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Development of a Unique Photothermal Interferometer

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LOYOLA UNIVERSITY CHICAGO

DEVELOPMENT OF A UNIQUE
PHOTOTHERMAL INTERFEROMETER

A DISSERTATION SUBMITTED TO
THE FACULTY OF THE GRADUATE SCHOOL
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

BY
MICHAEL HENRY

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CHAPTER 1
INTRODUCTION TO PHOTOTHERMAL SPECTROCOPY

Excitation of a chemical species with electromagnetic radiation can result in the generation of several forms of energy. The energy produced originates due to different relaxation mechanisms that a substance undergoes to rid itself of excess energy absorbed during excitation. These different de-excitation processes can be broadly classified as radiative, photochemical, photoelectric, and photothermal (1). With the exception of radiative de-excitation (in which a photon is produced), the other mechanisms represent different energy conversion pathways. For example, photoelectric de-excitation converts electromagnetic energy into electrical energy. Similarly, photochemical de-excitation changes electromagnetic energy to chemical energy (resulting in some chemical reaction). It should be noted that a molecule in the excited state is not restricted to one relaxation process. However, the sum of energy placed into the system must equal the energy produced. Therefore these pathways are complementary.

Photothermal de-excitation is the conversion of electromagnetic energy into heat. The term "photothermal effect" refers to physical manifestations that occur in the system due to optical heating. Some of the effects include temperature changes, refractive index variations, acoustical waves, volume changes, thermal emission variations, and reflectivity changes (1). These physical changes have become the basis for the development of many thermo-optical techniques and classified as photothermal spectroscopy.
Despite the thermal phenomena used for detection, all the various spectroscopic techniques can be categorized as either direct or indirect methods. Direct measurements involve detection of the temperature change that occurs within a sample matrix. Indirect methods typically measure the transformation of a light beam (usually a laser) due to the heated sample or the generation of a pressure wave. This discussion will only consider the thermo-optical indirect approaches.

DIRECT PHOTOTHERMAL MEASUREMENTS

In 1977 Brilmyer and coworkers described the first instrument design that directly measured the heat generated from an absorbing substance (2). Their approach relied on careful placement of thermistors onto a sample surface or into a sample solution. These thermistors were placed away from the path of the incident light with the fraction of light absorbed measured by the thermal diffusion of the heat. A xenon lamp and monochromator were employed as the excitation source. The signal was determined by the change in the resistance of the thermistor within one arm of a wheatstone bridge. These experiments were usually performed in a water bath. With this configuration, the spectrum of crystalline solids (TiO₂ and CdS) and chromophore solutions (methylene blue, aniline yellow, and rose bengal) were analyzed. Evaluation of the extinction coefficients for these materials was successful. However, the response time of the thermistor was slow due partly to the thermistor itself, as well as, the thermal conductivity of the sample. Problems with the thermistor placement were also encountered and affected the response of the system. Ideally, the thermistor should be placed at the point of excitation of the sample but the heat generated from the thermistor itself was a large
source of noise. However successful, the approach was limited because the reproducibility of locating the thermistor varied from sample to sample.

Bass et. al., attempted to improve the sensitivity of direct detection by using thermocouples as the heat sensing detector (3 & 4). Here the voltage difference between the junctions of two dissimilar metals as a function of temperature is recorded. With a Q-switched, Nd: YAG laser modulated at 5 hertz, the two photon absorption coefficients of solid CdTe and CdSe were evaluated. This proved to be a superior method of determining the extinction coefficients for both the linear and the two photon absorption processes when compared to conventional methods. Photothermal waveform profiles obtained using these sensors were the same as those obtained by Brilmyer. However, the increased sensitivity provided by this experimental setup allowed accurate evaluation of the two photon absorption process.

The sensitivity of directly measuring the heat generated at a sample was further improved with the use of pyroelectric detectors. Both Baumann et. al. and Coufal (5 & 6) utilized these transducers to construct an instrument to measure the absorbance of solid samples. Baumann modified a scanning electron microscope and used a modulated electron beam as the heat source. Two types of samples were analyzed with this instrumental configuration. The first was metal disks that had machined grooves to simulate defects in the surface structure. The second was wire grids which were covered with beryllium-copper. These samples were placed in thermal contact with the pyroelectric transducer (LiTaO₃ crystal), and the cross-sectional areas scanned in the instrument. Comparisons between normal electron microscopy and this approach of photothermal microscopy proved to be equivalent. In the case of the coated wire mesh, subsurface imaging was demonstrated.
Coufal also developed a direct photothermal photometer employing a pyroelectric detector to demonstrate the feasibility of measuring the powder absorbance spectrum of rare earth oxides. The ferroelectric polymer polyvinylidene difluoride was used as the heat transducer. Samples were prepared in which Nd$_2$O$_3$ in poly(methyl methacrylate) was spin coated onto the pyroelectric film. Both conventional sources (xenon arc lamp) and an excimer pump dye laser were modulated and used to optically heat the samples. The visible absorbance spectra for the samples were compared to the spectra produced by photoacoustic spectroscopy utilizing piezoelectric detection. The results demonstrated that both techniques had similar sensitivities and response times. It was noted that in the case of photoacoustic analysis, the system was more susceptible to acoustical noise and, that in this arrangement, greater care was needed to minimize this source of error.

**INDIRECT PHOTOTHERMAL MEASUREMENTS**

Indirect methods of measuring the photothermal response are done by sensing physical changes in the system. Typically, thermo-optical probing of the heated sample region is performed with a laser beam being transformed as if an optical element was present. Therefore, the names for these techniques usually reflect the type of optical element manifested. Although there are numerous variations and novel optical configurations, basically three techniques can be used to represent indirect thermo-optical detection. These include thermal lensing, photothermal deflection, and photothermal displacement.
Historically, the oldest indirect photothermal technique is thermal lensing which was first described by Leite and coworkers (7) in 1964. The original experiments involved placing an absorbing solution into the resonator cavity of a laser. It was observed that the beam diameter of the laser changed when a shutter was opened between the mirrors in the laser cavity. The change in beam size could be monitored with a photomultiplier tube and iris until a steady state power level was obtained at an oscilloscope. The phenomenon was termed "thermal blooming."

