PHOTOACOUSTIC EVALUATION OF SURFACES VIA PULSED EVANESCENT FIELD INTERACTION

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by
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EVANESCENT FIELD INTERACTION

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opinion, it is worthy of acceptance.

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DEDICATION

I dedicate this dissertation to my parents, Michael E. Goldschmidt and Susan R. Santoscoy, without whom I would not be the person I am today.

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ABSTRACT

In recent years, major research funding and commercial development has been going toward the production and characterization of increasingly useful nanomaterials. These materials such as quantum dots, nanoparticles, and thin films can increase the efficiency of solar panels, create new treatments for cancer, and vastly improve the detection capabilities for various optical sensors for biosensing. Unfortunately, to date, very few methods of characterizing these types of materials exist such as scanning electron microscopy and ellipsometry. These techniques are prohibitively expensive, cannot be used with all materials, and require rigorous preparation schemes before scanning. Therefore, a new method to characterize thin films and detect the properties of nanomaterials is needed. This study proposes a newly revived method, Total Internal Reflection Photoacoustic Spectroscopy, along with related techniques, to deliver cost effective characterization and detection for nanomaterials and thin films.

Chapter 1

Introduction

Over the course of human history there have been many technological revolutions, from the industrial revolution in the late 1700's to the information age that occurred after the early 1970's. In the 21st century, humanity has encountered another significant revolution with our discovery of the utility of nanomaterials. The discovery of materials below 100 nanometers in size with unbelievably useful properties, like superhydrophobicity, fluorescence, and strength and surface area hundreds of times higher than steel per gram, has led to the re-imagination of our medical devices, computers, and increasingly more scarce building materials. Current household products like Rust-oleum's Neverwet, which transforms any common surface into a superhydrophobic surface, stands to change every surface we touch by creating extremely simple methods of cleaning everything from clothing to cars, and stand to lessen our world's disease propagation by creating an impenetrable barrier to growing bacteria.

All of these technological marvels would not have been possible without a firm understanding of the underlying quantum, mechanical, electrical, optical, and thermal

properties of these classically strange materials. To understand these materials we must look at properties of the surface as shown in Figure 1.1 as opposed to the bulk material properties of the interior molecules and atoms. Our capabilities to probe these surface properties on the nano level are dependent upon a handful of technologies such as electron microscopy, ellipsometry, and atomic force microscopy. This is due to the fact that below 250 nanometers, traditional microscopy methods cannot resolve structures due to the optical diffraction limit. These exotic methods share the commonality that they can resolve structures below 200 nanometers, thus making them super resolution instruments, however, their ability to discern basic properties beyond imaging is extremely limited. Therefore, new methods to characterize materials in the UV/VIS/NIR region of the optical spectrum are needed to test these new nanomaterials at understandable conditions more akin to real life situations.

1.1 Mesoporous Materials

One class of materials that has generated a significant amount of interest for material characterization is mesoporous materials. These materials have nano sized pores from approximately 2-50 nm in size, which can be used for a variety of purposes, most obviously as a filter or catalyst support [1]. In addition to those traditional properties, mesoporous materials are being increasingly used in biomedical related fields like diagnostic optics [2,3], military applications such as antireflection coatings [4], gas sensors [5], and even as tri-modal imaging nano particles [6], which stand to revolutionize medical biosensing for cancer and other diseases.

Figure 1.1: Surfaces control the overall properties of materials.

1.2 Current Techniques

There are effectively three main techniques to help us characterize nanomaterials; electron microscopy, ellipsometry, and atomic force microscopy. Electron microscopy, invented in the early 1930's, gave the scientific community the ability to break the optical diffraction limit and look at structures below 200 nanometers. Traditional microscopy is limited by the optical diffraction limit given by

$$d = \frac{\lambda}{(2n \sin \theta)},$$

where d is the diameter of a diffraction limited spot, n is the refractive index of the medium being imaged, and θ is the half angle of the maximum cone of light that can enter the lens. However, due to the work done by deBroglie in the 1920's, it was recognized that the effective diffraction limit of non-light based particles such as electrons would be much smaller than that of a comparable visible wavelength of

light. Green light has a wavelength of approximately 532 nm that gives a theoretical optical diffraction limit in air of 190 nanometers. However, the deBroglie equation, shown in (1.1), calculates an electron's wavelength of approximately 5.4 picometers from (1.2) (assuming 100 kV microscope voltage). Thus, the diffraction limit of the electron microscope is approximately 0.24 nanometers given by

$$\lambda = h/p \tag{1.1}$$

and

$$\lambda = \frac{h}{2meV}, \tag{1.2}$$

where λ is the wavelength of the particle, h is Planck's constant, p is the momentum, m is the electron's rest mass, e is the electronic charge, and V is the accelerating voltage of the electron microscope. Therefore, using an electron microscope allows for an approximately 1000 fold increase in resolving power when compared to a traditional light microscope. Various modes of the electron microscope can be used for a variety of purposes suited to the sample and testing parameters needed for evaluation.

1.2.1 Transmission Electron Microscope

Electron microscopes work on the principle of interacting electron beams with the sample in much the same way that a traditional microscope exposes samples to light. In transmission mode, the electron microscope passes electrons through a micron-thin sectioned material of interest. As the electrons pass through the material image contrast is formed by the absorption and scattering of electrons as they encounter the

material. In transmission mode, electron microscopes, while extremely valuable for scientific research labs, do have several drawbacks that prevent their widespread use in industrial applications today. Although the resolution of TEMs is higher than that of scanning electron microscopes (SEM), the sample thickness is generally limited to micron-thin materials. Additionally, TEMs are very expensive (\$2,000,000), tend to be room-sized instruments, and require constant maintenance.

1.2.2 Scanning Electron Microscope

Figure 1.2: Gold nanoparticles attached to fluorescent beads have been used for their high optical absorption properties for the early detection of breast cancer. [7]

Electron microscopes can be used in the scanning mode that allows for physically larger samples to be used for scanning purposes. In contrast to transmission mode, scanning electron microscopes use secondary electrons for image contrast. Secondary electrons are electrons that have been removed from the material of interest by the interaction of the SEM's high speed electrons with the sample material. These secondary electrons generally have a mean free path on the order of a few nanometers and therefore the imaging of a material can only lightly penetrate the surface and

is therefore used primarily for imaging material surfaces. Metal nanoparticles, as shown in Figure 1.2, show up particularly well in electron microscopes due to their conductive properties. A schematic of a typical SEM is shown in Figure 1.3.

Figure 1.3: Electron microscopy is a complex series of condenser lenses similar to optical microscopy.

Unfortunately, SEM's tend to have drawbacks in terms of cost (\$100,000), intense sample preparation (most samples have to be coated in some type of metal), and most samples require a vacuum environment. However, there are some SEM models that have the ability to look at wet samples in a limited way.

1.2.3 Ellipsometry

Ellipsometry is an optical technique that measures changes in the linear polarization of an incident light beam reflected from sample surfaces in order to determine the optical properties of the material such as thickness and refractive index. As shown in Figure 1.4, a laser beam of known polarization is reflected off the surface of a sample. The reflection is then measured to determine the amount the polarized axis shifted after interacting with the sample.

Figure 1.4: Light interacts with a sample in ellipsometry via polarization changes from interacting with the surface layers. (Reproduced with permission from J.A. Woollam at http://www.jawoollam.com/tutorial_4.html)

Ellipsometry can be described by

$$\langle \tilde{\epsilon} \rangle = \sin^2 \phi \left[1 + \left(\frac{1 - \rho}{1 + \rho} \right)^2 \tan^2 \phi \right],$$

where $\langle \tilde{\epsilon} \rangle$ is the electromagnetic permittivity, ϕ is the angle of incidence of the beam relative to the sample, and $\rho = \sqrt{R_p/R_s}$, where R_p/R_s represents the reflected values for the P polarization and S polarization respectively.

Ellipsometry has the ability to measure many different types of properties, from thickness and refractive index to composition, crystallinity, surface roughness, and doping concentration. Unfortunately, ellipsometry suffers from several drawbacks. Most notably, the films that can be analyzed must be relatively non-pigmented materials in order to reflect back sufficient light for the ellipsometer to function. In addition, the surfaces of the films being analyzed must be polished before analysis in order to provide a high enough reflection to be analyzed. Finally, a complex Cauchy model must be used to fit the coefficients in the equation to find thickness and refractive index that requires extensive training on the user's side for appropriate ellipsometer functionality.

1.2.4 Atomic Force Microscopy

Atomic force microscopy as shown in Figure 1.5 is the final existing technology that can be used to evaluate surfaces and nanomaterials. Atomic Force Microscopy, invented in 1986 by Binnig, Quate, and Gerber, is a piezoelectric system that can probe the surface of a material by interacting the probe with the surface and recording the corresponding movement over the surface. The working principle of this device is that a very sharp probe is slid across the surface of the sample and small deflections of that probe are measured that can be used to create a topographical map or three dimensional image of the surface. There are three modes that an AFM is generally used in that can determine different properties about the surface of a material.

Figure 1.5: Atomic Force Microscopy driven by a piezo scanner can scan very small changes in surface roughness and can image materials at the nano level.

Contact Mode

In contact mode [8], the Atomic Force Microscope uses its AFM probe along with feedback from the piezoelectric element to keep the probe constantly in contact with the sample. In this way the AFM probe can follow the contours of the surface and respond to differences in height that translate to differences in piezoelectric voltage to recreate an image. This process is shown in Figure 1.6.

Contact mode is generally used to obtain the highest resolution imaging from the AFM to image atomically smooth materials like silicon and glass wafers. Unfortunately, contact mode suffers from the drawback that since it is in contact with the sample, some sample damage and image distortion may occur due to the contact of

the AFM tip with the sample. Additionally, as with all AFM techniques, imaging takes a very long time due to the device having to raster scan the film of interest over several minutes.

Figure 1.6: Several modes of atomic force microscopy exist such as contact and tapping modes.

Non-Contact Mode

In contrast to contact mode, non-contact mode [9] uses the AFM probe tip at 5-15 nm away from the surface of the sample for scanning. Van der Waals forces resulting from the proximity of the probe to the sample allow for topographical changes to be measured and thus an image to be formed by the device. Non-contact mode prevents the issues apparent with contact mode such as sample damage, tip damage, and image distortion. However, due to the fact that Van der Waals forces tend to be much smaller than the forces observed in contact mode, only relatively large changes can be seen in the three-dimensional image and therefore the technique has less resolution

than contact mode.

Tapping Mode

In tapping mode AFM [10], the strengths of both contact mode and non-contact mode are combined by modulating the contact of the probe tip with the sample. The probe is modulated by the piezoelectric element to vibrate at the probe's natural resonant frequency. At this frequency the probe stays in contact with the sample for a moment, then the probe is moved away from the sample out of the range of the contact forces. This process is repeated during the entire length of the scan. Since the probe tip is not in constant contact with the surface, no surface damage occurs, and since the probe is in contact with the surface for a short amount of time, high resolution images can be obtained with ease. Unfortunately, even this combined AFM method suffers from slow scan speed, and limited imaging area ($150\text{ }\mu\text{m} \times 150\text{ }\mu\text{m}$)

1.3 The Photoacoustic Effect

In 1880, with the invention of Alexander Graham Bell's photophone, the photoacoustic effect, or light induced sound, was created. The photoacoustic effect is the optical absorption of pulsed light that is converted into pressure waves, or sound. This effect was nearly forgotten by the scientific community until the early 1970s [11] with its rediscovery due to advancement in laser and microphone technology. Since then, the photoacoustic effect has been used in almost every field to fill niche applications from biomedical imaging to thin film analysis to non-destructive testing of armor in military vehicles.

1.3.1 Biomedical Applications of the Photoacoustic Effect

In the biomedical arena, there are effectively two main areas of application of the photoacoustic effect. One area of study has been cancer detection by Viator [7,12–27] and others [28]. Other types of cancers beyond metastatic melanoma have also been detected using this technique. Even the possibility of detecting semen [29] for fertility studies been investigated using this technique. Interestingly enough, two methods of circulating tumor cell detection have been used over the past few years, an *in vitro* method [15] and an *in vivo* method [30] have been used for circulating tumor cell detection.

The second area of application of the photoacoustic effect is photoacoustic imaging and optical property evaluation. Many papers have been published on photoacoustic imaging [25,31–47] and general optical evaluation [48–53]. However, several applications of photoacoustic imaging are currently being pursued such as burn depth imaging [32,40,52] and transcranial brain imaging [54]. Finally, even methods that use pulsed lasers that do not directly desire the photoacoustic effect can still affect measurements [55] through added effects for the permeabilization of cells.

1.4 Total Internal Reflection Photoacoustic Spectroscopy

A recently rediscovered technology, Total Internal Reflection Photoacoustic Spectroscopy, combines several different technologies together to form a device that has great capability for determining quantum, mechanical, electrical, optical, and thermal properties of materials at the nano scale. TIRPAS functions by gathering acoustic

waves that have been generated by the optical absorption of energy through the interaction of the sample with evanescently coupled photons through a prism/sample interface. TIRPAS has many advantages over traditional technologies such as limited cost, limited sample preparation, and optical spectroscopic evaluation. The overall working principle and functionality of TIRPAS is explained in Chapter 2.

Chapter 2

TIRPAS and SPRPAS

2.1 Total Internal Reflection

In order to understand Total Internal Reflection Photoacoustic Spectroscopy we must first explain the definition of total internal reflection. Total internal reflection refers to a state of light where an incident beam impinges upon an interface between two materials n_1 and n_2 such that 100% of the incident light is reflected away from the interface as shown in Figure 2.1. The condition needed for this reflection to occur comes from Snell's law given by

$$n_1 \sin \theta_1 = n_2 \sin \theta_2, \tag{2.1}$$

where the refracted portion of the light (θ_2 goes to 90 degrees so $\sin \theta_2 = 1$) disappears and the light is totally internally reflected away from the interface, which is described by

$$\theta_1 = \theta_{critical} = \sin^{-1} \left[\frac{n_2}{n_1} \right].$$

Total internal reflection is the effect that allows fiber optic waveguides to guide light large distances (km) for computational and telecommunication applications as in Figure 2.2. Additionally, total internal reflection is used in a variety of imaging technologies, most notably, the total internal reflection microscope as shown in Figure 2.4 that uses the interaction of the evanescent field with a fluorophore to see below the optical diffraction limit.

Figure 2.1: Total internal reflection at an interface can be described by this schematic.

Imaging below the wavelength of light is achieved with high contrast due to the confinement of the evanescent field to the interface between the sample and the prism. Beyond simple imaging, spectroscopic analysis and even dual modality microscopes have been created to utilize total internal reflection to image nanomaterials in a cost effective manner.

Figure 2.2: Optical fibers can only accept light within a given acceptance angle.

2.1.1 The Evanescent Field

The evanescent field is a non-propagating optical field [56] that is generated under the condition that a beam of light strikes an interface where the incoming medium has a higher refractive index than the outgoing medium and the light is striking at an angle larger than the critical angle as shown in Figure 2.3. This non-propagating field can be derived by starting with a few assumptions.

Figure 2.3: The evanescent field has a distinct optical penetration depth based upon factors such as excitation angle and material properties.

Figure 2.4: Total internal reflection fluorescence microscopy can examine materials on the nanometer level such as fluorescent dyes.

