two are discussed in the next section considering detectors saturation. The experimental results indicates that the during the cool down the photothermoelastic displacement had achieved $1/e$ the value of the maximum value of the displacement, after 6ms. The simulation results indicates relaxation to the $1/e$ value of the maximum displacement value after only 1.725ms.

4.4.2. Detectors Saturation
The quadrature signal plot indicates that during the Nd:Yag heating pulse the quadrature signal is lost. Fastidious investigation of the unwrapped angle plot shows that during the heating laser pulse the signal does not behave as expected as can be seen in Figure 4.11. Instead of showing the displacement increasing as the Nd:Yag laser pulse heats the optical zone, the plot shows a perfectly flat line with no noise suggesting that the detectors amplifiers are at theirs high voltage rail value. In Figure 4.12 a saturation process of the detectors is shown in details. The various stages in the process are described in different colors, the plot at left is the quadrature signal and the plot to the right is the corresponding displacement vs. time. At the beginning the signal is seen before the heating pulse, at approximately time zero. When the pulse is turned on the Nd:Yag laser pulse is reflected back from the aluminum in the sample and since the Nd:Yag laser beam is aligned with
Figure 4.11.: Detectors saturation, Unwrapped angle vs. sample number

Figure 4.12.: Saturation of detectors, Sample ID: 050-025-000, Nd:Yag laser voltage 600V, 5Hz Repetition Rate. Right – Quadrature signal, Left – Displacement vs. time
the interferometer laser beam, it will be transmitted through the glass plate into the BS and to the detectors. In the left plot of Figure 4.12 the saturating process is seen on the background of the signal for the entire frame of five pulses. The saturation of the detectors causes the signal to reach the highest voltage levels and the signal moves on both the \( x \) and \( y \) axis simultaneously. Then one of the detectors recover from saturation level while the other one is still saturated, causing the signal to move only on one axis, in Figure 4.12 this is the \( x \)-axis while the level of the signal in the \( y \)-axis is fixed. The signal return slowly to the circular pattern of the quadrature signal, and after approximately \( 600\mu s \) the signal is intact again. The time that is takes to the detectors to recover from saturation depends on the energy of the heating laser pulse and might be longer then \( 600\mu s \), during the analysis done in this work a \( 600\mu s \) saturation time is assumed.

Comparing the results of the simulation and experiment that were made in section 4.3.1 given the saturation of the detectors, i.e. measuring the simulation at \( 600\mu s \), will show different results then were obtained. The expansion that is measured in the simulation at \( 600\mu s \) is 373nm comparing to 483nm at the maximum value, a difference of 151nm and \(~23\%\), that is just less then a quarter of a wavelength. Accordingly the relaxation to \( 1/e \) of the value that is measured for \( 600\mu s \) is 2.2ms, comparing to the previous value of 1.75ms.

### 4.4.3. Quantitative Results

The simulation results that are shown in Figure 2.5 corresponds to the maximum value of the displacement that was measured during each simulation. The experiment results are measured when the detectors are out of saturation and the reading of displacement is re-
stored. Since the estimated time for the detectors to show reading is approximately 600µs according to some measurements, the measured values of the simulation results are taken at 600µs after the pulse starts.

Figure 4.13.: Simulation (dashed lines) and experimental results (solid lines) comparison for 10Hz Nd:Yag laser repetition rate, Top – Displacement vs. energy per pulse, Bottom – Displacement vs. absorption coefficient µa
The results of the comparison of the simulation vs. the experiment results are shown in Figure 4.13 for the 10Hz Nd:Yag laser repetition rate, and in Figure 4.14 for the 5Hz Nd:Yag laser repetition rate.