A year later Gordon, et. al. (coworker with Leite, 8) was the first to state that "thermal blooming" occurred due to temperature gradients present in the sample solution upon thermal de-excitation. He also related the phenomenon to density changes (and corresponding refractive index gradients) within the thermal gradient that mimicked a biconvex negative lens. The formation of a lens was based upon the gaussian nature of a laser beam operating in the TEM$_{00}$ mode. In this mode, the irradiance within the beam center is at a maximum and radially declines like a normal curve. Therefore, the thermal gradient has a refractive index gradient with the center being lower than the edges (for substances with a negative temperature coefficient of refractive index). The concept of a thermal lens best described the original observations.

In this article, the authors also provided an analytical expression to account for the deviation of the laser beam through the heated region of the sample solution. They based this mathematical model upon the fact that the center of a gaussian curve could be approximated by a parabola, therefore simplifying the expression.
They established a time (t) dependent focal length (F(t)) of the thermal lens as a function of a time constant (t_c) and the steady state lens (F_∞). The analytical expression was:

\[ F(t) = F_∞ \left( 1 + \frac{t_c}{2t} \right) \]

The steady state thermal lens (F_∞) is determined from the thermal properties of the material and the optical characteristics of the laser beam. This is defined as:

\[ F_∞ = \omega_o^2 \eta_o k \pi / 0.24 b P (dn/dt) \]

Where:

- \( \omega_o \) - Beam Waist Radius
- \( \eta_o \) - Initial Refractive Index
- \( k \) - Heat Conductivity
- \( b \) - Absorption
- \( P \) - Laser Power
- \( (dn/dt) \) - Derivative of Refractive Index versus Temperature

Although this equation only approximated the photothermal response, the primary success of the expression is that it established the first correlation for the thermal characteristics of the sample matrix and the photothermal signal obtained. In other words, the time required for a steady state lens to form in which the heat generated and the heat conducted out of the illuminated region are at equilibrium.
Hu and Whinnery removed the sample solution from the laser cavity by mimicking the properties of the laser beam within the cavity using an external focusing element (9). This approach will later be termed a single beam pump/probe configuration in which one laser is used to both thermally excite the sample and probe the thermal gradient. Their experiments still employed a shutter and measured the change in the irradiance of the laser until a steady state lens formed. From their experiments they noted that the absolute intensity change was a function of the distance between the sample and the external lens. They found that the largest fractional change in irradiance occurred when the sample was placed at a distance of one confocal length relative to the minimum beam waist of the focused laser beam.

The confocal length \((Z_c)\) of a focused laser beam is determined based upon the minimum beam radius (waist, \(\omega_0\)) by (10):

\[
Z_c = \frac{\pi \omega_0^2}{\lambda}
\]

This is the distance from the beam waist (either side) in which the beam radius is the \(\sqrt{2}\) times the minimum radius at the focus. Although this may seem to be an arbitrary constant, it represents the axial position along the focused optical path in which the beam wavefront is at a minimum. If the wavefront is at a minimum, the divergence of the beam due to the thermal lens is at a maximum (11).

Dovichi and Harris (12, 13, & 14) expanded the work of Hu and Whinnery providing an analytical expression in terms of the focused optical properties. Figure 1 is a reproduction of the response profile for thermal lensing as a function of sample distance relative to the waist. Using methylene blue solutions with a Helium Neon laser as the pump/probe and later measuring iodine concentration with an Argon Ion laser as the pump/probe, they established that their theoretical model fit the data.
Figure 1. Positional dependence of $\Delta I/I$
Their experimental arrangement also differed from previous work in that they used a chopper to modulate the laser beam. They noted an improvement in detection due to the reduction of the 1/f noise when compared to previous experiments in which a shutter was used to obtain a steady state thermal lens. The equation they derived to account for the response profile is:

$$\frac{\Delta I}{I} = \left(\frac{4.6 P (dn/dT)}{\lambda k}\right) \left(\frac{ZZ_c}{Z^2 + Z_c^2}\right) A$$

Where:

$$\frac{\Delta I}{I}$$ - Relative Change in Irradiance

P - Incident Laser Power

$$dn/dT$$ - Temperature Coefficient of Refractive Index

k -- Thermal Conductivity of Sample Matrix

$$\lambda$$ -- Excitation Wavelength

Z -- Distance of Sample from Beam Waist

$$Z_c$$ - Confocal Length

A - Optically Heated Area

Long and coworkers were the first investigators to use a dual beam pump/probe approach to investigate molecular absorbance (15). A general block diagram of the optical arrangement is presented in Figure 2. A modulated dye laser is used to heat the sample (pump) and a Helium Neon laser is used to probe the fluctuating thermal lens. The two lasers are collinearly aligned with a beam splitter and the pump energy rejected with an interference filter.
Figure 2. Block diagram for Thermal Lensing
A lock-in amplifier is employed to de-modulate the probe signal by frequency tracking. The intensity of the fundamental from the lock-in amplifier was obtained at a chart recorder. Their investigations involved measuring the fifth C-H stretching vibrational overtone of benzene. Kliger (16), in his review of thermal lensing, notes that there is almost an order of magnitude improvement in detection for this transition when compared to previous absorbance studies.