Deriving the Existence of the Evanescent Field

Maxwell's equations [57] are given by

$$\nabla \cdot E = 0, \quad (2.2)$$

$$\nabla \times E = \frac{-\partial B}{\partial t}, \quad (2.3)$$

$$\nabla \cdot B = 0,$$

and

$$\nabla \times B = \mu_0 \epsilon_0 \frac{\partial E}{\partial t}.$$

Since the evanescent field should be described from the perspective of the electric field I will use (2.2) and (2.3) to start the derivation. To simplify (2.3) it is necessary to take the curl that gives

$$\nabla \times (\nabla \times E) = \frac{-\partial}{\partial t} \nabla \times B. \quad (2.4)$$

Substituting (2.1.1) into (2.4) gives

$$\nabla \times (\nabla \times E) = \frac{-\partial}{\partial t} \mu_0 \epsilon_0 \frac{\partial E}{\partial t}. \quad (2.5)$$

Equation (2.5) can be simplified by using the curl of the curl identity given by $\nabla \times (\nabla \times E) = \nabla(\nabla \cdot E) - \nabla^2 E$ that gives

$$\nabla(\nabla \cdot E) - \nabla^2 E = \frac{-\partial}{\partial t} \mu_0 \epsilon_0 \frac{\partial E}{\partial t}. \quad (2.6)$$

Equation (2.6) can be simplified further using (2.2) that cancels a term to give

$$-\nabla^2 E = \mu_0 \epsilon_0 \frac{-\partial^2 E}{\partial t^2}. \quad (2.7)$$

The definition of the speed of light in a vacuum is $c = \frac{1}{\sqrt{\mu_0 \epsilon_0}}$ so (2.7) can be further simplified to

$$\frac{\partial^2 E}{\partial t^2} - c_0^2 \nabla^2 E = 0. \quad (2.8)$$

Equation (2.8) can be simplified still to (2.9), which is the electromagnetic wave equation given by

$$\frac{\partial^2 E}{\partial t^2} - c_0^2 \frac{\partial^2 E}{\partial \vec{r}^2} = 0, \quad (2.9)$$

where $\vec{r}$ is a position vector. A solution to (2.9) is given by

$$E(x, t) = E_0 \cos(\vec{k} \cdot \vec{r} - \omega t), \quad (2.10)$$

where $\vec{k}$ is a propagation vector. By using phasor notation it can be assumed that $\cos(\vec{k} \cdot \vec{r} - \omega t) = \Re\{e^{i(\vec{k} \cdot \vec{r} - \omega t)}\}$ and therefore we can rewrite (2.10) to get

$$E(x, t) = E_0 e^{i(\vec{k} \cdot \vec{r} - \omega t)}, \quad (2.11)$$

which is the equation to describe a plane polarized beam of light that is incident upon an interface as shown in Figure 2.5. The problem is set up as an interface between two dielectric media n_1 and n_2 such that $n_1 > n_2$ as shown in Figure 2.5, which can be described by a modified version of (2.11) given by

$$E_2 = E_{02} e^{-i(\omega t - \vec{k}_2 \cdot \vec{r})}. \quad (2.12)$$

Figure 2.5: This is a schematic representation of reflection and refraction to aid with evanescent field derivation.

The propagation vector in the second medium is given by

$$\vec{k}_2 = (k_2 \sin \theta_2, 0, k_2 \cos \theta_2).$$

The dot product of the propagation number, $\vec{k}_2$, and the position vector $\vec{r}$ is given by

$$\vec{r} \cdot \vec{k}_2 = k_1 x \sin \theta_2 + k_2 z \cos \theta_2, \quad (2.13)$$

where x and z are position. Equation (2.13) can be substituted into (2.12), which gives

$$E_2 = E_{02} e^{-i(\omega t - k_2 x \sin \theta_2 - k_2 z \cos \theta_2)}.$$

After applying the Pythagorean trigonometric identity we have

$$E_2 = E_{02}e^{-i(\omega t - k_2 x \sin \theta_2 \pm k_2 z (1 - \sin^2 \theta_2)^{\frac{1}{2}})}.$$

Equation (2.1), or Snell's law, can then be applied to get rid of the θ_2 so that the equation can be defined entirely by the incident angle, θ_1 , given as

$$E_2 = E_{02}e^{-i(\omega t - k_2 x \frac{n_1 \sin \theta_1}{n_2} \pm k_2 z (1 - (\frac{n_1 \sin \theta_1}{n_2})^2)^{\frac{1}{2}})},$$

where n_1 and n_2 are the refractive indices of the two materials. If we distribute the negative sign we get

$$E_2 = E_{02}e^{i(-\omega t + k_2 x \frac{n_1 \sin \theta_1}{n_2} \pm k_2 z (1 - (\frac{n_1 \sin \theta_1}{n_2})^2)^{\frac{1}{2}})}, \quad (2.14)$$

where the propagation number, k_2 can be defined as $k_2 = \frac{\lambda}{2\pi}$. In addition, the wavelength can be adjusted for different media refractive indices by using $\lambda = \frac{\lambda_0}{n_t}$ where λ_0 is the wavelength in a vacuum. Additionally, since $\sqrt{1-a} = i\sqrt{a-1}$ the exponential function in (2.14) can be split up into its constituent x and z components that allows the imaginary component to be removed to get

$$E_2 = E_{02}e^{i(-\omega t + \frac{2\pi n_2}{\lambda_0} x \frac{n_1 \sin \theta_1}{n_2})} e^{(\pm \frac{2\pi n_2}{\lambda_0} z ((\frac{n_1 \sin \theta_1}{n_2})^2 - 1)^{\frac{1}{2}})}.$$

After simplification we obtain

$$E_2 = E_{02}e^{i(-\omega t + \frac{2\pi n_2}{\lambda_0} x \frac{n_1 \sin \theta_1}{n_2})} e^{(\pm \frac{2\pi n_1}{\lambda_0} z (\sin^2 \theta_1 - (\frac{n_2}{n_1})^2)^{\frac{1}{2}})}. \quad (2.15)$$

Finally, we can define (2.15) in terms of z and, δ , the penetration depth of the

evanescent field given by

$$E_2 = E_{02} e^{i(-\omega t + \frac{2\pi n_2}{\lambda_0} x \frac{n_1 \sin \theta_1}{n_2})} e^{\frac{-z}{\delta}}, \quad (2.16)$$

where $\delta = \frac{\lambda_0}{2\pi n_1 (\sin^2 \theta_1 - (\frac{n_2}{n_1})^2)^{\frac{1}{2}}}$ and is a measure of the penetration depth of the electric field component of the evanescent field into the rarer medium. Since the electric field of the evanescent field is typically not measured during an experiment it is useful to form the equation in terms of incident number of photons or I and I_0 . To convert from the electric field to intensity we use

$$I = \epsilon_0 c E_2^2, \quad (2.17)$$

where I is the light intensity, ϵ_0 is the permittivity of free space, and c is the speed of light. Applying (2.17) to (2.16) gives the evanescent field's penetration depth in terms of light intensity given by

$$I = I_0 e^{\frac{-2z}{\delta}},$$

where $I_0 = \epsilon_0 c E_{02}^2 e^{2i(-\omega t + \frac{2\pi n_2}{\lambda_0} x \frac{n_1 \sin \theta_1}{n_2})}$. The factor of 2 in the exponentials is introduced due to the E_2^2 term in (2.17).

2.1.2 Photoacoustic Effect

The photoacoustic effect [58], is the generation of thermoelastic pressure waves via the localized optical absorption of light into a material. The photoacoustic effect was discovered in the early 1880's by Alexander Graham Bell as he was trying to invent

wireless communication in the form of the photophone. In essence the photoacoustic effect can be described by Figure 2.6.

Figure 2.6: Laser excitation to generate the photoacoustic effect in tissue has many uses including imaging blood vessels and cancerous tumors.

The photoacoustic effect in this case is a pulsed beam of concentrated laser light that can be absorbed by an object imbedded deep within tissue. The equation that describes the photoacoustic effect, surprisingly enough, is the same equation that describes the evanescent field, known as the wave equation. In the experiments conducted for this dissertation, all acoustic sources were assumed to be plane wave sources (that simplifies the mathematical description). The assumption of plane waves is valid due to the fact that plane waves are observed whenever the optical penetration depth of the incident light is less than $1/10$ the size of the diameter of the incident laser beam. Since only ~ 500 nm of optical penetration depth is possible using TIRPAS and related techniques, this assumption is valid since the size of the beam was at least 1 mm in diameter. Therefore, the photoacoustic effect in one dimension, x , can be described by (2.18) that is very similar to the general wave equation describing the evanescent field in (2.9).

$$\frac{\partial^2 p}{\partial x^2} - \frac{1}{c^2} \frac{\partial^2 p}{\partial t^2} = 0 \quad (2.18)$$

Equation (2.18) describes a 1 dimensional acoustic wave with pressure that varies in both position and time. The solution of (2.18) is given by

$$p(x, t) = f(x - ct) + f(x + ct). \quad (2.19)$$

In terms stress confined photoacoustic excitation, we obtain a final equation describing the plane waves as

$$p(x, t) = \frac{1}{2} \Gamma \mu_a H_0 e^{-\mu_a(x-ct)} + \frac{1}{2} \Gamma \mu_a H_0 e^{-\mu_a(x+ct)},$$

where p is pressure, Γ is the Grünesian coefficient, μ_a is the absorption coefficient, H_0 is the radiant exposure of the laser beam, c is the speed of sound in the excited medium, x is length, and t is time. Figure 2.7 shows the transducer signal corresponding to a detected plane wave.

Interestingly, the shape of the acoustic wave follows the photonic distribution within the material of interest for all plane wave excitation. Since photonic distribution into a material of interest follows Beer's law shown in (2.20), it is possible to apply a non-linear least squares curve fitting algorithm to a measured acoustic wave (Figure 2.7) and fit to the upward stroke of the acoustic wave to get an indication of the value of μ_a , or absorption coefficient, to estimate the concentration of the absorber via (2.20) and (2.21). Of course, without a measurement of the error with a single non-linear least squares fit this method is used in an exploratory manner to get a general idea of the optical absorption of a material rather than the exact value.

Figure 2.7: This electrical signal is an example of an acoustic plane wave generated from photoacoustic excitation.

The experimental details to obtain Figure 2.7 can be found in [59].

$$I = I_0 e^{-\mu_a x} \quad (2.20)$$

$$\mu_a = 2.303 \epsilon c \quad (2.21)$$

2.2 Total Internal Reflection Photoacoustic Spectroscopy

Total Internal Reflection Photoacoustic Spectroscopy, or TIRPAS, is the combination of both the total internal reflection effect and the photoacoustic effects together to form a new device. Specifically, the physical excitation achieved by TIRPAS can be described by Figure 2.8.

Figure 2.8: Optical penetration depth differences between PAS and TIRPAS are at least an order of magnitude different.

As can be seen from Figure 2.8, the excitation of the photoacoustic effect by absorption of light normal to an interface will excite the chromophores following the photonic distribution described by the Beer-Lambert law, or (2.20), that represents a penetration depth of 10-100 μm . TIRPAS, on the contrary, only excites chromophores that are within the evanescent field that is typically on the order of the wavelength (500 nm). This constrained optical excitation by the evanescent field gives rise to some interesting properties of TIRPAS induced acoustic waves. Most notably, since

the optical penetration depth is on the order of 500 nm regardless of the absorption of the material of interest, under most circumstances, we can treat the acoustic wave generation as nearly ideal plane waves. This is due to the fact that the spot size is usually at least 1000 times larger in diameter than the optical penetration depth of the evanescent field. This configuration, as shown in Figure 2.9, causes the acoustic energy to propagate primarily in the forward and backward directions causing a bipolar waveform as shown in Figure 2.7.

Figure 2.9: This is a diagram of an acoustic plane wave versus a mixed wave (spherical).

Since the energy under TIRPAS excitation is primarily contained in both a forward and backward acoustic wave regardless of the optical absorption of the material being investigated, TIRPAS has a major advantage over traditional photoacoustic spectroscopy in that a larger percentage of the total emitted acoustic energy can be captured for a given signal, since the energy is more directed toward a single source from the excited location. In this way, despite the fact that TIRPAS is inherently less

sensitive due to the excitation of less material (since the optical penetration depth is less), the technique is more efficient at detecting a material since per unit of material more acoustic waves can be captured for a given absorption.

TIRPAS was originally invented in the early 1980's by Hinoue et al. and others [60–66], in order to evaluate absorption at interfaces for a cyclic voltammetry project. In those days, high powered lasers above 1W per pulse were very expensive and difficult to come by, and therefore, the original inventors abandoned increasing the sensitivity of TIRPAS and other uses of the technology in favor of more sensitive technologies that were more appropriate. Since the 1980's, there have been two major sources of improvement in the technology in terms of sensitivity and utility. Notably, one of the improvements in sensitivity combines TIRPAS with a metallic layer to generate surface plasmons generated by the interaction of the light with an appropriately designed layer on a prism of interest. This method is called SPRPAS, or Surface Plasmon Resonance Photoacoustic Spectroscopy.

2.2.1 Surface Plasmon Resonance Photoacoustic Spectroscopy

Surface Plasmon Resonance Photoacoustic Spectroscopy was invented by T. Inagaki et al. [67] in the early 1980's alongside the development of TIRPAS by Hinoue et al. In contrast to TIRPAS where the primary mode of excitation is from the absorption of the evanescent field into absorbers close to the interface between a prism and a material of interest, in SPRPAS, the primary mode of excitation is from electron resonance absorption from excitation through a pulsed laser beam into the surface of a metal layer deposited on the prism surface. In this way, SPRPAS, increases the sensitivity of the TIRPAS technique by measuring the non-radiative decay of

the surface plasmons that form into an acoustic wave for detection. The SPRPAS technique has been used for the fundamental investigation of surface plasmons [67–84], for the measurement of Langmuir-Blodgett films [85, 86], and for the detection of nicotine in cigarette smoke [87].

2.2.2 Modern TIRPAS with Q-switched Lasers

Goldschmidt et al. [88] renewed interest in the technology by publishing a proceedings paper in a non-refereed journal regarding the use of TIRPAS with modern q-switched ND:YAG based lasers. Specifically, Goldschmidt et al. used nanosecond pulsed ND:YAG lasers with peak powers ~ 1 megawatt for the excitation of the chromophores with the evanescent field. This million fold increase in peak power allowed for the TIRPAS technique to become a useful tool for evaluating the absorption of a variety of materials at interfaces. In 2012, Goldschmidt et al. used the technology to show the potential for the early detection of malarial infection [89] by using an optically analogous nanomaterial known as β -hematin. The main advantage of modern TIRPAS with higher peak power excitation is its inherently high sensitivity for absorbing materials. With the high sensitivity it is possible to use TIRPAS for biosensing small concentrations of important biomarkers in the body for early detection of various disease states and conditions.

Chapter 3

Modern TIRPAS and Biosensing

Evanescent field sensing methods are currently used to detect many different types of disease markers [90] and biologically important chemicals such as the HER2 breast cancer receptor. Hinoue et al. used Total Internal Reflection Photoacoustic Spectroscopy (TIRPAS) as a method of using the evanescent field to detect an optically opaque dye at a sample interface. Although their methods were successful at detecting dyes, the results at that time did not show a very practical spectroscopic technique, which was due to the less than typical sensitivity of TIRPAS as a spectroscopy modality given the low power (~ 0.5 milliwatt) lasers being used. Contrarily, we have used an ND:YAG laser with a five nanosecond pulse that gives peak power of 1 megawatt coupled with the TIRPAS system to increase the sensitivity of this technique for biological material sensing. All efforts were focused on the eventual detection of the optically absorbing material, hemozoin, that is created as a byproduct of a malarial infection in blood. We used an optically analogous material, β -hematin, to determine the potential for detection in the TIRPAS system. In addition, four properties that

control the sensitivity were investigated to increase understanding about the sensor's function as a biosensing method.

3.1 Evanescent Sensing

Techniques using the evanescent field have become extremely useful in the past few years. Sensors have been created to detect a wide variety of materials such as to detect 2,4-dinitrotoluene [91], pesticides [92], pathogens [93], nucleic acids [94], gases [95], and disease markers such as the HER2 breast cancer marker [96]. Even lab on a chip microring lasers [97, 98] have been created using evanescent field techniques. These sensors function by measuring the interaction of the evanescent field, a non-propagating near field optical wave, with the target.

In the early 1980's, Hinoue et al. [60–64, 99, 100] developed a total internal reflection photoacoustic spectroscopy (TIRPAS) technique to detect dyes in a sample that used the evanescent field to create acoustic waves. Unfortunately, the sensitivity of the device was limited to the detection of highly absorbing dyes that could easily be seen with the eye and was unable to provide a meaningful detection of biological analytes due to the lack of high peak-power lasers. This technique was modified and expanded in order to excite the samples under 1 megawatt peak-power conditions as opposed to the high speed beam chopper with a 0.5 milliwatt continuous wave laser used previously. This giga fold increase in peak-power allows for the detection of microgram quantities of β -hematin that brings the TIRPAS technology into the useful realm of biological analyte sensing.