**Figure 4.13:** 5Hz displacement vs. energy.

**Figure 4.14:** Simulation (dashed lines) and experimental results (solid lines) comparison for 5Hz Nd:Yag laser repetition rate, Top – Displacement vs. energy per pulse, Bottom – Displacement vs. absorption coefficient $\mu_a$.
The top plots in both figures describe the displacement in meters as a function of the energy of the Nd:Yag laser per pulse, for the different absorption coefficient samples. The bottom plots describe the displacement in meter as a function of the absorption coefficient for the various samples Nd:Yag laser powers used in the experiments. In all of the plots the simulation results are described in dashed lines and the experimental results are shown in solid lines. The points of measurements along the plots are shown in asterisks for the simulated results and using small ‘x’ on the experimental results plots. The results in the lower plots, exhibit good agreement at the low absorption coefficients samples. As the absorption coefficients value increase the difference between the simulation and the experimental results are greater. The bottom plots in the figures shows that for the first two absorption coefficients the results show similar values and the simulated and experimental graphs exhibit similar slopes. The linear behavior of the displacement as a function of the energy per pulse for a given absorption coefficient, observed in the simulation results, can be seen in the experimental results to a good approximation, in the top plots in the figures. The measurements of the experimental results in the 650V charging voltage are all exhibit a lower displacement then expected and are out of the linear approximation of the curves. This anomaly is seen in all the experimental results, for each absorption coefficient and for the two repetition rates suggesting a problem in the Nd:Yag laser operation in this charging voltage.

The experimental results shown on the right plots suggests that the displacement in the samples starts to saturate at around 1.5µm-2µm. Figure 4.15 describes the photothermal
process in a sample. The heating laser beam is focused into the sample and heats the volume of the diffracted limited spot.

The assumptions in the computational model are that the optical zone is spherical and that the sample is infinite. The sample that is used in the experiments incorporates aluminum foil that is highly reflective. This causes the optical zone to be a half ellipsoid, the light pass through this optical zone twice, on the way to the aluminum foil, and after reflected from the aluminum foil it returns in the same path, providing good alignment and focus. The heating of the PVCP causes it to expand from the center of the hemisphere outwards.

*Figure 4.15.: Photothermal process in a sample*

The assumptions in the computational model are that the optical zone is spherical and that the sample is infinite. The sample that is used in the experiments incorporates aluminum foil that is highly reflective. This causes the optical zone to be a half ellipsoid, the light pass through this optical zone twice, on the way to the aluminum foil, and after reflected from the aluminum foil it returns in the same path, providing good alignment and focus. The heating of the PVCP causes it to expand from the center of the hemisphere outwards.
causing the aluminum foil to follow it and bend upward. The displacement of the aluminum foil is detected by the interferometer.

Figure 4.16 describes the photothermal process in greater details. Since the heating laser pulse is not short enough to meet with thermal confinement restriction, heat will diffuse out of the optical zone during the time the laser pulse is on. On the PVCP and aluminum boundary, shown in Figure 4.16 as $z_3$, boundary condition will require that the temperature in the aluminum side will be equal to the temperature in the PVCP side. Since the linear expansion coefficient for the aluminum, $\alpha_{Al}$, is greater than the linear expansion co-

\textit{Figure 4.16.: Photothermal process in a sample, zoom in}

Figure 4.16 describes the photothermal process in greater details. Since the heating laser pulse is not short enough to meet with thermal confinement restriction, heat will diffuse out of the optical zone during the time the laser pulse is on. On the PVCP and aluminum boundary, shown in Figure 4.16 as $z_3$, boundary condition will require that the temperature in the aluminum side will be equal to the temperature in the PVCP side. Since the linear expansion coefficient for the aluminum, $\alpha_{Al}$, is greater than the linear expansion co-
efficient of PVCP, $\alpha_{pvc}$, the horizontal linear expansion that the aluminum will experience, shown in Figure 4.16 in horizontal white arrow, on the PVCP aluminum boundary will be greater than the expansion of the PVCP, shown in Figure 4.16 in horizontal gray arrow. This will cause the aluminum and PVCP that are connected to each other to bend to a shape of a bowl as shown in white dashed line in Figure 4.16. This can explain the limit on the displacement that is observed in experimental results.