Since the early experiments with thermal lensing, numerous instrument configurations have been developed. A modulated square wave contains only odd harmonics in a Fourier series. Based upon this, a single laser pump/probe instrument was described by Pang and Morris (17). Using a lock-in amplifier, they measured the asymmetry induced into the square wave (second harmonic) due to the temporal thermal lens. Dovichi et al. (18) developed a thermal lens photometer in which the pump and probe beams were orthogonal. This produced a cylindrical thermal lens with high spatial resolution. A differential dual pump/probe configuration was constructed by Berthud (19) based upon earlier work by Dovichi (13). In this configuration two sample solutions were placed into the optical path of the collinear pump and probe beams. The first solution contained the sample of unknown absorbance while the second held a solution of known absorbance. The combination of the two thermal lenses was used to evaluate the absorbance of the unknown.

A single laser dual pump/probe configuration (20 & 21) was developed in which the light from a single laser beam is split into two beams. One beam is orthogonally polarized relative to the other and modulated to serve as the pump. The other beam is allowed to remain as a continuous wave and the two are combined and steered toward the sample with a polarizing cube. The pump beam is then rejected with a polarizer.

Polarization encoding was also used to produce another photometer in which a single pump/probe laser was employed. Again, the light from a single laser is split into
two beams, however the beams propagate through the sample in opposite directions (22). With this configuration, the light from the pump is never incident upon the signal photodiode. Jansen and Harris (23) reported a dual beam method in which a single modulated laser is split into a sample and reference beam (similar to modern double beam UV/VIS spectrophotometers). Each beam is imaged upon its own photodiode and the photo voltages are subtracted from each other. A lock-in amplifier is used to measure any difference in signal obtained.

Applications of thermal lensing have been extremely diverse since the first reported analytical use in 1979 (12). It has been used as a detector for liquid chromatography (24), gas chromatography (25), flow injection analysis (26) and electrophoresis (27 & 28). Reaction kinetics has been analyzed with this technique (29 & 30). The technique has also been combined with infrared spectroscopy in order to improve sensitivity (31). Although many references exist, no commercially available instrument exists which employs thermal lensing.

PHOTOTHERMAL DEFLECTION

In 1980, the first use of photothermal spectroscopy to measure the absorbance at a surface was reported by Boccara et al. (32). The basis of the approach was to irradiate a surface placed normal to a modulated laser beam (dye laser, pump beam). As the surface was periodically illuminated, it experienced a temporal change in temperature. The heat generated conducts away from the point of excitation through the material itself, but also into the air surrounding the surface. Thus the adjacent gas layer exhibits a temperature change with a corresponding change in refractive index. Orthogonal to the pump beam and as close to the surface as possible, a probe beam (Helium Neon laser) was aligned.
As the probe beam propagates through the heated air region the refractive index gradient deflects the beam. The amount of deflection can be measured with either a knife edge or a position sensor. They termed the photothermal effect observed as the "mirage effect" for obvious reasons. Figure 3 shows the experimental setup used.

In this original article, Bocacarra and coworkers were able to measure the spectra of Nd$_2$(Mo$_4$)$_3$ crystals and Cs$_3$Cr$_3$Cl$_9$ powdered samples. They also established a simple, one dimensional expression for the amplitude of deflection ($\phi$) as $\phi = (l/n)(dn/dT)(dT /dx)$, where $l$ is the interaction path length within the temperature gradient $(dT /dx)$, $n$ is the ambient refractive index of the gas, and $dn/dT$ is the temperature coefficient of refractive index for the gas.

Fournier and Boccara (33) described a photothermal deflection photometer in which the probe beam was aligned collinear and adjacent to the pump beam. By using this optical geometry they were able to increase the interaction path length. This increased the sensitivity of the technique and provided the ability to measure gas samples. Using a CO$_2$ laser as the pump, ethylene concentrations in nitrogen gas were measured. The detection limit was 5 ppb with a linear range over three orders of magnitude. The experiments performed also established the ability to measure gases in situ. Using different R branches of the 9.4 and 10.4 micrometer bands of the CO$_2$ laser, the spectrum of the laboratory air was obtained.

Jackson et. al. (34) derived a detailed analytical expression of the photothermal deflection effect. The approach was twofold: the temperature distribution was first determined and then the effect of the thermal gradient on the optical beam is derived based upon light propagation in a non-homogeneous medium. Jackson supported the mathematical treatment derived with experimental data and compared this analytical expression for both the transverse and collinear geometry with the well-established expression that defines thermal lensing.
Figure 3. Optical Configuration for Photothermal Deflection
The result of the rather complex derivation is:

\[
\frac{\Delta I}{I} = \frac{4\alpha P I_0^2}{\lambda k a^2 \sqrt{2\pi} dT} \left( \exp \left( -\frac{1}{(2+4t/t_c)} \right) - \exp \left( \frac{-1}{2} \right) \right)
\]

Where:

- \( \frac{\Delta I}{I} \) - Relative Change in Irradiance
- \( \alpha \) - Absorption Coefficient
- \( \lambda \) - Probe Wavelength
- \( k \) - Thermal Conductivity
- \( l \) - Interactive Path length
- \( P \) - Pump Power
- \( o \) - Probe Spot Size at \( 1/e^2 \) Intensity
- \( a \) - Pump Spot Size at \( 1/e^2 \) Intensity
- \( t \) - Time Sample is Illuminated by Pump
- \( t_c \) - Photothermal Time Constant
- \( dn/dT \) - Thermal Coefficient of Refractive Index

Theoretically, both techniques had equivalent sensitivities. This was substantiated by Yang and Ho (35) when the two techniques were compared.