Current commercial absorption-based evanescent field biosensors, such as internal

reflection spectroscopy elements pioneered by Harrick [101], are problematic with low absorbance materials. In internal reflection spectroscopy the measured absorption is determined from the difference between a reference reflection with no sample and the sample’s reflected light. This difference is often very small for nearly optically transparent materials in the visible wavelengths. Consequently, complicated and expensive multiple internal reflection systems must typically be created to overcome this limitation. Fortunately, however, since TIRPAS generates an active source of ultrasound with the interaction between the analyte and the evanescent field and there is no inherent ultrasonic background during sampling. Any absorption that can be distinguished by an acoustic transducer will result in detection of a low absorption analyte without the need for a reference reflection. It is important to mention that this technology of generating acoustic waves from total internal reflection is completely distinct from Paltauf et al. [102], who used a glass/sample interface to detect acoustic waves by the local disturbance in the index of refraction from the acoustic waves. Conversely, in TIRPAS, the acoustic wave occurs from the absorption of the evanescent field at the glass/sample interface by the sample.

3.1.1 Malaria Analytes

Hemozoin is a chemical byproduct found in human blood that has been infected with malaria. The formation of hemozoin by the malaria parasite *Plasmodium falciparum* begins once the parasite has infected an erythrocyte of the host. This stage of disease development is characterized by a period of growth for the parasite that is fueled by amino acids of the host cell’s cytoplasmic hemoglobin broken down inside a digestive vacuole [103]. The byproduct of hemoglobin digestion is molecular heme, a com-

pound that harms the parasite by inhibiting enzymes, peroxidizing membranes, and producing oxidative free radicals. *Plasmodium falciparum* is incapable of digesting heme, and must resort to polymerizing heme into hemozoin, an inert molecule made of heme monomers joined to one another by iron-carboxylate bonds. Infection causes the erythrocyte to eventually rupture and hemozoin is released into the blood stream, where some of it is engulfed by leukocytes via phagocytosis while some of it remains in the bulk blood.

Due to the difficulty in obtaining samples of hemozoin testing with an optically analogous [104, 105] material was preferred for these experiments. β -hematin is a viable analogue for hemozoin in detection systems based on spectroscopic methods, such as TIRPAS, because β -hematin has the same optical and structural properties as hemozoin [106]. β -hematin was used for testing the TIRPAS system due to the availability and cost compared to actual hemozoin crystals. As a synthetically derived material, β -hematin is created by the precipitation of haemin in an acetate buffer [106].

The reasonably high optical absorption of β -hematin in the visible spectrum allows for the use of a standard laser harmonic wavelength, 532 nm, for photoacoustic excitation. In addition, the 100 nm x 300 nm average size of β -hematin is on the order of the evanescent field penetration depth in the TIRPAS experiments. This should give rise to little background signal since the evanescent field cannot penetrate into larger absorbing structures such as red blood cells that are at least an order of magnitude larger than the crystals. β -hematin and chlorazol black dye, two optically similar materials, are used to show the potential of TIRPAS as a biosensor for the detection of malaria, while advantages and disadvantages of the system with regards to its detec-

tion capability are also discussed. Additionally, through the angle experiments and previous theory, the shape and size of the photoacoustic effect is shown to be related to absorption, index of refraction, and optical penetration depth. These variables affect any type of photoacoustic spectroscopy by changing the pressure maxima and distribution, however the exact understanding of how the excitation under TIRPAS conditions functions was more completely understood with the following experiments.

3.2 Materials and Methods

A schematic of the TIRPAS device is visualized by Figure 3.1.

3.3 Experimental Setup

All testing was performed on the same hybrid TIRPAS/oblique photoacoustic excitation setup. Both angle experiments were accomplished by selectively blocking the beam with a spatial filter as explained in Section 3.3.4. The dilution experiment was performed by spatial filtering the beam to leave a laser beam filtered below the critical angle. This left an excitation beam that was a semi-circular spot undergoing TIRPAS excitation at the highest radiant exposure and penetration depth available for TIRPAS in this setup.

3.3.1 Laser and Optical Fiber

A Q-switched ND:YAG laser (Surelite I-20 doubled to 532 nm, Continuum, Santa Clara, California) was coupled through 1000 μm 0.37 NA optical fiber (BFH37-1000,

Thorlabs, Newton, New Jersey). The laser was pulsed at 20 Hz at a duration of 5 ns with energies ranging from 5-6 mJ. The light from the optical fiber was collimated for TIRPAS excitation. We used 100 mm (LA1509, Thorlabs, Newton, New Jersey), 50 mm (LA1131, Thorlabs, Newton, New Jersey), and -30 mm (LC2679, Thorlabs, Newton, New Jersey) lenses to collimate the laser light from the optical fiber.

Figure 3.1: This is a schematic of the setup for TIRPAS and how the oblique photoacoustic excitation and TIRPAS excitation differ with angle.

3.3.2 Sample Holder and Transducer Electronics

The hemicylindrical lens was mounted to a rotational stage and to an XYZ stage (481-A, Newport, Irvine, California and LT3, Thorlabs, Newton, New Jersey) that allowed the hemicylindrical lens to be adjusted to position the total internal reflected

spot as well as to change the angle of incidence of the incoming laser light. The voltage response of a polyvinylidene fluoride (PVDF) transducer was amplified by a 350 MHz preamplifier (SR445A, Stanford Research Systems, Sunnyvale, California). The amplifier was then attached to a 200 MHz four-channel digital storage oscilloscope (TDS 2024B, Tektronix, Beaverton, Oregon) through 50 Ω coaxial cable (2249-C-24, Pomona, Everett, Washington). A silicon photodiode (DET10A, Thorlabs, Newton, New Jersey) was used to trigger the oscilloscope.

3.3.3 Ultrasonic Detector

A gold coated PVDF transducer was used as the acoustic sensor to detect ultrasonic waves. This sensor was similar to the designs used in Viator [107] except that the element was not etched and a layer of silicone was added to protect the sensing material and to prevent the sample from staining the transducer. This coating did not introduce significant reflections due to its near acoustic impedance match with the sample that was mostly water. The acoustic impedance of the silicone was 1.1 MRayls as compared to water's 1.5 MRayls. At normal incidence this boundary gives a reflection coefficient of $\sim 2\%$ and is explained in more detail in A.1. In addition, we used a brass mask to create an approximately 0.5 cm $\times$ 0.5 cm square active area in the center of the transducer to be used as the detection area.

3.3.4 Spatial Filter

Harrick [101] suggests a method of collimating the output to the interface by equation $d = \frac{r}{n_1 - 1}$. where d is the distance of the light source to the prism, r is the radius of

the lens, and n_1 is the index of refraction of the hemicylindrical lens. Unfortunately, due to the large radius of the lens and the large numerical aperture of the optical fiber, 0.37, less than 1 mJ of energy was possible for TIRPAS excitation under this configuration. Therefore, $d = \frac{r}{n_1-1}$ was not used and a spatial filter was used to block selected angles of excitation for the TIRPAS and photoacoustic spectroscopy experiments to compensate for the lack of collimation at the interface. An XYZ stage (PT3, Thorlabs, Newton, New Jersey) holding a spatial filter was used between light undergoing TIRPAS and oblique incidence photoacoustic excitation respectively as is seen in Figure 3.1. Since the optical setup caused a significant divergence angle across the beam, angles of light were blocked under photoacoustic excitation when TIRPAS was being studied and were blocked under TIRPAS excitation when oblique photoacoustic excitation was being studied. This setup introduced a continuum of angles that collectively gave rise to the overall photoacoustic signal in each experimental case.

3.3.5 TIRPAS and β -Hematin

The microstructure of β -hematin is an approximately 100 nm $\times$ 300 nm crystalline rod. The crystals have the ability to create a hydrogen bond to neighboring β -hematin crystals that allows for the crystals to aggregate into larger macrocrystals that may exceed several micrometers in size. The size of a typical red blood cell is several micrometers, at least an order of magnitude larger than the dispersed β -hematin crystals. The size of β -hematin is ideal for photoacoustic detection through TIRPAS because the crystals are approximately the same size as the optical penetration depth of the evanescent field. Therefore, absorption from hemoglobin inside red blood cells

will be reduced by appropriately choosing the optical penetration depth in TIRPAS by adjusting the angle of excitation.

Figure 3.2: Transmission electron microscopy of β -Hematin reveals the crystal size and shape.

3.3.6 β -Hematin Synthesis

40 g of NaOH pellets were dissolved into 60 ml of stirred distilled water to create a 12.9 M NaOH solution while covering the beaker to prevent evaporation. While rapidly stirring 75 ml of 17.1 M glacial acetic acid 12.9 M NaOH was added drop-wise until the pH of the titrated buffer solution was 4.5. Distilled water was added until the total solution volume became 100 ml. 34 ml of 0.1 M NaOH solution was heated to 60°C. 175.5 mg of haemin (Sigma-Aldrich, St. Louis, MO) was dissolved in the

NaOH solution while stirring the covered beaker. 20.7 ml of the acetic acid buffer was heated to 60°C. The haemin solution was neutralized with 3.4 ml of 1 M HCl solution. After stirring for 5 minutes the 20.7 ml of acetic acid buffer was added to the neutralized haemin solution. The resulting solution was covered and stirred at 60°C for 2 hours. The solution was then put in an ice bath for 10 minutes. The cooled solution was filtered over 8.0 μ m nitrocellulose filter paper (Millipore, Billerica, MA) and the filtered precipitate was washed with distilled water and dried for 5 minutes. The solid β -hematin was desiccated for 48 hours. Finally, β -hematin was stored in PBS suspension away from light exposure at 4°C until use.

3.3.7 Critical Angle Analysis

The divergent beam allowed for a simple visual determination of the critical angle as seen in Figure 3.1. Using 1.5+ OD safety goggles the reflected spot was observed undergoing TIR from a scattering surface irradiated by the light that had exited the hemicylindrical lens. The scattered laser spot had two visibly important components. One side of the spot is visibly darker due to the increased loss from the reflected light transmitting through the prism/sample interface due to the continuum of divergence angles. The other side is visibly brighter and is indicative of the reflected light that is undergoing evanescent interaction at the prism/sample interface. The interface between the brighter and darker sections of the spot indicates the reflected section of light where the critical angle occurs. Changing the angle of the hemicylindrical lens relative to the incident laser beam shifts the position of this line and thus the physical proportion of the reflected light that is undergoing TIRPAS and oblique photoacoustic excitation respectively. The resulting incident light is spatially filtered

with the aid of the reflected light to make the measurements of TIRPAS and oblique photoacoustic excitation independent of each other as shown in Figure 3.3.

Figure 3.3: **Left:** This figure shows the experimental setup for TIRPAS. **Right:** This figure shows reflected light after having interacted with the prism/sample interface. The critical angle is represented by the transition between the brighter and darker reflected portions of light. The darker shade represents the part of the beam under oblique photoacoustic excitation and the brighter shade represents the part of the beam under TIRPAS excitation

3.3.8 Angular Spectrum: Chlorazol Black

Chlorazol Black with an absorption coefficient of 55 cm^{-1} at 532 nm was used in order to assume a low absorption condition, less than 10% absorption per interaction, to simplify the equations as stated in Harrick [101]. The condition of less than 10% absorption per interaction is important because greater absorption may lead to non-linear optical effects that may lead to deviation from standard Beer's law and thus deviation from expected light interaction. The angle of the hemicylindrical lens was changed relative to the incident laser light by a rotational stage (481-A, Newport,

Irvine, California). The experiment was performed five times and the values were averaged to obtain a representative angular spectrum as seen in Figure 3.5.

3.3.9 Detection Limit of Chlorazol Black

Serial dilutions of an initial $\mu_a = 52.5 \text{ cm}^{-1}$ Chlorazol Black solutions were excited at 532 nm. The detection limit was defined by a signal to noise ratio of $\sim 3:1$. The incident light was spatially filtered to produce the largest excitation area of the laser beam at the critical angle for the samples so that the optical penetration depth of the evanescent field (and thus the PA signal) is the largest under this condition.

3.3.10 Data Collection

In order to rapidly analyze the photoacoustic signals recorded on the oscilloscope, a graphical user interface (GUI) was developed in MATLAB to import the data and determine the acoustic penetration depth of a given signal. The program determines the penetration depth as a user-defined percentage of the maximum value of the desired photoacoustic waveform. Using cursor placement, the user is able to define the time domain of the photoacoustic signal prior to the percent-maximum calculation in order to avoid bias due to noise and extraneous acoustic effects that may have been recorded in the data file. The program displays the location of the percent-maximum on the plot of the original waveform in addition to displaying its value for user verification of correct penetration depth.

3.4 Evaluation

Figure 3.4: **Left:** Acoustic penetration depth shows that there is an acoustic penetration depth shift versus angle. **Right:** Signal width differences can be clearly seen between TIRPAS and oblique photoacoustic excitation at the critical angle. Overall, acoustic waves qualitatively seem different between TIRPAS and oblique photoacoustic excitation.

On the right side of Figure 3.4 the comparison between the two types of excitation, TIRPAS and oblique photoacoustic excitation, can be seen. The angular spectrum on the left of Figure 3.4 illustrates how the acoustic penetration depth reduces in magnitude versus an increasing excitation angle. The measured acoustic penetration depth values under TIRPAS excitation and the measured acoustic penetration depth values under oblique photoacoustic excitation never meet in value for this representative sample. The measurements, despite using some of the same angles and both being excited using some form of the photoacoustic effect, show different acoustic penetration depth values where oblique photoacoustic excitation and TIRPAS excitation are contrasted. Of course, since this is a single example there is no measurement of the

error and therefore it cannot be stated absolutely that this will always be the case, so further testing is needed to confirm any hypothesis regarding differences in the acoustic penetration depth. The graph on the right shows the physical effect of the sample's difference in acoustic penetration depth on single waveforms between oblique photoacoustic excitation and TIRPAS excitation. The TIRPAS waveform rises much faster than its corresponding oblique photoacoustic excitation counterpart.

Figure 3.5: **Left:** Four angular spectra as seen in Figure 3.4 were averaged to create a point wise averaged spectra graph. **Right:** An outlier in the angle measurement is displayed for completeness. As can be seen in both the outlier and the averaged graph there is a visible transition in acoustic penetration depth at ~ 67 degrees. The reduction corresponds to the theoretical optical penetration depth reduction expected from oblique photoacoustic excitation and TIRPAS excitation.

On the left side of Figure 3.5 the point wise average of penetration depths at various angles are used to illustrate a comparison between the acoustic penetration depth in both oblique photoacoustic excitation and TIRPAS excitation as shown. There is a substantial reduction in measured acoustic penetration depth at the critical angle between oblique photoacoustic excitation and TIRPAS excitation. The right side

of Figure 3.5 shows an anomalous reading that the author believes may have been caused by setup movement during the experiment, but is displayed for completeness. A detection limit experiment was performed with a solution of Chlorazol black dye to estimate the approximate detection limit of the system. The approximate detection limit was $\sim 1.1 \text{ cm}^{-1}$. Such a low absorption would be difficult to detect using oblique photoacoustic excitation due to the very large optical path length needed for excitation.

Figure 3.6: **Left:** This figure shows an angular spectrum with β -hematin and Chlorazol black under oblique photoacoustic excitation and TIRPAS excitation. **Right:** This figure shows an angular spectrum of the 2% Intralipid/Chlorazol black solution that was made to approximate the oblique photoacoustic excitation optical penetration depth obtained by β -hematin.

Figure 3.6 shows the results of an experiment to determine the penetration depth of an optically absorbing and optically scattering material. The angular spectrum, without point wise averaging, on the left shows the acoustic penetration depth of standard chlorazol black dye and β -hematin for both oblique photoacoustic excitation and TIRPAS excitation. The β -hematin acoustic penetration depth values are much closer

in value for both oblique photoacoustic excitation and TIRPAS excitation. The figure on the right shows Chlorazol Black with a 2% intralipid optically scattering solution. Both TIRPAS and oblique photoacoustic excitation measured acoustic penetration depths are much closer to each other, similar to the figure on the left for β -hematin despite the only difference being the addition of optical scattering.