The heat that is induced in the sample may change the properties of the material causing local changes in the index of refraction of the material, that in turn will effect the path of the He-Ne and Nd:Yag laser beams. This can change the location of the optical zone and also cause the He-Ne laser to defocus. This effects are not studied in this research and are left to future work.
5. DISCUSSION

The image obtained by photothermal spectroscopy imaging techniques, is related to the power of the light that is absorbed in the sample, while the scattered light does not contribute to the image and is basically lost. In confocal microscopy the scattered (or transmitted) light is the light that contribute to the image formation, while the absorbed light is lost. Imaging of highly scattering turbid opaque samples with a confocal microscope impose a problem on the depth of which the microscope can penetrate. This is caused mainly because light that is scattered from locations in front of the focal spot in the sample will cause power lose, and will clutter the signal. Imaging transparent types of tissue will impose problems on the confocal microscope, since a small amount of signal is scattered back to the detectors, the sample will show little details unless the image formation decodes phase information detection, similar to OQM. The suggested technique in this work combines an interferometer signal beam, with a heating laser beam coaxially, to heat the volume at the diffraction limited spot in which the interferometer measures displacement. Expansion of this volume due to the photothermal process will cause small scatterers within this small volume to change their location, this small displacement will then be detected by the interferometer. Since the displacement is related to the power of the laser and the absorption coefficient, as well as to properties of the sample, then assuming constant power in the laser output will produce mapping of the absorption coefficients in the sample.
5.1. Scanning

In this research a basic setup was demonstrated for proof of concept purposes. No scanning was made of the sample not in the $x$, $y$ plane nor in the $z$ axis. In further studies this apparatus needs to be improved to implement features that will allow it to scan in 2D in the $x$, $y$ axis and to section the sample in the $z$-axis. To implement $x$, $y$ scanning either a standard two-mirror scanner or a dual wedge technique [35], can be implemented. The $z$-axis sectioning can be made either by moving the stage along the $z$-axis or by moving the objective. Since the two laser beam enter the objective collimated in this design, then the objective can be moved, and the focal point of the two beams will be aligned as long as a good quality apochromatic objective will be used that is corrected for both wavelengths.

5.2. Heating Laser

In this work a high power Nd:Yag laser, with a $10W$ maximum output power capability, was used to initiate the photothermal process. Only a small fraction of the power of the laser was used to combine coaxially with the interferometer beam and to the microscope objective. The highest Nd:Yag laser beam power used in this experiment to go through the objective of the microscope to the sample was $0.449W$. Using a diode laser, and replacing the plate glass that was reflecting only 5% of the power of the Nd:Yag laser with a suitable dichoric, that will reflect the IR and will transmit visible light, will allow the same powers to be achieved, and will increase the control of the user on the heating laser pulse. The Nd:Yag laser allows very limited control on the laser pulse that basically limited to controlling the output power very coarsely. There is no control on the duration
of the output pulse, which is important in this type of study. Using short laser pulses that will meet the thermal confinement restriction will allow to maximize the displacement for a given level of energy, and will eventually enable using lower laser output powers, which is always a desirable feature in biomedical applications. Small diode laser do not require cooling and therefore will remove the largest noise source in the given system and will allow the interferometer to function with greater sensitivity and therefore will increase the accuracy of the apparatus in detecting small differences between absorption coefficients. In order to minimize noise that is raised by vibrations of the sample the reference beam can divert to reflect back from a small highly reflective surface that is attached to the sample next to the area that is scanned. This arrangement will promise that the reference and signal beams will experience the same variations of the optical path, caused by the sample. The Nd:Yag laser that was used in this research have a 1064nm wavelength beam. Because of the structure of this apparatus after the two laser beams are combined coaxially at the glass plate, they will follow the same path, to a good approximation. Therefore not only the interferometer laser beam is reflected back to the detectors of the interferometer, but also the heating laser beam is reflected back and will reach to the detectors. This was enhanced further by using high reflecting samples in this work.