A year before the Jackson contribution, Murphy and Aamodt (36) developed a mathematical expression for photothermal deflection. It was extremely complicated and somewhat limited (e.g., see 37). Therefore, Jackson has typically been given credit for a concise expression. However complicated, Murphy's mathematical treatment took into account the positional dependence of the probe laser beam within the thermal gradient (e.g., the distance of the beam from the surface). Based upon a geometry in which the
sample surface lies in the XZ plane, the modulated pump beam in the YZ plane, and the probe beam in the YX plane, the refractive index gradient was probed by displacing the probe beam along the Y axis incrementally further from the sample. For a constant pump power and modulation frequency, a linear relationship was determined in which the magnitude of the deflection decreased as the distance increased. The following expression was derived:

$$\ln(S) = K - \left(\frac{\omega}{2 \alpha_g}\right)^{1/2} Y_0$$

Where:

- $S$ - Magnitude of the Deflection Signal
- $\omega$ - Probe Beam Size
- $\alpha_g$ - Thermal Diffusivity of Gas Layer
- $Y_0$ - Offset Distance from Surface
- $K$ - Parameter of $y_0$

Measuring the temperature change directly, the thermal diffusivity of nitrogen gas was accurately determined. It should be noted that no significance was discussed for the variable $K$ except that it is the $Y$ intercept of the linear response.

After these initial reports, several instrument design variations were published. In 1983 Johnson et. al. (38) placed the absorbing surface within the resonator cavity of a low power Argon Ion laser. While the sample was excited with a chopped dye laser, the Argon Ion laser experienced power modulations that were proportional to the absorption coefficient of the sample. The resolution of this configuration for powder samples was significantly improved when compared to the work of Boccara (32). According to the
authors, the spectral resolution was limited only by the line width of the dye laser. Although not discussed by the authors, this configuration is essentially a Fabry-Perot interferometer. By pumping an absorbing sample within the cavity, the optical path length varied as the laser beam propagates between the mirrors. This variation produced constructive and destructive interference within the tube. Therefore the output power level is modulated.

Yang and Ho (35) described a single laser cross beam photothermal photometer to measure liquid samples. An Argon ion laser which is predominately vertically polarized (approximately 1% horizontal polarization) was used. The two polarizations were split with a polarizing cube and the vertical polarization modulated to pump the sample while the horizontal polarization was used to probe the refractive index gradients. Their investigations (as discussed briefly above) involved comparing the sensitivity of thermal lensing and photothermal deflection. The results showed that the techniques were equivalent. The authors also suggest that the fundamental difference between the two techniques is that thermal lensing probes the center of curvature for a lens, while photothermal deflection probes a prism type optical element.

In 1987 and 1988 Bialkowski (39 & 40) constructed a cross beam photothermal deflection photometer in which an etalon was used to contain various concentrations of CFC-12 in an argon matrix. A CO₂ laser was repetitively pulsed at 3.75 Hertz and operated at the P(32) line of the 10.6 micrometer transition (933 cm⁻¹). A Helium Neon laser was used to probe the refractive index gradients within the etalon. Theoretically, as the probe beam makes multiple reflections within the etalon, the interference from the beam deflection due to the refractive index gradient, would be a sensitive means of detection. The authors did show that a position sensor (knife edge, aperture detector, or dual photodiode detector) was not necessary to measure the photothermal response. However, their theoretical calculation of a 100 fold increase in sensitivity was not
achieved. Experimentally, they found only an increase in sensitivity by approximately 40%. They did not present a discussion for the discrepancy.

A simple modification to transverse photothermal deflection photometry was developed by Horita and coworkers (41). Their instrument employed a double pass system in which the modulated pump energy, after propagating through a transparent sample, was re-focused back onto the sample with a concave mirror. In this configuration, the sample was illuminated twice and the amount of heat produced (after some attenuation of the beam from the first passage) is nearly doubled. Using a high intensity halogen lamp and monochromator for the pump, the spectrum of n type Si and InP wafers were taken. The refractive index gradient was probed with a Helium Neon laser and the deflection determined with a dual photodiode position sensor. In regions of the spectrum where lower optical absorption occurred, a factor of 1.9 improvement was obtained relative to the conventional technique. However, as the absorbance in other regions of the spectrum increases, little or no improvement was noted. The investigators did provide a mathematical description that fit the experimental results accurately.

Analytical applications of photothermal deflection have been applied to various detection needs. As discussed above, it has been applied to measure the absorbance spectrum and extinction coefficients of solid and powder materials. The technique has been used to build sensitive densitometers (42 & 43) for the analysis of thin layer chromatography plates. Detectors for liquid chromatography (35 & 44) have been developed. Masujima et. al. (45) investigated the corrosion of titanium metal by perfluoropolyether lubricant. The authors used the lubricant itself as the thermally conducting medium. In order to better understand the combustion process, Rose et. al. (46) investigated products formed in flames by this approach. The enthalpy of a reaction at a liquid/liquid interface was studied by Wu and coworkers (47). In this case, no pump beam was necessary and the steady state deflection was measured directly.
Photothermal deflection for bioanalytical detection of IgE and carcinoembryonic antigen was done by Matsuzawa (48). Although there are a large number of other references in the literature, the applications discussed are intended to provide a survey of applications that would interest chemists. As was the case with thermal lensing, no commercially available instrument exists.

PHOTOTHERMAL DISPLACEMENT

Amer and Olmstead (49, 50, & 51) introduced a new approach of photothermal detection at a solid surface and termed it photothermal displacement. This technique relies on a periodic surface distortion induced by optical heating from a modulated source. The change in surface geometry occurs due to the thermal expansion of the material as heat is generated from the absorption of light. The surface displacement at the incident location on the sample is sensed through the change in the reflection angle of a continuous probe laser. Figure 4 is a diagram of the optical arrangement used for this approach. This figure illustrates the typical optical arrangement used for photothermal displacement in which the change in the reflection angle is measured with an aperture detector. Amer and Olmstead referred to this approach as the "laser beam deflection scheme". However, in these first papers, two other detection schemes were discussed. An interferometric approach was evaluated in which the sample was used as one arm of a conventional Michaelson interferometer. An attenuated total reflection configuration was also evaluated in which a prism was placed in close proximity to the sample surface. The space between the surface and the prism served as a light guide for the probe laser beam. The irradiance of the probe laser would vary as the width of the gap between the prism and surface changed during displacement.
Figure 4. Optical Configuration for Photothermal Displacement
Amer determined the surface displacement height \( h \) and mathematically expressed it as:

\[
h = \frac{\alpha B P}{(2A \rho C_p)}
\]