3.5 Discussion

3.5.1 How to Relate Optical Absorption to Refractive Index

It is well known for both evanescent field sensing [108–111] and for TIRPAS based sensing [60–66, 88, 89, 99, 100] that the electric field decays with an exponential according to

$$E_z = E_0 e^{-z/\delta_p},$$

where E_z and E_0 refer to the electric field intensity at a sample depth, z , and initial intensity at the interface ($z=0$), respectively, with δ_p as the optical penetration depth. The ability for the light to be absorbed into an analyte very close to the interface is dependent upon four fundamental properties: penetration depth, electric field strength, sampling area, and how well the sample and prism indices are matched [101]. Since the photoacoustic effect is caused by absorption of photons into materials that causes localized thermoelastic expansion to induce an acoustic wave, the penetration depth is one of the factors that controls the sensitivity of TIRPAS since this determines photonic absorption into a material and thus the intensity of the resulting acoustic

wave. The penetration depth is controlled by the following factors given by [112,113]

$$\delta_p = \frac{\lambda_1}{2\pi(\sin^2\theta - n_{21}^2)^{1/2}}, \quad (3.1)$$

where $n_{21} = \frac{n_2}{n_1}$, n_2 and n_1 are the indices of refraction of the hemicylindrical lens and the sample, where θ = angle of excitation, and $\lambda_1 = \frac{\lambda}{n_1}$ according to Muessig et al. [66] The laser used did not have a short enough pulse duration to fall within a stress confined condition, however, a reasonable proportion to explain the amount of power absorbed can still be obtained when the underlying causes of the absorption and the relationship of the absorption to the index of refraction, are considered.

3.5.2 Optical Absorption Under TIRPAS Excitation

In Muessig et al. [66] the authors describe an equation to relate the absorbed power to both the penetration depth and the intensity of the electric field. A similar expression can be obtained by substituting the electric field with an average of the parallel and perpendicular polarizations of the electric field. This does not completely explain our results due to the assumption that polarization interaction does not change with the angle of incidence. However, it does give a general overview to the interaction if each polarization is assumed to contribute equally to the absorbed power. If the terms $E'_{\parallel}$ and $E'_{\perp}$ are known in the experiment being conducted their proportional contribution can be calculated by the amount each contributed to the acoustic signal. The variables relating to photoacoustic pressure can be explained by

$$P \propto \delta_p \left[\frac{[E'_{\parallel}]^2}{2} + \frac{[E'_{\perp}]^2}{2} \right], \quad (3.2)$$

where $E'_{\parallel}$ is the electric field intensity of the parallel polarization and $E'_{\perp}$ is the electric field intensity of the perpendicular polarization and P is the acoustic pressure. Absorption is directly related to the amount of acoustic pressure generated in a sample under photoacoustic excitation. From Muessig et al. [66], it has been shown that the electric field parallel to the interface is

$$E'_{\parallel} = (E'^2_x + E'^2_z)^{\frac{1}{2}}, \quad (3.3)$$

where the individual components E'_x and E'_z are

$$E'_x = \frac{2E_{\parallel}(\sin^2\theta - n_{21}^2)^{\frac{1}{2}}\cos\theta}{(1 - n_{21}^2)^{\frac{1}{2}}[(1 + n_{21}^2)\sin^2\theta - n_{21}^2]^{\frac{1}{2}}}$$

and

$$E'_z = \frac{2E_{\parallel}\sin\theta\cos\theta}{(1 - n_{21}^2)^{\frac{1}{2}}[(1 + n_{21}^2)\sin^2\theta - n_{21}^2]^{\frac{1}{2}}},$$

respectively. In addition, the electric field perpendicular to the surface is

$$E'_{\perp} = \frac{2E_{\perp}\cos\theta}{(1 - n_{21}^2)^2}. \quad (3.4)$$

By using (3.3)-(3.4) and substituting them into (3.2) we get

$$P \propto \frac{\lambda_1}{2\pi(\sin^2 \theta - n_{21}^2)^{\frac{1}{2}}} \times \quad (3.5)$$

$$\left[\frac{\left\{ \left[\frac{2E_{\parallel}(\sin^2 \theta - n_{21}^2)^{\frac{1}{2}} \cos \theta}{(1-n_{21}^2)^{\frac{1}{2}}[(1+n_{21}^2)\sin^2 \theta - n_{21}^2]^{\frac{1}{2}}} \right]^2 + \left[\frac{2E_{\parallel} \sin \theta \cos \theta}{(1-n_{21}^2)^{\frac{1}{2}}[(1+n_{21}^2)\sin^2 \theta - n_{21}^2]^{\frac{1}{2}}} \right]^2 \right\}}{K_1} + \frac{\left[\frac{2E_{\perp} \cos \theta}{(1-n_{21}^2)^2} \right]^2}{K_2} \right],$$

where θ is the angle of incidence, $n_{21} = \frac{n_2}{n_1}$, $\lambda_1 = \frac{\lambda_0}{n_1}$, K_1 and K_2 are the constants to indicate the proportion of each polarization used in excitation; in this case $K_1 = K_2 = 2$ since each polarization is assumed to contribute half the photoacoustic response to the system, and $E_{\parallel}$ and $E_{\perp}$ are the electric field intensities for parallel and perpendicular polarization relative to the prism. Beyond pressure, the effective thickness of the evanescent field is controlled by four factors, electric field intensity, penetration depth, index matching of the prism/sample interface, and the sampling area. The equation above shows variables for three of the four factors, penetration depth, electric field, and index matching that agrees well with our experiments. The sampling area should be included as a multiplication constant for the above equation since the acoustic pressure should be proportional to the area of excitation.

3.5.3 Acoustic Plane Waves in TIRPAS

In contrast to normal incidence photoacoustic excitation, our observation was that every waveform regardless of the optical absorption seemed to be a bipolar plane wave. This observation can be understood with a more thorough analysis of the in-

interaction between the evanescent field and the target absorber. Plane waves can be formed in the acoustic under normal incidence photoacoustic excitation. The plane wave assumption is appropriate to apply when the transducer is closer to the incident absorber than the boundary where $w = \frac{d^2\mu_a}{8}$, or in the acoustic near field, as found in Viator et al. [59] where w is the distance from the transducer to the near field boundary, d is the beam diameter, and μ_a is the absorption coefficient. Under this condition an acoustic piston is approximated as a plane wave where almost half the acoustic energy generated moves forward toward the transducer. With TIRPAS, a similar effect is observed as seen in Figures 3.4 and 3.5 that can be explained by the fact that the evanescent field only optically penetrates a very small amount, ~ 500 nm, into the medium and so an acoustic piston is nearly guaranteed for almost all absorbing materials. The acoustic piston phenomena made detection much less ambiguous than normal incidence photoacoustic excitation where plane waves are only obtained if the sample is significantly absorbing and the spot size is very large according to $w = \frac{d^2\mu_a}{8}$. In the TIRPAS system an acoustic plane wave will be obtained for all absorbing materials unless the spot size for excitation is as small as the optical penetration depth or thickness of the sample, whichever is smaller, that would necessarily be a diffraction limited beam for excitation in the visible wavelengths.

3.5.4 Optical Penetration Depth

The optical penetration depth of the evanescent field exponentially decays away from the interface of total internal reflection. The distance where the evanescent field decays to 37% ($\frac{1}{e}$) of the incident energy is defined as the optical penetration depth and can be used as one factor to predict evanescent field interaction that is described

by equation 3.1 [108,109]. The penetration depth is important for TIRPAS excitation that uses the interaction of the evanescent field with absorbers to detect the analyte. Physical sizes of analytes can range from an order of magnitude larger to an order of magnitude smaller than the penetration depth of the evanescent field (~ 500 nm) in typical biological systems. An example of the large distribution of sizes would be a cell that may be $10\text{ }\mu\text{m}$ in size compared to the size of a single hemazoin crystal that is $100\text{ nm} \times 300\text{ nm}$. By tuning the penetration depth of the evanescent field by exciting the prism/sample interface at increasing excitation angles, analytes of ever smaller sizes can be detected since the field will interact most strongly with the material based upon its size along with its inherent optical absorption.

3.5.5 Stress Confinement

In normal incidence photoacoustic excitation, stress confinement, described in (3.6), is a condition where the pulse duration of incident light is shorter in duration than the time it takes for the induced acoustic wave to propagate away substantially. Under stress confinement the resulting acoustic wave represents how the incident light optically decays through the media. This effect allows for optical properties such as the absorption coefficient to be estimated using an exploratory non-linear least squares curve fitting algorithm as shown in Figure 3.7 from the acoustic wave. This is explained in more detail by Viator et al. [59] and Viator [107].

$$\tau \leq \frac{\delta}{c_s} \tag{3.6}$$

Equation (3.6) is the stress confinement condition where $\delta = \frac{1}{\mu_a}$, μ_a is the absorption

coefficient of the sample, and c_s is the speed of sound in the sample medium.

Figure 3.7: A Non-linear exponential least squares curve fit can be used to estimate μ_a .

Meeting the stress confinement condition and inputting the acoustic wave in Figure 3.7 and using a non-linear exponential least squares curve fit it, it is possible to gain some insight into the optical properties such as an estimate for the absorption coefficient of the material of interest. This does not take into account statistical uncertainty, therefore, this is used as more of an exploratory method to get a general idea of the value of the absorption coefficient rather than an exact value for further testing. The author used $m1=1$ and $m2=150$ for the initial guesses with 0.1% tolerance and

more information about this method and experimental setup for collecting this type of acoustic wave can be found in [59]. Assuming that TIRPAS waveforms are stress confined, the experimental evidence should show that the waveforms' penetration depth reduces with a reduction in the evanescent field penetration depth. However, our data shows a reasonably insensitive measurement compared to theoretical values as seen in Figures 3.4, 3.5, and 3.6.

Choi et al. [114], studied how the laser pulse duration can affect the determination of μ_a computed from curve fitting waveforms collected by normal incidence photoacoustic excitation. It has been shown that as the stress confinement condition was no longer satisfied, the μ_a estimation was increasingly underestimated until the limiting value of μ_a is reached. In that case, one would expect the penetration depth, which is the inverse of μ_a , to hit a minimum value if the stress confinement condition is not met. Our results seem to agree with non-stress confined photoacoustic excitation, as seen in Figures 3.4 and 3.5. This result suggests that TIRPAS excitation is not in stress confinement and is limited by the duration of the laser pulse. If laser light is delivered to a sample within the stress confinement condition, no substantial acoustic energy has time to propagate away before the pulse duration of the laser ends. Since TIRPAS has a very small penetration depth, ~ 500 nm, then calculating with $\tau = \frac{\delta}{c_s}$ the stress confinement estimate for TIRPAS is at maximum 0.33 nanoseconds around the largest optical penetration depth attainable.

3.5.6 Signal Acquisition

Since the penetration depth of the evanescent field, even near the critical angle, is within hundreds of nanometers of the interface, most of the frequency components

expected under stress confined TIRPAS would be in the low GHz range. Since the duration of the laser pulse was 5 ns, this limited the frequency range to the maximum obtainable via a 5 ns pulse. To approximate this frequency range, we used a simple method [107] and assumed a pulse duration of 5 ns and used

$$f = \frac{1}{p_d},$$

where f is the approximate frequency maximum of the resulting wave and p_d is the pulse duration of the laser. The PVDF sensors used were reliable at frequencies up to approximately 100 MHz. Far beyond 100 MHz, the PVDF does not respond linearly to inputs. The maximum sampling rate of the oscilloscope used was 200 MHz that would not be enough sampling to fully capture the signals appropriately if a picosecond laser were used in order to stay within the regime of stress confinement. However, due to the nanosecond pulse duration mentioned previously, the maximum frequency threshold was far below the maximum frequency of the oscilloscope and therefore, no distortion was expected. In Figure 3.4 the difference in frequency components can be qualitatively inferred when the acoustic penetration depth of oblique photoacoustic excitation and TIRPAS excitation are compared. Of course, a more rigorous statistical evaluation would be needed to show this for certain, however, TIRPAS contains laser pulse limited frequency components due to its optical penetration depth being on the order of a few hundred nanometers. The measured acoustic penetration depth (roughly corresponding to optical penetration depth) of TIRPAS corresponds to a rise time of 5 ns in Figure 3.4.

3.5.7 Differences in Penetration Depth

With the above limitations in mind, it is important to note the differences detected between the TIRPAS acoustic wave and the oblique photoacoustic excitation acoustic wave at compared angles of incidence. Figure 3.5 shows the measured acoustic penetration depth corresponding to the optical penetration depth versus the angle of incidence of the incident light. It should be noted that due to the beam divergence the angles listed are a continuum of angles from the critical angle to the most grazing angle.

The measured acoustic penetration depth under oblique photoacoustic excitation levels off as the incident light is tuned/spatially filtered very close to the critical angle. Incident light exciting any sample will always have a lower measured acoustic penetration depth with larger angles of incidence. At the critical angle the measured acoustic penetration depth reduces to the laser pulse limited acoustic penetration depth for all angles at or beyond the critical angle. This huge reduction in acoustic penetration depth is due to the fact that in oblique photoacoustic excitation the optical penetration depth is solely a function of the absorber's optical properties, but in TIRPAS, the optical penetration depth is a function of the wavelength, index of refraction of the sample and substrate, and the angle of incident light at the prism/sample interface, that gives rise to the evanescent field that substantially limits the optical penetration depth.

3.5.8 Advantages of TIRPAS

There are three advantages to TIRPAS compared to conventional photoacoustic spectroscopy and other evanescent field spectroscopies:

1. Optical size filtering can limit the material being excited.
2. Simple, obvious detection methods are obtained using this method.
3. Taking an optical spectrum is possible with the use of tunable lasers.

With regard to 1, since the excitation comes from the interaction of the evanescent field with the interface up to only a few hundred nanometers, biological analytes of interest can be size filtered for detection. This type of size filtering utilizing the evanescent field's angular dependent optical penetration depth allows for the analysis of harmful chemicals in biological fluids without the substantial background from cellular components. Relating to 2, TIRPAS produces easily discernible signals since all generated acoustic waves are plane waves as discussed previously. Moreover, the absorbed energy is converted to acoustic energy making the need to change detectors based on wavelength unimportant and therefore, allowing the method to be utilized over the large optical spectrum without expensive or time consuming alterations to the setup [58, 115]. Finally for 3, TIRPAS has the potential to determine the absorption spectrum of a material in a similar way to internal reflection spectroscopy as discussed by Harrick [101]. This type of spectroscopy may be much less expensive due to the lack of need to change detectors as mentioned.

3.5.9 Optical Scattering of β -Hematin

In Figure 3.6, the penetration depth for Chlorazol black is typical for what is expected with oblique photoacoustic excitation and TIRPAS excitation. However, for β -hematin the same setup parameters provide the same results for TIRPAS but generate very different results for oblique photoacoustic excitation. From transmission

electron microscopy images of β -Hematin in Figure 3.2 it is observed that the length of individual crystals is on the order of the wavelength of the incident light used, 532 nm. Due to β -hematin's size and shape the component for Mie scattering as well as Raleigh scattering should be very strong with the sample's crystal size and incident light wavelength.

Chlorazol black dye was mixed with intralipid at 2% (v/v) of the same optical absorption coefficient and was tested as a comparison to β -Hematin. This mixture increased the optical scattering of the Chlorazol black to visually approximate that of β -Hematin. In Figure 3.6 the results of this test show that the same trend of the measured acoustic penetration depth under oblique photoacoustic excitation as compared with highly absorbing Chlorazol black solution is no longer observed. Instead a very similar result to the β -Hematin is observed where the measured acoustic penetration depth changes differently from all absorbing materials along with the overall measured acoustic penetration depths being much closer to those observed in TIRPAS.

This observation can be explained by two factors. First, oblique photoacoustic excitation under stress confinement displays the distribution of photons in a material. Therefore, a scatterer, by changing the distribution of photons, can alter the measured acoustic penetration depth associated with it. Also, the addition of optically scattering material tends to make the optical penetration depth smaller since the distribution of photons is affected by both μ_a and μ_s . Specifically, the distribution of photons in Chlorazol black solution can be described by

$$I = I_0 e^{-\mu_a z}.$$

$$I = I_0 e^{-(\mu_a + \mu_s(1-g))z} \quad (3.7)$$

However, if optical scattering is introduced, the effective attenuation of the light is increased due to the addition of the scattering coefficient, μ_s , and the anisotropy of the material, g as shown in (3.7).