The detectors that were used in this work are silicon detectors, and the sensitivity of silicon to 1064nm wavelength is strong. Using a longer wavelength laser, such as 1300nm or 1500nm, will place the heating laser outside of the detectors responsivity curve, preventing detectors saturation during the times that the heating laser pulse is on.
5.3. Computational Model

The computational model that was used in this work is very basic. Ignoring boundaries and different types of materials, as well as sample thickness, results in difference from the case that is study in this work. The approach that is taken in this work is to find an analytical solution using Green's function and integration. This does not allow complex simulations with different materials and boundaries, and therefore infinite, homogeneous medium is assumed. In the model a 1D displacement and stress were simulated, assuming spherical symmetry. This assumptions are a first order approximations and in order to achieve better simulated results, in future works, will have to be to be abandon.

Other methods for simulation such as Finite Difference Time Domain (FDTD), or Finite Elements Method (FEM), will do better for inhomogeneous and finite samples, and will allow to define the source with greater accuracy. But this models will have other restrictions regarding solution stability.

5.4. Samples

The samples used in this work were simple uniform PVCP samples that incorporated only absorption, while the scattering coefficient was not controlled. In further work the samples need to include scattering by adding TiO2 to the solution of the sample during preparation. Small structures can be constructed to mimic features in the tissue such as blood vessels [36]. In further work once a scanning method is implemented in the apparatus it is desirable to take in vitro images. Eventually the objective of developing this apparatus is to image in vivo, and produce a 3D model of a tissue that is deeper then standard confocal
microscope image and holds resolution as a confocal microscope, or better then OCT imaging technique.
6. CONCLUSIONS

In biomedical imaging it is important to achieve a good, non invasive image, with suitable depth of penetration, while maintaining good resolution and contrast of the image. This research suggests an imaging method that combines coherent confocal microscopy with photothermoelastic displacement detection. The objective is to enhance the coherent confocal microscope capabilities to image deeper than a standard confocal microscope, while preserving the resolution of the confocal microscope, and applying a new mode of contrast that is phase related. In this research the potential of the optophotothermoelastic displacement detection using a coherent confocal microscope, is demonstrated for the first time. Further work needs to be made to improve the apparatus as well as the computational model that is used to simulate the process. The goal is to produce in-vitro and eventually, in-vivo imaging of tissues, using this technique.
APPENDIX A: ALIGNMENT

Optical systems are highly sensitive to the alignment of their components. Misalignment of the system components can result in significant degradation of the apparatus performance. In this appendix a procedure for the alignment of the optophotothermoelastic detection microscope is given. This procedure is a good basic introduction to the user that is not yet familiar with the system.

Alignment of coherent confocal microscope:

1. Make sure that the laser beam is collimated by either using a shear plate or measuring it diameter over a long distance.

2. Make sure that the polarizer is tilted 45° to the vertical and that the plate glass is tilted at 45°.

3. Remove the Objective.

4. Place a mirror at the sample plane.

5. Using a semi transparent piece of plastic, check that the reference beam returns to same position by comparing the beam spots positions on the piece of plastic, while it is placed on the BS side. Correct using reference arm mirrors.

6. Repeat step 5 for the signal beam.
7. Check that the signal and reference beams are aligned by examining the interference pattern after combined in the BS, the interference pattern needs to be uniform, fringes indicates that the wavefronts are not matched, correct using the reference beam alignment mirrors.

8. Check the alignment by gently vibrating the optical table and observing the magnitude of the quadrature signal on the oscilloscope, align the reference beam to achieve largest quadrature signal magnitude.

9. Align the QWP to get a circular quadrature signal.

10. Return the objective to place and check the focus by comparing the sizes of the signal beams on the side of the BS using the semi transparent piece of plastic, correct by the changing the tilt of the microscope objective.

11. Repeat steps 7-8.

12. Adjust the focus by gently vibrating the optical table and observe the magnitude of the quadrature signal on the oscilloscope, align the reference beam to achieve largest quadrature signal magnitude.

**Alignment of Nd:YAG laser:**

1. Make sure that the coherent confocal microscope is aligned using the procedure detailed here above. Place a mirror as a sample in the coherent confocal microscope.
2. Adjust the Nd:YAG laser charging voltage to 400V, 1Hz repetition rate. Use IR detection paper to see the Nd:YAG laser beam.

3. Remove the collimating lens in the Nd:YAG laser path and make sure that the first alignment mirror is set to the height of the laser beam.