Where:

\( \alpha \) - Linear Thermal Expansion Coefficient
\( B \) - Fraction of Incident Power Absorbed
\( P \) - Incident Laser Power
\( A \) - Optically Heated Area
\( f \) - Modulation Frequency
\( \rho \) - Density
\( C_p \) - Heat Capacity

The deflection of the continuous probe beam by the surface deformation and the resulting displacement \( S \) within the detector plane (see Figure 5) is determined by:

\[
S = 2h_x \cos \theta + 2g_x L
\]

The subscript \( x \) represents the location of the probe beam at the surface, where \( h_x \) and \( g_x \) are the displacement height and gradient respectively. The term \( L \) is the distance from the surface to the detector plane (i.e., the optical lever arm) and \( \theta \) is the grazing angle of the probe beam.
Figure 5. Optical Configuration for Surface deformation for Photothermal Displacement
Another important characteristic expressed during their derivation is the thermal diffusion length \( L_{th} \) which is defined as the depth within the sample from which the surface distortion will occur and is given by:

\[
L_{th} = (k/\pi \rho C_p)^{1/2}
\]

Where \( k \) is the thermal conductivity of the sample. The authors suggested that due to the thermal diffusion length, sub-surface imaging of a sample could be performed.

Yang (52) developed a simplified mathematical model for photothermal displacement. In a manner similar to thermal lensing, the deformation of the surface was considered to mimic an optical element (in this case a thermal mirror). The model is derived in the limit of low absorbance (analytically useful range) in which a non-aberrant thin convex mirror would account for the change in the probe beam reflection angle. At low absorbance, the mirror is confined to the dimensions of the waist of the laser beam, and its curvature approximated by a parabola. The change in the probe beam irradiance in the far field is represented by the beam radius \( w_0 \), the confocal length \( Z_c \), and the relative distance in which the sample is located about the waist \( Z \). The propagation transformation of the probe laser due to the presence of this optical element is expressed as:

\[
\frac{\Delta I}{I} = \phi \left( \frac{Z/Z_c}{1+(Z/Z_c)^2} \right)
\]

\[
\phi = \left( -\frac{4\alpha BP}{2\lambda w_0^2 \rho C_p} \right)
\]
Therefore the displacement height is governed by the same parameters discussed by Amer. The model predicts a response profile as the sample is placed at various distances from the waist which is similar in form to that of thermal lensing. It differs from thermal lensing in two significant ways. First, the optimum signal position is determined by differentiating the above equation and found to be \( Z_{\text{opt}} = Z_c \sqrt{3} \). In the case of thermal lensing, the optimum signal location is at the confocal length. The other difference between the response profiles is the rate of signal decay as samples are positioned through the Raleigh range. In the thermal mirror model the decay rate occurs much faster. Figure 6 shows the response profile for the thermal mirror approximation. This has been verified experimentally (53).

Although this work has never been published, a similar derivation by Zimmerman and Welsch in 1994 was done in which a geometric optical model for photothermal displacement based upon the gaussian energy profile of a TEM\(_{00}\) laser was performed (54). In order to avoid confusion, it should be noted that they refer to the technique as photothermal deflection. However, inspection of their optical design and their derivation is the same as the displacement technique. With gold films deposited onto glass substrates, they established the same response profile.

Amer and Olmstead initially used photothermal displacement as a means to determine the band gap energy of semiconductors. Other material applications for the technique ensued. Rosencwaig et al. (55) used the technique to measure thin film thickness. The technique is well suited for this application as it is a nondestructive, noncontact approach, and can be performed in a host of environments. Films of Al and SiO\(_2\) deposited on Si substrate could be measured with a 2 micrometer resolution ranging from 500 to 25,000 Å in thickness. The optical arrangement used for their analysis varied from that of Amer and Olmstead. This system irradiated the sample with the pump and probe beam normally incident.
Figure 6. Positional dependence of $\Delta I/I$

Thermal Mirror Response

Distance from Waist (units of confocal length)
The two laser beams were aligned such that they partially overlapped in a manner similar to collinear photothermal deflection. Therefore, the signal deflection occurred from only one side of the gaussian surface distortion.

Three years later Rosencwaig and coworkers (56) modified their instrumental approach to photothermal displacement. The new configuration had the pump and probe beams collinear and incident normal to the sample surface. A fiber optic light guide was placed perpendicular to the beam and located close to the incident spot location. Light that scattered as a result of the surface bulge could then be detected through the light guide with a photomultiplier tube. The system also included two additional detection schemes that they had reported earlier (57). These two methods involve the change in the transmittance and reflectance of a material when heated.

Karner et. al. (58) demonstrated the use of a pulsed laser as the pump. In their experiments, the thermal diffusivities of various metals were established by the decay time of the signal when the pump laser was turned off. Although they were able to establish a correlation between their results and the reported thermal diffusivities of the metals, it was noted that the signal decay was not a pure exponential. It was convoluted with some other function. Therefore, quantitative determinations of thermal diffusivity could not be calculated. However, with the use of a pulsed laser, a time resolved photothermal displacement technique was established.