3.6 Conclusion

TIRPAS has the ability to detect Chlorazol black dye at an absorption coefficient of 1.1 cm^{-1} . In addition, the results demonstrate through Figure 3.5 how the acoustic (and by extension optical) penetration depth changes with the angle of incidence. Finally, the angular spectrum of β -hematin shows the potential for TIRPAS to be used as a biosensor for the early detection of malarial infection.

Chapter 4

Photoacoustic Refractometry (PAS/TIRPAS) and Biosensing

Current methods of determining the refractive index of chemicals and materials, such as ellipsometry and reflectometry, are limited by their inability to analyze highly absorbing or highly transparent materials, as well as the required prior knowledge of the sample thickness and estimated refractive index. Here, we present a method of determining the refractive index of solutions using the photoacoustic effect. We show that a photoacoustic refractometer can analyze highly absorbing dye samples to within 0.006 refractive index units of a handheld optical refractometer. Further, we use myoglobin, an early non-invasive biomarker for malignant hyperthermia, as a proof of concept that this technique is applicable for use as a medical diagnostic. Comparison of the speed, cost, simplicity, and accuracy of the techniques shows that this photoacoustic method is well-suited for optically complex systems.

4.1 Introduction

The refractive index of a material describes the group velocity of light as it propagates through that medium. This property is highly correlated with both the density of the medium, and the absorptive properties of the medium. Using Kramers-Kronig analysis (mathematical relationships that relate the complex and real parts of a physical system) it is possible to estimate many material properties, including refractive index. However, in recent years, the legitimacy of this analysis has been questioned for heterogenous biological materials, such as hemoglobin, due to the complex nature of the analysis [116]. Measurement of the refractive index is based upon the fundamental Fresnel equations that describe the refraction, reflection, and transmission of light at an interface between two media with non-matching refractive indices. In recent years, the gold standard of measurement of this property has been either reflectometry or ellipsometry, depending upon various sample properties, such as its transparency, and thickness, as well as overall cost, and the necessary data precision. However, for highly absorbing materials, partially transparent materials, or entirely unknown materials, both techniques demonstrate significant drawbacks that prevent the widespread adoption of refractive index measurements beyond typical materials characterization. Here, we explore a low cost, simple, alternative method based on the photoacoustic effect. We demonstrate that our photoacoustic method, Photoacoustic Spectroscopy/Total Internal Reflection Photoacoustic Spectroscopy (PAS/TIRPAS) refractometry, can be successfully used to determine the refractive index of both dyes, as well as a physiologically relevant concentration of myoglobin [117,118] in phosphate buffered saline.

4.1.1 Reflectometry

Reflectometry is a technique where a polarized beam of light, operating in two linear polarizations, S and P, optically interrogates a sample. The reflected light from the sample is then measured by a photodetector. Both S and P polarizations, along with the incident angle, are recorded in order to obtain the real component of the refractive index. Equation (4.1) shows how the refractive index of a material is related to the reflection coefficients and angle of incidence as

$$n_R = \sin \theta_i \left[1 + \left(\frac{1 - \rho}{1 + \rho} \right)^2 \tan^2 \theta_i \right]^{\frac{1}{2}}, \quad (4.1)$$

where $\rho = \sqrt{\frac{R_p}{R_s}}$ and $n_R = \frac{n_2}{n_1}$. Equation 4.1 is used to determine the real part of the refractive index of a material using reflectometry methods [119].

4.1.2 Critical Angle Analysis

In a special case, the real component of the refractive index can be determined by measuring the critical angle, or the angle where total internal reflection occurs. This angle can be determined using equation 4.6. This method simplifies the analysis of Fresnel's equations and allows the refractive index of the sample to be found directly, if the refractive index of the substrate, n_1 , and the angle of incidence, θ_1 , are known.

4.1.3 Ellipsometry

Ellipsometry, a closely related technique to reflectometry, can be described with a slight alteration to Equation 4.1. The relative permittivity of a material, $\tilde{\epsilon}$, can be

found, and is directly related to the refractive index of a material by (4.2) and (4.3). This is explained in more detail at http://www.rp-photonics.com/refractive_index.html. The equations are given by

$$\langle \tilde{\varepsilon} \rangle = \sin^2 \phi \left[1 + \left(\frac{1 - \rho}{1 + \rho} \right)^2 \tan^2 \phi \right] \quad (4.2)$$

and

$$\langle \tilde{\varepsilon} \rangle = n^2 = \kappa, \quad (4.3)$$

where $\tilde{\varepsilon}$ and κ are the relative permittivity of a material, n is the refractive index of the sample material, ϕ is the incident angle on the sample, and $\rho = \sqrt{\frac{R_p}{R_s}}$ is the ratio between the two reflected values for both linear polarizations, P and S. For simple bulk substrate systems, ellipsometry uses the same equation as reflectometry, albeit formatted differently, to determine the refractive index of a material.

4.1.4 Limitations

Each of these methods has limitations. Reflectometry's main limitation lies in the fact that the technique does not take into account surface roughness or optical scattering effects. Therefore, the actual measurement of the refractive index is a combination of the optical contributions from both refractive index and surface or substrate roughness. Additionally, reflectometry typically has less success with materials that have many highly absorbing layers, due to the light being unable to be measured from being completely absorbed. Ellipsometry has similar issues with measuring materials that are highly absorbing for the same reasons. Additionally, ellipsometry has the addi-

tional limitation of being unable to determine a single optical property independently of the thickness of a material.

This can lead to erroneous results if the operator is not appropriately trained and makes incorrect assumptions or initial guesses for thickness and refractive index in the curve fitting program to determine refractive index and layer thickness. Model fitting in ellipsometry attempts to minimize the mean squared error or (MSE) in order to achieve a global minimum to determine the values of thickness and refractive index at a particular wavelength. More information can be obtained at http://www.jawoollam.com/tutorial_5.html.

Critical angle analysis is highly accurate and inexpensive, however, it requires a prism setup that will be optically complicated and physically large. Additionally, the material of the prism must be of a larger refractive index than the sample in order for the condition for total internal reflection to be fulfilled that poses problems for unknown samples and increases cost.

4.1.5 The Photoacoustic Effect

An alternative to all of these methods relies on the photoacoustic effect. The photoacoustic effect is generated by the absorption of light into a sample that induces pressure in the form of an acoustic wave. The simplest equation to describe photoacoustic excitation is equation 4.4 from Viator et al. [59] that describes the pressure distribution of a generated photoacoustic plane wave. The magnitude of the resulting acoustic wave relies directly upon the optical absorption coefficient of the material which can be explained by

$$p_0(z) = \mu_a \Gamma H_0 e^{-\mu_a z} \quad (4.4)$$

and

$$\mu_a = \frac{1}{\delta}, \quad (4.5)$$

where z represents physical depth, μ_a is the optical absorption coefficient, Γ is the Grunesian coefficient, H_0 is the incident laser energy per area, δ is the optical penetration depth, and p_0 is the photoacoustic pressure produced. The inverse of the optical absorption coefficient as shown in (4.5) is the optical penetration depth or measure of the distance the light travels until it decays to $\frac{1}{e}$ of its initial optical intensity. Equation 4.4 with various experimental techniques are used to analyze the thickness, absorption, and imaging of many materials from blood vessels to thin films. [58, 115, 120–123]

4.1.6 Total Internal Reflection Photoacoustic Spectroscopy

When the refractive index of one medium is larger than that of a second medium, Snell's law, shows that beyond the critical angle, θ_c , light is totally internally reflected back into the first medium given by

$$n_1 \sin \theta_1 = n_2 \sin \theta_2.$$

When $\theta \geq \theta_c$, and $\sin \theta_2 \rightarrow 1$

$$\theta_c = \sin \left(\frac{n_2}{n_1} \right)^{-1}. \quad (4.6)$$

This configuration produces total internal reflection, and generates a special type of nearfield optical effect called the evanescent field. The evanescent field can be perturbed to reduce the reflected coefficient of light if a sample material of appropriate optical absorption or scattering is sufficiently near the field. This type of frustrated total internal reflection is the basis of many characterization systems including attenuated total reflectance [101].

Figure 4.1: This figure shows a schematic of the setup showing how laser light interrogates sample to give an acoustic effect in TIRPAS.

Recently, a method of generating a photoacoustic response from optical absorption

through a pulsed evanescent field was rediscovered by Goldschmidt et al. [88, 89], who used the technique in conjunction with modern lasers to improve the method's sensitivity. This technique is graphically demonstrated in Figure 4.1. The method has been used successfully to detect β -hematin to demonstrate the technique's potential as a new type of biosensor [89].

In the 1980's, this technique [60–66, 75–78] was explored as an alternative method to normal incidence photoacoustic spectroscopy, due to total internal reflection photoacoustic spectroscopy's (TIRPAS) ability to determine information about materials at an interface. This type of information was never achievable with normal incidence photoacoustic spectroscopy (PAS), due to the generally large optical penetration depth obtained with low absorption materials using the PAS method as can be seen in Figure 4.2. TIRPAS was used to determine dye adsorption properties at a glass/solution interface for interfacial phenomena studies as part of a cyclic voltammetry project.

Figure 4.2: This figure illustrates the vastly different optical penetration depths between the PAS and TIRPAS excitation.

The excitation of the photoacoustic effect using TIRPAS is fundamentally different than that by typical PAS experiments due to the fact that the evanescent field is interacting with absorbers close to the interface as opposed to the photoacoustic effect being excited by propagating photons through normal incidence. This type of excitation limits the optical penetration depth to the penetration of the evanescent field that is nearly always much less than the optical penetration depth of propagating photons through a typical material. The evanescent field component in polarization parallel to the optic is given by (4.7) that comes from (4.8) and (4.9), as presented by Muessig et al. [66] is given by

$$E_{\parallel}' = (E_x'^2 + E_z'^2)^{\frac{1}{2}}, \quad (4.7)$$

$$E_x' = \frac{2E_{\parallel}(\sin^2 \theta - n_{21}^2)^{\frac{1}{2}} \cos \theta}{(1 - n_{21}^2)^{\frac{1}{2}} [(1 + n_{21}) \sin^2 \theta - n_{21}^2]^{\frac{1}{2}}}, \quad (4.8)$$

and

$$E_z' = \frac{2E_{\parallel} \sin \theta \cos \theta}{(1 - n_{21}^2)^{\frac{1}{2}} [(1 + n_{21}) \sin^2 \theta - n_{21}^2]^{\frac{1}{2}}}, \quad (4.9)$$

where $E_{\parallel}$ is the component of the electric field parallel to the plane of incidence, $n_{21} = \frac{n_2}{n_1}$, θ is the angle of incidence of the laser on the prism/sample interface, and $E_{\parallel}'$ is the parallel component of the evanescent field. Aside from the electric field components, which may affect the TIRPAS signal, the evanescent field's near field interaction can be described by a parameter called the optical penetration depth, as noted by Goldschmidt et al. [89]. The optical penetration depth shown in (4.10) describes the point where the non-propagating evanescent field decays to $\frac{1}{e}$ of its

original intensity. This distance can be roughly used as the depth that can be analyzed with the photoacoustic effect in TIRPAS as given by

$$\delta_p = \frac{\lambda_1}{2\pi(\sin^2 \theta - n_{21}^2)^{\frac{1}{2}}}, \quad (4.10)$$

where $\lambda_1 = \frac{\lambda}{n_1}$, λ is the vacuum wavelength of the laser used and δ_p is the optical penetration depth of the evanescent field. This relationship is important because it implies that the signal magnitude in TIRPAS is proportional to the amount of material excited assuming the material is sufficiently thicker than the penetration depth of the evanescent field.

4.1.7 Surface Plasmon Resonance Photoacoustic Spectroscopy

Another method, related to TIRPAS, was explored in the early 1980s that used the resonant effect of surface plasmons to induce a photoacoustic effect for the detection of materials at an interface as well. Several groups [67–74, 79–87] used this approach to understand the technique and to detect various materials, such as nicotine, and various gases of interest. The optical setups varied substantially, however, these methods generally used a laser pulse not meeting the excitation requirement for thermal confinement in the samples of interest. The entire systems were enclosed in photoacoustic cells for the detection and characterization of materials with properties related to and including refractive index. This technique, however, differs from TIRPAS in that TIRPAS uses pulsed laser light that interacts with a sample directly by the evanescent field. The related surface plasmon technique uses the generated evanescent field with an intervening metal layer to generate surface plasmons for detection

of materials adsorbed on the surface of the metal layer.

4.1.8 PAS/TIRPAS Refractometry

PAS/TIRPAS refractometry is a new technique that combines the advantages of both the photoacoustic effect and the evanescent field to determine the refractive index of materials. It should be noted that a similar, but more computationally and physically complicated non-thermally confined photoacoustic refractometry technique was accomplished in the early 1990s. In that case, researchers relied upon the analysis of resonant optical modes in Langmuir-Blodgett films [124–127] and were able to produce refractive index estimates on the order of 0.01. In traditional reflectometry, light is measured after its interaction with a sample in two linear polarization states to determine refractive index. In PAS/TIRPAS refractometry, a laser beam is scanned across a sample at changing angles of incidence to collect acoustic waves from both the PAS and TIRPAS regimes, as well as any location inbetween. This experiment allows for the direct observation of the light-induced acoustic wave that changes drastically in peak to peak voltage between the PAS and TIRPAS regimes. This was shown experimentally in previous data from Goldschmidt et al. [89], and can be physically shown to be different by Figure 4.2. The drastic change in peak to peak voltage can be easily explained by comparing (4.5) and (4.4) with (4.10), which for typical values gives an order of magnitude or more difference in photoacoustic pressure. Here we demonstrate a simple, robust technique based upon the large changes in optical penetration depth for the determination of the real component of the refractive index of dyed liquids and the biologically relevant material, myoglobin, for potential disease detection.

4.2 Materials and Methods

4.2.1 Direct Red 81 Dye Solutions

The dye solutions were made with a 50% polyethylene glycol (PEG, m.w. 200) 50% H₂O solution along with the dye at 125, 250, 500, and 750 $\mu\text{g}/\text{ml}$ respectively. The PEG was added in order to shift the refractive index closer to the middle range of the ATAGO R-5000 refractometer to provide easier measurement and comparison with the PAS/TIRPAS refractometer. Each solution was mixed for 5 minutes or until visually homogenous before testing using a hot plate/stirrer (Corning PC 220). The mixing directly preceded each test to reduce the effects from dye settling on the prism surface.

4.2.2 Myoglobin Solution

The myoglobin solution was made by mixing 460 μg of dry horse skeletal myoglobin (Sigma Aldrich #M0630-250MG) per 1 ml of phosphate buffered saline (Fisher #BP2438-4). As with the dye solutions, the myoglobin was mixed for at least 5 minutes before testing using a hot plate/stirrer. The myoglobin solution did not have added PEG to it since the refractive index was within the readable scale on the handheld refractometer.

4.2.3 PAS/TIRPAS Refractometer

The device, shown in Figure 4.3, works on the principle of exciting a photoacoustic effect in the sample at different angles of incidence. The samples were interrogated

with vertically polarized 532 nm laser light generated by a Q-switched Nd:YAG laser (Surelite I-20 Continuum). The acoustic signal was detected using a 15 MHz transducer with a 1" focal length (Olympus #U8472001).

Figure 4.3: **Left:** This figure shows the full system including laser, stepper motor stage, and optical train. **Right:** This is a closeup of the PAS/TIRPAS refractometer sample holder used for testing.

4.2.4 Laser Leveling

The PAS/TIRPAS holder was leveled relative to the optical table by a Micromark digital level (Micromark #: 84519). The interrogating laser beam was leveled relative to the table as well by a setup similar to a Michaelson interferometer except that the output of the setup was placed on a fluorescent card where the two beams could be made coincident to level the beam. Thorlab's mirrors, a beam splitter, and cage system connectors were used to create this setup (Thorlabs #: CM1-BS013 and #: PF10-03-G01). The PAS/TIRPAS refractometer was aligned by viewing the incident and reflected spots on a sheet of fluorescent paper and adjusting the incident reflected

laser to overlay it on that of the reflected beam.