4. Turning off surrounding light, the beam of the coherent confocal microscope, reflected from the highly reflective target and the plate glass can be seen. Adjust the third alignment mirror, the one in front of the plate glass, to be the height of the coherent confocal microscope. There will be two beams use Figure 3.10 to figure which beam to use.

5. Check that the coherent confocal and ND:Yag laser beam are aligned after the plate glass, in front of the beam stopper.

6. Replace the lens in the Nd:YAG laser path. Make sure that the lens is centered by the coherent confocal microscope beam, that is reflected from the target.

7. Check that the Nd:YAG laser is collimated, correct with the lens.

8. Check that the reflected beam from the target of the coherent confocal microscope, focused at the center of the pinhole placed in the the Nd:YAG laser path. Align using the first alignment mirrors.

9. The system is aligned.
APPENDIX B: PVCP SAMPLE PREPARATION

PVCP (M-F Manufacturing Co., Fort Worth, TX, USA), is a white opaque solution of monomers that polymerizes and becomes clear, at visible wavelengths, when heated to approximately 200°C. PVCP does not absorb light at the 1064nm that is a wavelength used for heating in this work. Black plastic color (BPC) (M-F Manufacturing Co., Fort Worth, TX, USA), composed of CI Pigment Black 7, is added to the PVCP to add an absorbing material to the compound, and control its absorbing coefficient.

The process of making a PVCP phantom sample, that was used in this research is specified here, to allow reconstructing of samples that have the same optical properties.

1. Measure 50ml of PVCP using a 10ml syringe, and pour into a 500ml glass container that can be heated.

2. Measure the amount of BPC in [µl] to add to the PVCP using a pipette (Pipetman® P, Gilson Inc. USA), and add to the PVCP in the glass. Read the Pipetman® P operating manual, before using it, to ensure proper measurement. Mix the BPC with the PVCP until the solution color is uniform.

3. Heat the solution, slowly, stirring it gently. Avoid stirring fast to prevent bubbles from forming. Heat should be increased slowly and gradually, allowing the entire solution volume inside the glass container to heat before increasing the temperature.
4. It is good practice to use a thermometer for both stirring and measuring the solution temperature. When the solution approaches ~140°C-150°C, small lumps of gray translucent gel will start to appear in the solution. Increase the heat and continue stirring slowly.

5. When the solution has completely turned from opaque to translucent, and reaches pouring consistency which will be clear and thin, pour the liquid to a mold of the sample.

6. Allow the plastic to cool for about 30 minutes, and then remove it from the mold.

7. Do not store the samples in a plastic container because the PVCP will react with and, dissolve, other plastic materials.

This was originated from the instructions of M-F Manufacturing Co. and the procedure that is described in [16]. It is a result of learning through a repetitive process of phantom preparing. This recipe is a good starting point for making the first attempts to make a phantom. However practice will eventually allow one to acquire the skill of making a proper phantom.
APPENDIX C: EXPERIMENTAL RESULTS

The experimental results of the maximum displacement is given in this appendix in the following tables. For each sample the two repetition rates 5Hz and 10Hz, are measured. The Nd:Yag laser is turned on and data is acquired by the oscilloscope, synchronized by the Nd:Yag laser beam. Each measurement is made over a period of time that allows for 4-5 Nd-Yag laser pulses to be recorded, so that data of 4-5 cycles of expansion and contraction is recorded. The maximum displacement for each of the cycles is seen in the table under the respective measurement number. For every repetition rate measurement are made for four Nd:Yag laser charging voltages 550V, 600V, 650V and 700V. The respective powers and energy per pulse for this charging voltages are detailed in Table 4.1.
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Table 6.1: Experimental results of maximum displacement for sample ID: 050-025-000
## 5Hz

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## 10Hz

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Table 6.2: Experimental results of maximum displacement for sample ID: 050-050-000
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Table 6.3: Experimental results of maximum displacement for sample ID: 050-100-000
Table 6.4: Experimental results of maximum displacement for sample ID: 050-250-000

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