Current publications about photothermal displacement usually involve modification of the mathematical model used to describe the technique. Saito et.al. (59) in 1992 extended the work of Amer and redefined the expression for the surface displacement height. Their model accurately determined the dependence of surface displacement as a function of both the incident pump power and the modulation frequency. Carolyn and coworkers (60) revised the analysis of thermal diffusivities in metals first discussed by Rosencwaig and later by Karner (above). They considered that the effect of the
supporting substrate for a thin film coating produced the signal decay anomalies. In order to eliminate this problem, their experiments were performed on a picosecond time frame. Therefore, the photothermal response could be measured before the influence of the supporting substrate had an effect on the signal. Jaurequi and then Cheng (61 & 62), have used photothermal displacement to measure the transient thermal grating that exists on an optically heated surface due to thermo-elastic waves.

The above discussion of photothermal displacement is meant as an overview of the topic. There are significantly fewer publications in the literature about this technique. To date, applications for this approach are restricted to the areas of material science. These areas typically involve analysis of band gap energies in semi-conductors, thermal optical imaging of surfaces, measurements of film thickness, and analysis of the thermal properties of different bulk materials. No reference exists in which the technique has been applied to the determination of analyte concentrations at a surface. No commercial instrument is currently available.
CHAPTER 2

OBJECTIVE OF THE DISSERTATION

As discussed in the historical section, several thermo-optical spectroscopic methodologies have been developed based upon the non-radiative relaxation process of excited molecules. However, to date no analytical application of photothermal displacement spectroscopy has been cited in the literature and the technique has remained a curiosity for physicists. We have postulated that the use of photothermal displacement spectroscopy as a basis for analytical detection has several substantive advantages. First, the origin of signal change is proportional to the incident power of the pump laser. Therefore, like fluorescent techniques, sensitivity is enhanced to some practical limit by increasing the source power (in this case the pump laser). Required sample sizes are small since the laser beam can be focused to an extremely small waist size (on the order of microns). Optical arrangements can be designed which reduce solvent and matrix interference effects. Consider Figure 7 as an example of such an arrangement in which variations in the amount of absorbance at the second surface is determined by the intensity of the reflected light from this interface. As illustrated, the measured reflected intensity has never interacted with the sample solution. This is particularly important since turbidity and solvent absorbance are major sources of error for spectroscopic instrumentation (63). Finally, the apparatus is easily adaptable to current analytical methodologies (e.g., flow cells, chromatographic detectors, electrophoretic gels, etc.).
Figure 7. Proposed geometry for Photothermal Spectrometer
An unexpected phenomenon was observed during the investigation of various instrument design configurations that might be employable for such a spectrophotometer. When the probe laser was aligned normal to a sample surface and the reflected light imaged onto a field stop, a highly symmetrical pattern of concentric rings was noted. This pattern was reminiscent of an Airy distribution which typically occurs due to diffraction or interference of light. As no apertures were in the system, it was concluded that multiple reflections between the two sample surfaces formed interference fringes. This is not unusual in the strictest sense as most optical textbooks discuss multiple reflection interference within glass slides. However, the symmetry of these fringes is normally poor and distorted. As with most interferometers, the quality of the reflecting surfaces (flatness) is crucial in obtaining a good fringe pattern and float glass (i.e., microscope slides, pane glass, etc.) do not have that degree of flatness. The system we designed utilizes an extremely short focal length objective lens which produces beam spot sizes at the sample on the order of microns. Apparently within these small dimensions, the surfaces were essentially flat enough to form symmetrical interference fringes.

The purpose of this dissertation is fourfold. The first is to design a photothermal spectrophotometer that utilizes the interferometric nature of multiple beam reflections. The second is to verify that the signal measured during the course of analysis of a sample can be attributed to interference. The third purpose is to derive a mathematical framework which will establish a model for the effect. The last is to develop a sensor which can be used to measure a particular analyte in solution thereby demonstrating feasibility for this approach to detection.
CHAPTER 3

DERIVATION OF GAUSSIAN BEAM MULTI-REFLECTIONS

In order to establish a mathematical foundation for the interferometric effect that occurs during photothermal interferometric displacement, several general optical expressions need to be defined. One of these is the propagation of a focused laser beam through a material which exhibits multiple beam reflections. After exhaustive searching of the literature, to this author's knowledge, no such derivation exists. Within this section a general expression has been derived. Several sources discussing the physics of laser beam propagation (64, 65, and 66) are available and is used for the following discussion. The overall approach to the derivation is loosely based upon a derivation for the transmitted radiation of a collimated laser beam through a Fabry-Perot Interferometer (67). Throughout the following, an assumption is made in ignoring the glass polymer interface. The refractive index difference between these materials is relatively small when compared to an air or a solvent adjacent to the polymer. Therefore, the light reflected from the polymer/glass transition is a small fraction of that reflected at the other interfaces.

A description of the Hermite-Gaussian nature of a laser beam can be derived using the time independent paraxial approximation. This approximation relates to a plane wave propagating nearly parallel along only one axis (z axis, optical axis) and gives a relative complex electric field amplitude expressed as follows:

\[
\frac{E(x,y,z)}{E_0} = H_B \left( \frac{\sqrt{2x}}{w(z)} \right) H_L \left( \frac{\sqrt{2y}}{w(z)} \right) \frac{w_0}{w(z)} \exp \left( -i \frac{x^2+y^2}{w_0^2} \right) \exp \left( -i \left[ Kz - (B+L+1) \arctan \left( \frac{z}{z_0} \right) \right] \right) \exp \left( -i \frac{K(x^2+y^2)}{2R(z)} \right)
\]
where \( H_B \) and \( H_L \) are Hermite polynomials of order \( B \) and \( L \) for the two transverse modes, \( K \) is the wave number, \( w(z) \) is the beam radius at the \( z \) axial distance, \( R(z) \) is the radius of curvature of the wave front at \( z \), and \( i = \sqrt{-1} \). \( w(0) \) relates to the beam waist which is the radius of the smallest spot size at \( 1/e \) of the transverse radial distance and contains \( 1/e^2 \) of the beam intensity. \( z_0 \) is a constant based upon the refractive index of the propagation medium and is given by:

\[
z_0 = \frac{\pi w_0^2}{\lambda_0}
\]

where \( \eta \) is the refractive index and \( \lambda_0 \) is the wavelength in vacuo. For a laser beam operating in the TEM\(_{00}\) mode (i.e., radially symmetric), the above equation reduces to:

\[
\frac{E(x,y,z)}{E_0} = \left( \frac{w_0}{w(z)} \right) \exp \left\{ -i \left[ \frac{x^2+y^2}{w(z)^2} \right] \right\} \exp \left\{ -i \left[ Kz - \arctan \left( \frac{z}{z_0} \right) \right] \right\} \exp \left\{ -i \left[ \frac{K(x^2+y^2)}{2R(z)} \right] \right\}
\]