4.2.5 Labview Automation

A Sherline stepper motor XY base (Sherline 5200-CNC) was used, along with a 4" rotary table (P/N 8700), right angle attachment (P/N 3701), and 3-jaw chuck (P/N 1041) to hold and position the rotational mount made of a prism and custom machined PTFE rodstock. The rotary table was controlled by Labview. The angular spectrum settings were set to scan a range of angles where the critical angle was initially assumed to be. The Labview program stepped the motor at 0.01 degree increments across the entire range of angles. At each increment, the Labview program measured as stated the photoacoustic wave's peak to peak voltage.

4.2.6 Exploratory Analysis

The angular spectra were analyzed by taking the numerical derivative of the angular spectrum data to find the local minima corresponding to the reduction in photoacoustic signal amplitude. The process of taking the numerical derivative of the angular spectrum data produced visually noisy graphs that needed data smoothing to illustrate the existence of the critical angle. Kaleidagraph 4.1.0 was used with the *smooth* curve fit function to smooth the data so that the critical angle could be estimated from the data. This *smooth* curve fit is based upon a combination of Stineman interpolation [128] with a weighting structure to smooth the data. The exact weighting structure is described further in Appendix A.3.

4.2.7 PAS/TIRPAS Measurement

First, the laser beam was leveled using the technique described in Section 4.2.4. Next, the sample holder was filled with sample liquid and the sample holder was leveled using the Micromark digital level by balancing the level on the unconnected acoustic sensor that was previously inserted into the sample holder. Finally, the Labview software described in Section 4.2.5 was used along with the Sherline stepper motors to obtain the angular spectrum scan of the sample material. The angular scan was then used to determine the critical angle as explained in Section 4.2.6.

4.2.8 Optical Refractometer Measurement

Figure 4.4: This figure shows the refractometer setup used to compare refractive index with PAS/TIRPAS refractometry.

We used an ATAGO R-5000 refractometer along with a 532 nm line filter (Thorlabs #: FL532-10) and a polarizing beam splitter cube (Thorlabs #: CM1-PBS251) to limit the polarization and wavelengths measured to find the refractive index of the samples for comparison with our PAS/TIRPAS refractometry technique as shown in Fig. 4.4.

4.3 Results

A typical result from the dye is shown in Figure 4.5 and demonstrates a clear transition in photoacoustic peak to peak voltage at approximately 66 degrees. The numerical derivative of the angular spectrum data was taken along with smoothing to obtain a computable measurement of the transition as demonstrated by the arrow in Figure 4.5. Figure 4.6 shows a second smoothing applied to the data in order to obtain the minima corresponding to the critical angle. Finally, it is straightforward to determine the critical angle from finding the minima in Figure 4.6.

Figure 4.5: **Left:** This figure shows a typical angle spectrum (Direct red 81 at 125 $\mu\text{g/ml}$) that shows the characteristic drop [129] in peak to peak voltage from the PAS to TIRPAS regime. **Right:** This figure shows the numerical derivative of the figure on the left showing a smoothed Stineman function fit (Kaleidagraph 4.1) that displays a local minima corresponding to the critical angle.

In order to demonstrate the utility of PAS/TIRPAS refractometry, we compared the results obtained with optical reference measurements with the ATAGO R-5000 refractometer setup. We show illustrative agreement between the two techniques with the samples' refractive index values using PAS/TIRPAS refractometry and the

ATAGO R-5000 refractometer as shown in Table 4.1. We took five measurements with PAS/TIRPAS refractometry and one measurement with the ATAGO R-5000 refractometer for comparison.

Figure 4.6: **Left:** This figure shows the second smoothing of Figure 4.5 that shows a second smoothed Stineman function fit (Kaleidagraph 4.1) that displays a local minima corresponding to the critical angle. **Right:** The second smoothed Stineman function (Kaleidagraph 4.1) displays a local minima corresponding to the critical angle.

PAS/TIRPAS	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	ATAGO R-5000
Direct Red 125 $\mu\text{g/ml}$	1.395	1.395	1.395	1.395	1.395	1.395-1.397
Direct Red 250 $\mu\text{g/ml}$	1.390	1.390	1.390	1.390	1.390	1.390-1.396
Direct Red 500 $\mu\text{g/ml}$	1.388	1.389	1.389	1.389	1.389	1.381-1.395
Direct Red 750 $\mu\text{g/ml}$	1.382	1.382	1.387	1.387	1.387	1.372-1.395
Myoglobin 460 $\mu\text{g/ml}$	1.330	1.329	1.331	1.330	1.331	1.335

Table 4.1: PAS/TIRPAS refractometry is compared to a handheld refractometer and demonstrates that our method is in good agreement with standard techniques.

4.4 Discussion

As was illustrated in the previous section, PAS/TIRPAS refractometry seems to be a fairly accurate method that compares favorably to ellipsometry and reflectometry. Every dye solution's refractive index measurement fell within the measurement range of the handheld refractometer. In the case of the Direct Red, 750 $\mu\text{g}/\text{ml}$, the sample-to-sample variability seemed to be more significant (0.005) than the other dyes. We believe this was due to dye settling over time. The myoglobin concentration seemed to have some divergence from the measured value with our handheld refractometer, however, this variability can be explained by the illustratively lower signal-to-noise ratio as shown in Figure 4.7. Of course, more research is needed to determine the error between these two conditions to determine quantitatively different values in signal to noise ratio. However, variability of 0.006 is still fairly reasonable compared to the differences between reflectometry and ellipsometry for many materials and therefore this is not substantial for our current demonstration of the technique's capabilities. Therefore, PAS/TIRPAS refractometry has the capability to analyze both dyes and a biologically relevant analyte, myoglobin, for potential biosensing and biomedical monitoring. Our technique opens up significant potential for biomedical monitoring and adsorption state monitoring through the refractive index at a sample/prism interface.

One interesting feature about PAS/TIRPAS refractometry is the generation of plane waves, which are acoustic waves, that deliver most of their energy in a forward and backward direction. The initial pressure rise due to these waves can be described by equation 4.4. In order to obtain plane waves in photoacoustics the following equation must be satisfied: $z = \frac{d^2 \mu_a}{8}$ where d is the laser beam diameter and z is

Figure 4.7: There are some acoustic differences between the dye solution and myoglobin solution under oblique photoacoustic excitation.

the acoustic near field/far field boundary as discussed by Viator [59]. In the PAS regime this is generally followed since the pigment of the dyes combined with the low optical penetration depth along with the geometry of the incoming beam create these conditions. However, with myoglobin, the solution is much more transparent and therefore, plane waves are not necessarily always obtained at every angle of incidence. In the TIRPAS regime, plane waves are always obtained due to the penetration depth of the optical radiation being so small compared to the relative size of the beam, 500 nm vs. 1 mm beam size or more. This can create issues when peak to peak voltage is compared in the PAS and TIRPAS regimes since it is possible for the TIRPAS regime to have similarly sized peak to peak voltage as the PAS regime, despite the PAS regime having more optical penetration depth due to the differences between comparing plane waves in the TIRPAS regime to non-plane waves in the PAS regime. Our data with myoglobin shown in Figure 4.7 suggest that this had an effect on our refractive index

resolution since the acoustic waves from the PAS region with myoglobin do not show the typical plane wave configuration due to the low concentration of myoglobin used. An alternative method based upon integrated pressure may be more appropriate in the future.

Beyond the peak to peak differences, the two signals' relative shapes are worth mentioning. The myoglobin wave's complex shape comes from the fact that a cylindrical segment of sample is being excited that is giving rise to the acoustic wave. First, since angles of incident light smaller than the critical angle are used for the PAS regime, there is an effectively planar wave generated for both Direct Red and myoglobin when the refracted light transfers into the sample region at an angle close to 90 degrees from normal to the interface. This effect is due to the fact that the optical penetration depth is limited since the light is traveling nearly perpendicular to the sample/prism interface. However, at angles of incidence further away from the critical angle in the PAS regime, as shown in Figure 4.7, the myoglobin's signal starts to resemble a wave with more acoustic diffraction due to larger boundary waves being generated from the laser pulse incident at a lower angle of incidence from normal to the interface.

Table 4.2: This table shows a comparison of current technologies.

	Resolution	Speed	Opaque materials?
PAS/TIRPAS refractometry	0.006	54 seconds	Yes
Ellipsometry	0.001	1 second	No
Reflectometry	0.001	1 second	No
Hand held refractometer	0.001	1 second	Yes

4.5 Conclusion

As can be seen from Table 4.2, PAS/TIRPAS refractometry should have several uses for materials that are heavily opaque with unknown optical properties. This suggests the use of PAS/TIRPAS refractometry with metals as well as less homogenous materials for quick and simple critical angle analysis. PAS/TIRPAS refractometry has two major advantages over current methods that include the ability to analyze deeply opaque materials along with a true single wavelength determination of the refractive index without the need for complex model fitting needed for other techniques.

PAS/TIRPAS refractometry has shown promise in its ability to determine refractive index values for myoglobin at a diagnostically relevant concentration. This opens the technique to biological monitoring of myoglobin levels before and after surgery to aid doctors in the diagnosis of potential renal problems occurring from the accumulation of myoglobin after acute hyperthermia or myocardial infarction. In addition, PAS/TIRPAS refractometry, if combined with an automated syringe pump, could be used for the monitoring of levels of important biological analytes in blood, urine, and for the determination of binding kinetics similar to surface plasmon resonance.

We have demonstrated a new photoacoustic technique that can be used in place of ellipsometry and optical reflectometry for the determination of the real part of the refractive index of liquid solutions. Additionally, we have demonstrated the refractometer's capability to determine the refractive index of myoglobin at a diagnostically relevant concentration. This new technology has been shown to complement standard technologies such as ellipsometry and reflectometry for future biomedical testing.

Chapter 5

QTPAS and Thin Films

Thin films of sol-gel or mesoporous silicas for optoelectronic use currently require several types of analytical instruments for proper characterization of crucial parameters, such as refractive index, porosity, thickness, and homogeneity. Currently, spectroscopic ellipsometry is the standard method for determining refractive index of such films; however, this technique has difficulty analyzing non-reflective materials, such as structured or porous films of micrometer or larger thickness. Moreover, ellipsometry produces values for refractive index that are dependent on thickness and knowledge of the film's substrate or other layers it is packaged with. Here we present an alternative method, using the photoacoustic effect, to non-destructively determine the refractive index of thin films independently of thickness using well-understood mesoporous silica thin film systems as a proof of concept.

5.1 Photoacoustic Evaluation of Thin Films

There is a substantial amount of existing literature regarding using the photoacoustic effect to evaluate surfaces and thin films [130,131] for a variety of physical parameters. Types of parameters studied with this configuration include spectroscopic evaluation, thickness or depth profiling evaluation, and even thermal diffusivity and even thermal conductivity could be studied for particular films [132]. Spectroscopic evaluation is simple to employ on standard substrates such as deposited films on silicon since optical methods [102, 133–135] exist for the detection of acoustic waves in a variety of configurations. The primary limitation with spectroscopic evaluation of thin films with the photoacoustic effect is to scan a material with effectively zero absorption over the optical wavelengths studied. With many mesoporous silica materials, there are very few absorption bands even into the near-IR portions of the spectrum compared to other materials. This optical transparency limits traditional photoacoustic evaluation of these materials for any properties unless a mid-IR laser or mesopore introduced chemical can be used for absorptive contrast [136] of the films.

5.2 Quantum Tunneling Photoacoustic Spectroscopy

Quantum Tunneling Photoacoustic Spectroscopy (QTPAS), is a technique that uses the photoacoustic effect coupled with optical tunneling to determine thickness and refractive index of nanofilms. QTPAS works on the same principle to the classical optical tunneling experiment where light can traverse a small gap through interaction of a high refractive index material with the extending evanescent field created through total internal reflection.

Figure 5.1: Quantum tunneling photoacoustic spectroscopy can be described by a schematic of four layers of material with laser interaction through them as shown above.

Figure 5.1 shows that light becomes delocalized and can “tunnel” through a thin film when the light is under total internal reflection. Equations [137] to describe optical tunneling with S and P polarization states for light are shown below as

$$T = (\alpha \sinh^2 y + \beta)^{-1}, \quad (5.1)$$

which describes what percentage of light will cross the thin film.

$$y = 2\pi \frac{n_1 d}{\lambda} \sqrt{N^2 \sin^2 \phi_0 - 1} \quad (5.2)$$

Equation (5.2) describes the optical penetration depth of the evanescent field into the film of interest.

$$\alpha_s = \frac{(N^2 - 1)(n^2 N^2 - 1)}{4N^2 \cos \phi_0 (N^2 \sin^2 \phi_0 - 1) \sqrt{n^2 - \sin^2 \phi_0}} \quad (5.3)$$

$$\beta_s = \frac{(\sqrt{n^2 - \sin^2 \phi_0} + \cos \phi_0)^2}{4 \cos \phi_0 \sqrt{n^2 - \sin^2 \phi_0}} \quad (5.4)$$

Equations (5.3) and (5.4) refer to the attenuation constant and the propagation constant of the system assuming S polarized light.

$$\alpha_p = \frac{\alpha_s}{n^2} \{(N^2 + 1) \sin^2 \phi_0 - 1\} \{(n^2 N^2 + 1) \sin^2 \phi_0 - n^2\} \quad (5.5)$$

$$\beta_p = \frac{(\sqrt{n^2 - \sin^2 \phi_0} + n^2 \cos \phi_0)^2}{4n^2 \cos \phi_0 \sqrt{n^2 - \sin^2 \phi_0}} \quad (5.6)$$

Equations (5.5) and (5.6) refer to the attenuation constant and the propagation constant of the system assuming P polarized light where $n = \frac{n_2}{n_0}$ and $N = \frac{n_0}{n_1}$. These equations describe how the light tunnels through a thin film of interest based upon different film/substrate properties. In Figure 5.2, it can be seen that differences in thickness of the film with a given refractive index (1.4) affect the quantum tunneling by shifting the transmission curves lower with higher inflection as the thickness of the film increases. This makes sense because the thicker the film becomes, the less the evanescent field can penetrate into a guiding material thus leading to less optical tunneling. Additionally, it can be seen in Figure 5.2 that increases in the refractive index of a film with given thickness (300 nm) tend to shift the critical angle closer to 90 degrees but more or less keep the shape of the spectrum intact unless the refractive index becomes very close to the value of the substrate.

Figure 5.2: **Left:** Optical tunneling transmission curves for different film thicknesses show that thicker films reflect more light than thinner films of the same refractive index. **Right:** Optical tunneling for different refractive indices shows that higher refractive indices shift the curves to higher angles

5.2.1 QTPAS Derivation

The transmission of light through a film due to quantum tunneling is insufficient to completely describe the inner workings of QTPAS since there is of course a photoacoustic component to it and not just optical. The photoacoustic effect's working principle is that sound is generated through localized transient optical absorption of light. According to [137] we define (5.1) at zero film thickness (Fresnel reflection) as

$$T_0 = \beta^{-1}. \quad (5.7)$$

Therefore, we can introduce a conversion factor, K , that can describe the conversion of light absorbed to acoustic transmission. Therefore, we can create (5.8) to explain how acoustic transmission in volts, P , can be transformed into normalized transmission

from 0 to 1, T . K is a function of the sensitivity of the transducer, the optical absorption of the acoustic emitter, and weakly on the temperature of the material being irradiated.

$$\frac{T}{T_0} = \frac{pK}{p_0K} \quad (5.8)$$

If we use (5.8) and set it equal to (5.1) and (5.7) we can write

$$\frac{T}{T_0} = \frac{pK}{p_0K} = \frac{(\alpha \sinh^2 y + \beta)^{-1}}{(\beta)^{-1}}.$$

With a little cancellation and rearranging we have

$$T = \frac{p}{p_0\beta} = (\alpha \sinh^2 y + \beta)^{-1}.$$

This fully reduces to

$$P'\beta^{-1} = (\alpha \sinh^2 y + \beta)^{-1}, \quad (5.9)$$

where P' is the normalized acoustic voltage obtained by scanning a substrate with thin film divided by a blank substrate. In the case of matching refractive index between the prism and the acoustic emitter $\beta = 1$ and the equation can be simplified further to

$$P' = (\alpha \sinh^2 y)^{-1}. \quad (5.10)$$

5.2.2 Assumptions/Limitations of the QTPAS Derivation

There are several assumptions that must be mentioned before the QTPAS equations, (5.9) and (5.10), can be applied to a real situation.