The physical interpretation of this expression can be broken into three parts. The first two bracketed terms on the right relate to the amplitude of the electric field vector. The second exponential term relates to the phase of the radiation at some axial position, and the last term emerges from the paraxial wave approximation.

Radiation reflecting at an interface between two refractive indices has a unit complex electric field intensity defined by the following:

\[
E(x,y,z)_{R1} = r_1 E(x,y,z)
\]

where the term on the left is the electric field amplitude for the reflected light and \( r_1 \) is the complex reflectivity of the surface. Subsequent reflection after refraction through the
interface as the beam is multi-reflected between the two surfaces (consider Figure 8) and is defined as $E_{R2}$, $E_{R3}$, $E_{Rn}$, with $n$ being the number of reflections. The subscripts 1 or 2 refer to the first or second interface with the primes distinguishing whether the radiation is originating from $\eta_1$ to $\eta_2$ (no prime) or $\eta_2$ to $\eta_1$ (primed). The electric field amplitudes for these multiple reflections are:

$$E(x,y,z)_{R2} = r_2' t_1 t_1' E(x,y,z)$$
$$E(x,y,z)_{R3} = (t_2')^2 t_1 t_1' E(x,y,z)$$
$$E(x,y,z)_{Rn} = (t_2')^{n-1} (t_1')^{n-2} t_1 t_1' E(x,y,z)$$

Solving for two reflections from the support, the total electric field amplitude becomes:

$$E(x,y,z)_{RT} = E(x,y,z)_{R1} + E(x,y,z)_{R2} = r_1 E(x,y,z) + r_2' t_1 t_1' E(x,y,z)$$

The irradiance of the radiation reflecting from the two surfaces is determined by multiplying the total unit complex field amplitude by its complex conjugate.

$$I_R = E(x,y,z)_{RT} E(x,y,z)_{RT}^*$$

Substituting the complex electric field amplitude for both reflections gives:

$$I_R = \left\{ r_1 \left( \frac{w_0}{w(z_1)} \right) \exp \left\{ -i \left( \frac{x_1^2 + y_1^2}{w(z_1)^2} \right) \right\} \exp \left\{ -i Kz_1 \arctan \left( \frac{z_1}{z_0} \right) \right\} \exp \left\{ -i \frac{K(x_1^2 + y_1^2)}{2R(z_1)} \right\} + \right.$$  

$$r_2' t_1 t_1' \left( \frac{w_0}{w(z_2)} \right) \exp \left\{ -i \left( \frac{x_2^2 + y_2^2}{w(z_2)^2} \right) \right\} \exp \left\{ i Kz_2 \arctan \left( \frac{z_2}{z_0} \right) \right\} \exp \left\{ i \frac{K(x_2^2 + y_2^2)}{2R(z_2)} \right\} \right\} \times$$

$$\left\{ r_1 \left( \frac{w_0}{w(z_1)} \right) \exp \left\{ -i \left( \frac{x_1^2 + y_1^2}{w(z_1)^2} \right) \right\} \exp \left\{ -i Kz_1 \arctan \left( \frac{z_1}{z_0} \right) \right\} \exp \left\{ -i \frac{K(x_1^2 + y_1^2)}{2R(z_1)} \right\} + \right.$$  

$$r_2' t_1 t_1' \left( \frac{w_0}{w(z_2)} \right) \exp \left\{ -i \left( \frac{x_2^2 + y_2^2}{w(z_2)^2} \right) \right\} \exp \left\{ i Kz_2 \arctan \left( \frac{z_2}{z_0} \right) \right\} \exp \left\{ i \frac{K(x_2^2 + y_2^2)}{2R(z_2)} \right\} \right\}$$
Figure 8: Schematic representation of multiple beam reflections
Simplification of the above equation is accomplished by defining new variables for each reflection (n) such that:

\[ I_R = [A_1 \exp(-i\{B_1\})\exp(-i\{C_1\})\exp(-i\{D_1\}) + A_2 \exp(-i\{B_2\})\exp(-i\{C_2\})\exp(-i\{D_2\})] X [A_1 \exp(i\{B_1\})\exp(i\{C_1\})\exp(i\{D_1\}) + A_2 \exp(i\{B_2\})\exp(i\{C_2\})\exp(i\{D_2\})] \]

where:

\[ A_1 = r_1 \left( \frac{w_0}{w(z_1)} \right) \]
\[ A_2 = r_2 \left( \frac{w_0}{w(z_2)} \right) \]
\[ B_n = \frac{\rho_n^2}{w(z_n)} \]
\[ C_n = \left[ Kz_n - \arctan \left( \frac{z_n}{z_0} \right) \right] \]
\[ D_n = \left[ \frac{K\rho_n^2}{2R(z)} \right] \]

and:

\[ \rho_n^2 = x_n^2 + y_n^2 \]
By simplification, the above equation and calculation of the cross terms produces the expression:

\[ I_R = (A_1)^2 \exp(-2(B_1)) + (A_2)^2 \exp(-2(B_2)) + A_1A_2\exp(-1(B_1+B_2))\{\exp[i(C_2-C_1+D_2-D_1)]+\exp[-i(C_2-C_1+D_2-D_1)]\} \]