1. Temperature of acoustic emitter is constant
2. Laser energy per pulse and distribution is constant
3. Refractive index of prism, substrate, thin film, and acoustic emitter are constant
4. Optical absorption into acoustic emitter is constant
5. Optical coupling must be constant between scans
6. Optically transparent thin film
7. Film thinner than 1500 nm

With regard to 1, the temperature of the acoustic emitter needs to be relatively constant because the photoacoustic effect will increase or decrease depending upon the temperature of the absorber. This can be shown to describe photoacoustic plane waves [107] and (5.11) as

$$p(z, t) = \frac{1}{2}\Gamma H_0 \mu_a e^{-\mu_a(z-ct)} + \frac{1}{2}\Gamma H_0 \mu_a e^{-\mu_a(z+ct)}$$

and

$$\Gamma = \frac{\beta}{\kappa \rho C_v}, \quad (5.11)$$

where β is the thermal coefficient of volume expansion, κ is the isothermal compressibility, ρ is the density, and C_v is the specific heat capacity at constant volume. Since β , κ , ρ , and C_v are functions of temperature Γ also varies with temperature. Although experimentally it is important to keep the acoustic emitter at a constant temperature, this is truly difficult since a laser is constantly impinging upon the surface causing acoustic waves to be emitted. Luckily, steady state heating is achieved with a few laser pulses and therefore no overall correction is needed for practical testing.

In addition to constant temperature, the laser energy's distribution (Gaussian) and pulse energy needs to be held constant in order to compare the light transmission from one pulse to the next. Unfortunately, Nd:YAG lasers are very poor lasers for optical stability since at specifications energy fluctuation may be as high as $\pm 3.5\%$. Since the photoacoustic effect works by linear thermoelastic expansion this suggests that a $\pm 3.5\%$ fluctuation in laser energy would lead to a $\pm 3.5\%$ fluctuation in acoustic signal that, due to the normalization, P' , would create an even higher error in the overall measured transmission. To correct for this fluctuation in laser energy, the QTPAS system performs a dual photoacoustic measurement to measure not only the voltage from the transducer coupled to the substrate/film system, but additionally to a transducer with the same acoustic emitter with the laser light normally incident into it. The additional transducer/acoustic emitter system provides the function of measuring the "laser energy" and allowing for the photoacoustic signal to be normalized based upon small fluctuations in laser energy. Additionally, since both transducers are measuring laser light incident upon the same type of acoustic emitter, the fluctuations from changes in temperature based upon the Grunesian coefficient are approximately cancelled out since both materials heat similarly.

With regard to 3, we must make the assumption that the refractive indices of the thin film, substrate, prism, and acoustic emitter are constant. If the refractive indices are not constant, the properties of both refractive index and thickness (since they are interdependent) will change based upon (5.2)-(5.6). Changes in temperature as well as the presence of an acoustic wave can change the refractive index of these materials. However, generally temperature only needs to be taken into account when doing a refractive index measurement when precision beyond 0.001 refractive index units is needed that, in the case of our preliminary study, is not the case. Additionally, the refractive index of these materials will only change during the presence of an acoustic wave which, since there are no interfering acoustic waves until after the light has interacted with the materials, the assumption of no acoustic interference still holds, however, this no interference assumption means that previous iterations of photoacoustics (where a Hemholtz cavity is used to increase signal to noise ratio) will not work due to the temporal and spatial limitations of the system.

With regard to 4, we make the assumption that the optical absorption of the acoustic emitter stays constant. This assumption means that we cannot excite the acoustic emitter so much that it starts to chemically change or burn. If the material burns or changes chemical properties dramatically, this can affect the signal by changing the emitter's acoustic impedance and by changing the emitter's optical absorption (typically burned materials turn black and so the photoacoustic signal increases). In order to prevent this effect, the laser energy must simply be lowered so that burning or chemical changes do not occur.

With regard to 5, it is important to keep the optical coupling the same from one scan to the next (particularly from the p_0 scan to the p scan). The reason is that if the

optical coupling is not the same from one test to the next, there may be nanoscopic air gaps in-between the film and the acoustic emitter. If there are gaps then the overall thickness and refractive index will be incorrect since the system will calculate them based upon the combination air gap/thin film.

With regard to 6, the films being interrogated need to be transparent. The reason for this is that the optical tunneling equations given in [137] do not take into account absorption due to the evanescent field interacting with absorbers in the film. Since the evanescent field has to exist in the film for optical tunneling to occur, any optical absorption within the film will change the overall measurement of the tunneling in the resulting acoustic wave and thus change the tunneling measurement.

Figure 5.3: Optical transmission is shown for a 1500 nm film.

Finally, with regard to 7, it is important that the thin film under investigation be thinner than approximately 1500 nm in size. The reason for this is that as the thickness of the film is increased more reflection occurs beyond the critical angle through optical tunneling. Although the probability of finding a tunneled photon is not truly ever zero (since the probability decays exponentially from the interface), there is a physical limitation of being able to measure whether or not a photon tunnels far away from the interface due to the fact that to get a measurable photoacoustic signal there must be at least several hundreds of thousands of photons exciting the acoustic emitter to generate a measurable sound. In the current system it is difficult to determine changes much smaller than 5% transmission. Therefore, if we use 5% as our cutoff we can calculate the tunneling profile of an example film (1.4 RI, 1500 um) and show that this film only tunnels approximately 5% of the initial light at the extreme value near the critical angle as shown in Figure 5.3.

5.3 Labview Automation: QTPAS.vi

In order to run the QTPAS system it became necessary to fully automate the rotation of the prism/sample interface as well as to automatically collect signals through a digital acquisition system. A schematic of that setup is shown in Figure 5.4. Beyond the physical setup, a Labview program was created to run the system called “QTPAS.vi”. Figure A.8 shows that the program has three main modes that can be user selected, *Setup*, *Angular Spectrum*, and *Curve fitting*.

- **Setup:** Allows the user to see signals at a single angle of incidence to confirm correct system operation

- **Angular spectrum:** Allows the user to run an “angular spectrum” test for later evaluation under TIRPAS, PAS/TIRPAS refractometry, or QTPAS analysis techniques
- **Curve fitting:** Allows the user to fit a function to collected data using (5.9) to estimate n_1 and thickness of thin films using an exploratory method to get a general range of the properties.

Figure 5.4: QTPAS schematic shows how electronics and physical components fit together.

Beyond selecting the modes, the user must input parameters such as the refractive index of the surrounding air, the prism, the acoustic emitter, and set the band pass signal filter boundaries, etc. Once the initial parameters and defined cursor regions are set, the user can run the program to get the desired result of setup running, an

angular spectrum scan, or can perform a non-linear least squares curve fit to (5.9) to estimate the parameters n_1 and thickness using this exploratory method.

5.3.1 Refractive Index and Thickness Estimation

Initial estimation of refractive index and thickness is accomplished by using an un-weighted constrained non-linear curve fitting algorithm developed by National Instruments. The method is the Levenberg-Marquardt algorithm [138] that is used to find a local minima given an initial guess for thickness and refractive index based upon fitting to equations describing light transmission given in [137] for S and P polarization. Specifically, (5.1) is the equation that is fit to for determining the refractive index and thickness for QTPAS material property estimation. Since (5.9) only describes optical tunneling given the total internal reflection assumption it is important to use the constrained non-linear curve fit. The author originally tried using the non-constrained non-linear curve fitting algorithm for simplicity, however, depending upon the initial guesses it was possible for the algorithm to guess values of n_1 and thickness that would give rise to imaginary numbers that could cause an error in the virtual instrument. More information about the non-linear curve fit can be found at: http://zone.ni.com/reference/en-XX/help/371361G-01/gmath/nonlinear_curve_fit/#parent

5.4 Exploratory Analysis and Simulated Example

Although QTPAS has not yet demonstrated an accurate evaluation of the thin film properties, thickness and refractive index, I have included experimental data as well as

a simulation that qualitatively explores the current state of the art in the developing field of QTPAS. Simulated data such as Figure 5.5 was created using Matlab code to add random noise to a function and is found in Appendix A.6. In the simulation the standard deviation or (σ)=10 and mean or (Baseline)=1. This simulation provides an illustration of a typical signal.

Figure 5.5: Optical transmission based upon $n_1 = 1.4$ and 100 nm thickness shows substantial change in light transmission over a range of angles of incidence.

5.4.1 Substrate Scans

In order to calibrate the QTPAS system one must scan a substrate (NBK-7 glass) and then scan another substrate with the film on it to divide one scan by another and apply (5.12). Since the substrate and acoustic emitter are the same refractive index, $\beta = 1$, so the equation can be simplified to

$$P' = \frac{P}{p_0} = (\alpha \sinh^2 y)^{-1} = 1. \quad (5.12)$$

Ideally, the equation reduces to equal 1 because the same substrate is being scanned twice and therefore, the same acoustic effects should be obtained over the same scanning angles.

Figure 5.6: This figure shows a representative scan of a substrate divided by itself.

In practice, the graph obtained from scanning the same substrate twice can be represented by Figure 5.6. To show that this is the case, a simulation with the introduction of Gaussian white noise to a predicted optical tunneling curve for a film $n_1 = 1.4$ of 100 nm thickness can be seen in Figure 5.7. In order to reduce the Gaussian noise inherent with all electronic systems several techniques have been employed with the QTPAS system. These techniques include averaging waveforms together at each angle of incidence 128 times as well as to use a bandpass Chebyshev 5th order infinite impulse response filter with a range from 1 Hz-5 MHz to reduce noise.

Figure 5.7: This figure shows a simulated data of a substrate scan with Gaussian white noise.

5.4.2 Laser Energy Interaction

Interestingly enough, the most recent testing with QTPAS indicates that the results obtained from an angular spectrum depend upon the laser's energy intensity per pulse. In Figure 5.8 a representative sample shows that the slope of the angular spectrum graph seems to be qualitatively different when high energy (Q-200) and low energy (Q-270) are compared. Of course, more testing is needed to conclude this with any statistical significance, however, with a hypothetical understanding of this effect one would think that this should not be occurring since energy differences are accounted for by measuring the relative laser energy at every pulse. However, since the two curves seem to be different this suggests that there may be some interaction between the laser beam's intensity and the resulting acoustic wave obtained for the scan. In order to determine what possible effects may occur over time angular spectra were

taken on the same substrate over a seven hour period of time to get an idea of how the spectra would change as shown in Figure 5.8.

As can be seen from the spectra, the curves seem to increase in peak to peak voltage with every additional test bringing up the possibility that overall sample heating may be occurring that would affect the refractive index and Grunesian coefficient of the sample or some complex form of photobleaching may be occurring that may increase the efficiency of photoacoustic generation.

Figure 5.8: **Left:** Differences in angular spectra shape can be seen for two laser q-switch values for the same substrate. **Right:** This figure shows angular spectra taken over a seven hour period to illustrate changes in spectra over time.

There is ongoing research to investigate this effect to determine what the effect is from and how strongly it affects the resulting data.

Chapter 6

Future Work

Due to time constraints it was not possible to explore all possible avenues for research regarding TIRPAS, QTPAS, and related techniques. However, a review of the possibilities may be helpful for future researchers to explore new technologies and techniques.

6.1 Birefringence Determination

Provided is a schematic drawing of a method to simultaneously determine the refractive index of a material for both S and P polarizations. The incident beam from the laser is passed through a $\frac{\lambda}{2}$ wave plate to rotate the polarization of the incident beam to split the beam into two components (S and P polarized). The P polarized component will pass through the polarizing beam splitter cube into another polarizing beam splitter cube where it will recombine with the S-polarized beam that has either been delayed by mirrors or by a coil of optical fiber with a third polarizing beam splitter

(since the fiber won't preserve polarization). By splitting the optical beams into two polarizations to excite the material or thin film of interest at different points in the time domain it is possible to cut the time of typical TIRPAS and QTPAS testing in half to test both polarization states. Once the two refractive index values are known we will have an idea of the birefringence of the material that adds an additional layer of characterization to most materials.

Figure 6.1: This idea uses dual polarizations of light to estimate birefringence.

6.2 Optically Resonant TIRPAS

Although TIRPAS has been shown to be able to detect as low as 1.1 cm^{-1} there are some materials that may have a very limited quantity of optical absorption such as dilute absorbers dissolved into water or biological fluids that are diagnostically important. In order to create an excellent biosensing platform the sensitivity of

TIRPAS must be increased with the primary method of doing this being to create a resonant cavity to perform TIRPAS.

6.2.1 100% Absorption

Figure 6.2: This figure shows a thin film resonator which can achieve a theoretical 100% optical absorption.

The major advantage of depositing a thin film resonator on a prism is that in principle it is possible to achieve 100% absorption of light into an analyte of interest. This is extremely important since it allows for the highest possible signal to noise ratio for a material. In addition, it is possible to optically saturate a material in absorption, which can be useful for optically pumping thin films for lasing studies. The important idea behind a 100% absorption of the incident light is that this process would make TIRPAS as sensitive as it can physically be regardless of the absorbing material of interest. Since TIRPAS already constrains the optical penetration depth

via the evanescent field ~ 10 times tighter than that obtainable in normal incidence photoacoustic excitation, it is in principle possible to increase the sensitivity of the photoacoustic effect by approximately 100 fold compared to standard photoacoustic excitation simply by adding a few thin films to a prism or substrate surface.

6.3 Porosity Measurements

Beyond simple characterization of the refractive index and thickness it is important to characterize other properties of the films that may be of interest such as the volume of the pores to calculate the porosity. This is particularly important for the development of mesoporous and microporous thin films with low dielectric values since porosity is a key factor in reducing the dielectric value for standard materials. Using the equation obtained from [139] we can find the porosity, ρ given by

$$\frac{n_c^2 - 1}{n_c^2 + 2} = (1 - \rho) \frac{n_b^2 - 1}{n_b^2 + 2},$$

where n_b is the refractive index of the non-porous material, n_c is the refractive index of the porous material, and ρ is the porosity. The physical experiments necessary to do this calculation would be simple since the refractive index of most non-porous materials are already known in the visible spectrum and are available online. Once we calculate the porosity of the thin films we can do a variety of things such as monitor the absorption of materials into the films by measuring the refractive index over periods of absorption.

6.4 Optical Detection

TIRPAS, although currently limited to liquid and solid materials due to the large difference in refractive index between gases and ultrasonic transducers, can in principle excite gases with the evanescent field the same way that the excitation occurs in liquids or solid samples. Over the years, several different optical methods [133–135] of photoacoustic detection have been created to overcome acoustic impedance limitations [140] between typical materials like PZT transducers and gases. However, since TIRPAS uses a prism/sample interface for excitation, it makes sense to use an optical method that includes a prism as well due to convenience.

Paultauf et al. [102] created a method using the change in refractive index induced due to acoustic wave propagation to optically detect photoacoustic excitation. The ideal quality of the optical detector is that the acoustic wave generated at the interface between the prism and sample is picked up by the photodetector sensing a change in light intensity rather than an acoustic wave activating a piezoelectric element that makes the differences in acoustic impedance meaningless. Therefore, optical detection of gases using TIRPAS should be feasible with this technique.

6.5 Absorbing Thin Films

Optical tunneling can happen in both transparent films as well as highly absorbing films. While I have been able to create a fully functional Labview program to characterize the optical tunneling effects through thin films, one of the assumptions in QTPAS is that the films do not absorb any of the evanescent field created in the film itself. In highly absorbing thin film materials such as solar cells there is significant

absorption within the thin film and therefore more general equations need to be used to account for the absorption within the films as well as the optical tunneling effects.

Chapter 7

Summary and Concluding Remarks

I have presented several new photoacoustic technologies based upon the interaction of the pulsed evanescent field with everything from highly absorbing dyes to biologically relevant analytes for heart attacks and malarial infection to mesoporous nanofilms. The importance of developing new characterization technologies, such as TIRPAS/(PAS/TIRPAS) refractometry/QTPAS, to probe the material properties of thin films, and biomarkers has been discussed at length in Chapter 1. In Chapter 2, using Maxwell's equations, the derivation of the existence of the evanescent field was presented in tandem with equations describing the photoacoustic effect and the qualitative effects of combining both into TIRPAS. Chapter 3 presents several experiments to show the potential to use TIRPAS as a biosensing technique for the detection of hemozoin, an optically pigmented waste product of a malarial infection in humans. A simple photoacoustic critical angle analysis technique, (PAS/TIRPAS) refractometry, was developed and tested on highly absorbing dyes and myoglobin in Chapter 4. Chapter 5 detailed the derivation of, current experiments, assumptions,

and modeling of a technique that could determine the refractive index and thickness of nanofilms by combining the photoacoustic effect with optical tunneling (QTPAS). Finally, future directions for TIRPAS, (PAS/TIRPAS) refractometry, and QTPAS are presented with substantial background for reproduction in Chapter 6.

7.1 Modern TIRPAS and Biosensing

Experiments showing the first example of the TIRPAS technique with nanosecond lasers were performed with the explicit purpose of detecting and characterizing the response of Chlorazol black dye and β -hematin for the purpose of using the TIRPAS technique as a biosensor for malarial infection. A proportion for the changes in photoacoustic signal was given an update for dual polarization excitation for the signal. It was shown through experiments comparing the oblique photoacoustic excitation regime that β -hematin and Chlorazol black give substantially different optical responses due to optical scattering.

These responses were compared with the responses of Chlorazol black dye and β -hematin for TIRPAS excitation and it was illustrated using representative examples that the acoustic response was very similar between the two materials suggesting that optical scattering could be somewhat dismissed due to the lower inherent optical penetration depth in TIRPAS. It was important to note that all TIRPAS acoustic waves imparted a plane wave like response regardless of the optical absorption/scattering tested. This was explained by the much larger aspect ratio of the photoacoustic excitation than typical techniques since the optical penetration depth is on the order of 500 nm while the irradiation spot size is nearly always 1000 fold larger for experi-

ments. It is currently unknown if it is even possible to obtain a spherical wave from TIRPAS experiments.

7.2 Photoacoustic Refractometry (PAS/TIRPAS) and Biosensing

Experiments were conducted to show the predictive ability of a photoacoustic refractometer (PAS/TIRPAS refractometry) to predict the refractive index of dye solutions and myoglobin for biosensing applications. The refractometer was compared to a standard handheld refractometer to correspond to the results. A Labview program was created to run the refractometry testing and reduce overall system noise through the use of a computational filter and waveform averaging. The PAS/TIRPAS refractometry technique allowed the photoacoustic effect to estimate the real component of refractive index of a material despite the photoacoustic effect being used traditionally to determine the complex component of the refractive index by finding μ_a . This represented a step forward in technological development for photoacoustic technologies as it will allow the technique to find more optical properties beyond absorption and performing imaging. Additional modifications to the setup used the direct beam from a laser so that minimum distortion from optical divergence would be observed, eliminating the need for the spatial filter in Chapter 3. Finally, a process for finding initial estimates for the critical angle from the angular spectrum using a Stineman smoothing function and a numerical derivative from Kaleidagraph 4.1.0 allowed for the estimation of the refractive index of dyes and myoglobin.

7.3 QTPAS and Thin Films

A technology (QTPAS) showcasing the potential to estimate the refractive index and thickness of a thin film was created and data was compared to a representative sample simulation for continued research in ongoing work. The potential to use this photoacoustic technique to evaluate the materials at wavelengths where the material is optically transparent and traditional photoacoustic evaluation would be unsuitable explains the true nature of the technique in revolutionizing the way the scientific community uses the photoacoustic effect. To date, there is no other photoacoustic technique that could scan a material without inherent optical absorption and potentially estimate material properties. Optical tunneling coupled with the photoacoustic effect seems to suggest the potential to expand the photoacoustic technique to optically transparent materials. Ongoing experimental evidence shows additional research is needed to fully realize the potential of the technique due to currently unknown complex effects involving the laser pulse energy and measured refractive index of the film of interest.

7.4 Conclusion

Traditional photoacoustic spectroscopy has illuminated many areas of biomedical research by reducing the importance of optical scattering for tissue and burn depth imaging. Additionally, the photoacoustic effect has changed the landscape of label-free detection of circulating melanoma cells for fast and inexpensive diagnosis, characterization, and capture to usher in a new age of personalized medicine. However, biomedical imaging and flow cytometry are not the only avenues of research for the

photoacoustic effect. With the combination of the pulsed evanescent field and the photoacoustic effect, I have revealed that there are still substantial unexplored avenues for the photoacoustic effect for the detection and characterization of materials at substrate/film or substrate/sample interfaces. The ability to detect the surface properties of materials while ignoring the bulk properties allows for the photoacoustic effect to evaluate material properties that it has never had access to before. The photoacoustic effect has shown the exceedingly helpful ability to evaluate materials at all scales except substantially at the nanoscale due to laser pulse duration limitations. With the invention of TIRPAS, (PAS/TIRPAS) refractometry, and QTPAS, the nano world can finally be substantially evaluated with photoacoustics without the need for expensive light sources or high frequency ultrasonic equipment. The potential to scan a transparent material with the photoacoustic effect without exogenous chromophores or destructive dielectric breakdown techniques will help bring into focus the nano world of photoacoustics by allowing the effect to work at scales from imaging an entire human body to evaluating the thickness of a nano film.

Appendix A

Various Important Issues

There are several important experimental procedures or mathematical descriptions that are given here for completeness.

A.1 Electrical Impedance Matching

In all electrical systems mentioned in this dissertation it is important to have appropriate impedance matching for the systems and cabling. Electrical impedance refers to the real value and complex value of the resistance in wiring or devices as given by

$$\Gamma_{12} = \frac{Z_2 - Z_1}{Z_2 + Z_1},$$

where Γ_{12} is the voltage reflection from impedance 1 to impedance 2, and where Z_2 and Z_1 are the characteristic impedances. A typical reflection that occurs in laboratory equipment is between 50 Ω and 75 Ω BNC cables. Even with a small difference

between the two 20% reflection will result which can affect measurements from all sorts of devices. With regard to TIRPAS and QTPAS, the impedance matching is particularly important since the signals generated by the evanescent field and those signals generated by oblique absorption may have substantially different frequency responses which can distort the measured voltage values and give biased results. All cables (except the transducer which is assumed to be approximately 50Ω) and connecting devices are 50Ω unless otherwise specified for the experiments.

Figure A.1: Electrical impedance matching for signal integrity is very important to achieve an acceptable signal to noise ratio.

A.2 Acoustic Impedance Matching

Acoustic impedance is mathematically analogous to electrical impedance and to optical refractive index. In order to have a high signal to noise ratio for measurements it is important to match the impedance of the medium being tested and the transducer/any materials in-between given by

$$\Gamma_{12} = \frac{Z_2 - Z_1}{Z_2 + Z_1}.$$

Typical materials such as water have an acoustic impedance of 1.5 Mrayls whereas stainless steel is 45.7 Mrayls. The reflection from a water/stainless steel interface is $\sim 93\%$. Since there is such a large difference between the acoustic impedance of liquids and solids, photoacoustic experiments have to take into account this difference and use materials that approximately match each other to reduce acoustic reflections and thus increase signal to noise ratio.

A.3 Stineman Smoothing Algorithm from Kaleidagraph 4.1.0

The *smoothing* function in Kaleidagraph's software is based upon a combination of the Stineman interpolation method [128] with a weighting function. The step by step method is given below:

1. Fit the Stineman interpolation function to the data.
2. Apply a weighting function to the data to smooth the curve.

The weighting function takes into account the surrounding 10% of the data points during smoothing which can be understood better by a plot of the distribution in Fig. A.2. This is only true if 10% or more of the data is remaining on either side. If less is remaining than 10% of the data the function uses all data that is left until at the final point on the edge of the dataset the value of the Stineman interpolation is used. An array is then created using the following code:

```
coeff[wsize-1]=1.0
```

```
coeff[kk]=coeff[kk+1]*magic
```

where wsize is 10% of the number of data points, $magic = \frac{1}{10^{abs(wsize)}}$ and kk goes from 0 to (wsize-2). From there the weighting function is applied to the data and the smooth curve is output to the program.

Figure A.2: This figure shows the weighting structure for the Stineman *smooth* function.

A.4 Electromagnetic Noise Considerations

Due to the very sensitive nature of TIRPAS and QTPAS, our setups had to be designed in such a way to reduce electromagnetic noise in as many ways as possible. For completeness, a more comprehensive explanation of our methods is given here.

A.4.1 Ground Loop Noise

Both TIRPAS and QTPAS use National Instruments' Labview programming environment to scan materials. In order to completely automate the system so that no optical movement is needed, a stepper motor driver, the MID 7604, along with a Sherline rotational stage is needed to measure optical tunneling and evanescent absorption. Traditional photoacoustic experiments simply rely upon a single-ended amplifier for signal acquisition. However, due to the fact that the high current stepper motors are directly connected to the same circuitry as the transducer, amplifier, computer, and PXI system, any electronic noise generated by the stepper motors will result in measured output noise in the signal due to ground loops.

A.4.2 What is a Ground Loop?

Figure A.3: This figure shows the circuit diagram of a ground loop.

A ground loop, as shown in Figure A.3, occurs when two devices are grounded to two

potential grounds that are assumed to be at the same potential, but in fact are not. When the two grounds are at different potentials it creates the situation where an unwanted current can flow between them causing signal contamination in the form of common mode noise, it can cause component damage, and in some extraordinary cases even human harm in the form of electrocution.

In terms of how ground loops affect overall measurement in the TIRPAS system, there is a noisy signal trace versus angle which is particularly emphasized at higher angles where the signal to noise ratio is lower. The noise presents itself as semi-random spikes of peak of peak voltage as shown in Figure A.4.

Figure A.4: TIRPAS angular spectra sometimes receive noise via ground loops.

A.4.3 How does a Differential Amplifier Help?

A differential amplifier, as explained in detail in [141], helps alleviate the issue of ground loop noise by only amplifying the difference between two inputs + and -. Since both inputs share the ground loop noise or common mode noise caused by the unavoidable interconnections in the system the differential amplifier simply cancels them out and the resulting signal is free from that form of noise.

A.4.4 Inductive Noise

Figure A.5: Noise can occur via magnetic induction as well as ground loops.

Inductive noise is noise generated by the interaction of two conductors with overlapping magnetic fields as shown in Figure A.5. This process is the basis of electrical transformers, however, this presents the issue of increased signal noise for typically configured equipment that is within close proximity to other noisy equipment. For TIRPAS and QTPAS, the motor that allows for the automation of the system is physically close to the sensing element (transducer) and can inductively couple motor noise into the system. Unfortunately, the electronic noise generated through this pro-

cess has a similar frequency spectrum to the electrical representation of the acoustic signals and therefore, computational filtering is not possible. In Figure A.6 we see the effect of inductive noise on a typical spectrum. At the lower angles of incidence, the noise seems to be large but as the angles increase the noise seems to subside. This fluctuation of the noise versus angle of incidence has to do with the fact that at lower angles of incidence, the transducer and wiring is nearly parallel with the motor and wiring whereas at higher angles of incidence the transducer and the motor are nearly perpendicular to one another. This difference in alignment is important because the most efficient induction of charge from one wire into another occurs whenever the two wires are parallel to each other. Almost no inductive coupling occurs between two wires when they are perpendicular to each other due to the increased distance between the wires everywhere but at the intersection of the perpendicular.

A.4.5 Quantization Noise

The final major source of noise in the system comes from the nature of converting an analog signal to a digital signal in a real world application. Quantization noise is the effect where an analog signal (which can take on an infinite number of values) is digitized into discrete levels that contain fewer values than that in the set of numbers that correspond to an analog signal. This compression causes information loss in A/D converters by rounding off a value such as 3.445631 to 3.4 by the nature of how many digitization levels can be obtained by a digitizer. In the case of the PXI-5152, only 8-bit is available, thus only 256 levels can represent any one signal. With the smallest voltage range enabled, this means that the smallest value of voltage that can reliably be measured is 0.390625 mV out of a possible 100 mV. What this means

is that a small measured signal may only be recreated with a few points which may introduce distortion in the overall shape of the waveform due to only discrete voltage levels being possible values as shown in Figure A.7.

Figure A.6: This figure shows an angular scan with inductive noise.

A.4.6 Dithering

Many researchers have used a technique called “dithering” to reduce or eliminate quantization noise for signals that can be averaged. Dithering intentionally introduces a random source of noise to the analog signal in order to make the value fluctuate

Figure A.7: Dithering can increase vertical digitizer resolution. (Reproduced with permission from National Instruments at <http://www.ni.com/white-paper/3016/en/>)

between two adjacent quantization levels. This process is demonstrated in Figure A.7 where the sinusoidal waveform without dithering is shown averaged and without averaging to show substantial artifacts introduced as steps via quantization. However, when random noise is added to the signal (dithering) after averaging the sinusoidal signal is clearly more accurately depicted. This is due to the fact that the noise is random such that after averaging the step quantization effect is cancelled out and a waveform closer to the analog signal is reproduced as shown in Figure A.7.

A.5 QTPAS Flow Chart

Figure A.8: This flow chart explains the QTPAS Labview/experimental program from the beginning to the end of the experiment.

Following is the flow chart for the Quantum Tunneling Photoacoustic Spectroscopy virtual instrument. Three separate modes, *Setup*, *Angular Spectrum*, and *Curve fitting* are available for operating the system.

A.6 Signal Simulation: ModelingQTPAS.m

Following is the code used to generate the Gaussian simulated signal for QTPAS.

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Benjamin S. Goldschmidt, PhD Bioengineering%
%    Contact: bsgn74@mail.missouri.edu    %
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%Clear variables and screen
clc
clear all
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%Define system variables
n0=1.519;
n1=1.4;
n2=1.519;
d=100;
lambda=532;
n=n2./n0;
N=n0./n1;
crit=asind(1/N);
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%Define angles of interest
theta=crit:.05:90;
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

```

%Coefficients for transmission equation
A=sqrt((n.^2)-sind(theta).^2);

B=(n.^2)*cosd(theta);

C=((N.^2)-1);

D=((n.^2).*(N.^2))-1);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%P polarization for Beta
beta_p=((A+B).^2)./(4.*B.*A);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%S polarization for Beta
beta_s=((A+cosd(theta)).^2)./(4.*cosd(theta).*A);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%Optional to plot Beta
%plot(theta,(beta_s).^-1)
%axis([75 85 0 1]);
%pause

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%S polarization for alpha
alpha_s=(C.*D)./(4.*(N.^2).*cosd(theta).*((N.^2).*(sind(theta).^2)-1).*A);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%P polarization for alpha

```

```

alpha_p=((alpha_s./(n.^2)).*((N.^2)+1).*(sind(theta).^2)-1).*((n.^2).*(N.^2)+1).*(s
%%%%%%%%%%

%Optional to plot Alpha
%plot(theta,alpha_s)

%pause

%%%%%%%%%%

%Calculate Y versus angle of incidence
Y=((2.*pi)./lambda).*n1.*d.*sqrt((N.^2).*(sind(theta).^2)-1);
%%%%%%%%%%

%Optional to plot Y
%plot(theta,Y)

%pause

%%%%%%%%%%

%Calculate the total optical tunneling transmission
Tunnel=100.*(alpha_p.*(sinh(Y).^2)+beta_p).^-1;
%%%%%%%%%%

%Calculate baseline
Baseline=100*ones(1,length(theta));
%%%%%%%%%%

%Generate waveform with noise and average
t=0;

for ii=1:1:128 %128 is the # of averages%

```

```

sigma=10; %Define the standard deviation of the noise
t = (Baseline + sigma*randn(size(Baseline)))+t;
end

Average=t/128;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%Generate signal by dividing average waveforms by baseline p/p0
%In this case beta_p=1 when prism and rubber stopper match so beta_p=1
Signal=Average/Baseline*beta_p;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%Plot the simluated data
plot(theta,Average)
axis([74.5 80.5 90 110])

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

```

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