Utilizing Euler's formula, the cross terms are further simplified and the formula reduces to:

\[ I_R = (A_1)^2 \exp(-2(B_1)) + (A_2)^2 \exp(-2(B_2)) + A_1A_2\exp(-1(B_1+B_2))2\cos(C_2-C_1+D_2-D_1) \]

Inspection of the above equation quickly reveals a general solution for multiple reflections as follows:

\[ I_R = \sum_{j=1}^{n-1} (A_j)^2 \exp(-2(B_j)) + \sum_{q=1}^{n-1} \sum_{k=q+1}^{n} A_qA_k\exp(-1(B_q+B_k))2\cos(C_k-C_q+D_k-D_q) \]

Based upon Huygen's law of reversibility (68 p., 326), the complex transmissivities at the first interface (\(\eta_1\) into \(\eta_2\)) are the same, irrespective of which direction the light is traveling. Therefore, the scalar transmission is used and determined by Fresnel's equation at normal incidence (\(\theta_1 = \theta_2 = 0\), from Snell's law) such that:

\[ t_{11}^t = T_{11} = \frac{4\eta_1\eta_2}{(\eta_1+\eta_2)^2} \quad (69 p., 102) \]
In a similar manner, the complex reflectivity's for each individual interface is combined to determine the scalar reflectance of the interface providing (68 p., 326):

\[(r_1')^2 = R_1 = \left(\frac{n_2 - n_1}{n_1 + n_2}\right)^2\]
\[(r_2')^2 = R_2 = \left(\frac{n_3 - n_2}{n_3 + n_2}\right)^2\]

Further reduction of this equation is done by noting that neither surface absorbs the probe beam energy (a non-conducting medium). Therefore, the cross reflectivity's \((r_2'\text{ and } r_1)\) can be evaluated in a similar manner and the complex refractive index (68 p., 80) does not need to be taken into account.

So:

\[r_2'\cdot r_1 = \left(\frac{n_2 - n_1}{n_1 + n_2}\right)\left(\frac{n_3 - n_2}{n_3 + n_2}\right) = R_{12}\]

Using the above equations, an expression for two reflections originating from the sample surfaces shows the total irradiance upon interference to be:

\[I_R = \]
\[R_1 \left(\frac{w_0^2}{w(z_1)^2}\right) \exp\left(-\frac{1}{w(z_1)^2}\right) + R_2' T_1^2 \left(\frac{w_0^2}{w(z_2)^2}\right) \exp\left(-\frac{1}{w(z_2)^2}\right) + \]
\[R_{12} T_1^2 \left(\frac{w_0^2}{w(z_1)^2 w(z_2)^2}\right) \exp\left(-\frac{1}{w(z_1)^2} + \frac{1}{w(z_2)^2}\right) * \]
\[2 \cos K \left[ \left(\frac{\rho_2^2}{2R(z_1)} - \frac{\rho_1^2}{2R(z_2)}\right)^2 + \arctan\left(\frac{z_2}{z_{02}}\right) - \arctan\left(\frac{z_1}{z_{01}}\right) \right]\]
The overall interference profile can be approximated by the interference experienced within the center of the two reflected beams (67). Therefore, the derivation simplifies because the exponential terms become 1 (i.e., \( \rho_n^2 = 0 \)). The resulting equation is then:

\[
I_R = R_1 \left( \frac{w_0^2}{w(z_1)^2} \right) + R_2 T_1^2 \left( \frac{w_0^2}{w(z_2)^2} \right) + R_{12} T_1 \left( \frac{w_0^2}{w(z_1)w(z_2)} \right) 2\cos \left[ K(z_2-z_1)+\arctan \frac{z_2}{z_0}-\arctan \frac{z_1}{z_0} \right]
\]

Evaluation of the cosine function reveals that part of the interference is based upon the distance between the two reflecting surfaces \((z_2-z_1)\) and is defined as \(\Delta z\). Also, the interference is a function of the change in the phase of the light at both surfaces \((\arctan(z_2/z_0) - \arctan(z_1/z_0))\). Lastly, the arctangent functions are phase angles at either surface and the cosine term then becomes:

\[
2\cos \left[ K(z_2-z_1)+\arctan \frac{z_2}{z_0}-\arctan \frac{z_1}{z_0} \right] = 2\cos[K\Delta z+\Delta \Phi]
\]

Upon expansion of the cosine:

\[
\{2\cos(K\Delta z)\cos(\Delta \Phi) - 2\sin(K\Delta z)\sin(\Delta \Phi)\}
\]

Where:

\[
\arctan(z_2) = \Phi_2
\]

\[
\arctan(z_1) = \Phi_1
\]

\[
\Delta \Phi = \Phi_2 - \Phi_1
\]
The overall equation then becomes:

\[ I_R = R_1 \left( \frac{w_0^2}{w(z_1)^2} \right) + R'_2 T_1 \left( \frac{w_0^2}{w(z_2)^2} \right) + R_{12} T_1 \left( \frac{w_0^2}{w(z_1)w(z_2)} \right) \{ 2 \cos(K \Delta z) \cos(\Delta \Phi) - 2 \sin(K \Delta z) \sin(\Delta \Phi) \} \]

At this point in the derivation the formulation is simplified enough to understand how various parameters affect the change in interference during pump modulation. As shown above, part of the signal generated arises from the change in the distance between the two sample surfaces and the phase angle shift of the light caused by the different distance the light has traveled. Similarly, the radial spot size \( w(z_2) \) varies with distance. During thermal expansion, the second surface density change (within the polymer) occurs and causes a proportionate refractive index change. Therefore, the cross reflectance \( R_{12} \) and the reflectance at surface 2 \( R'_2 \) become temperature dependent.
Figure 9 represents the block diagram of the optical layout of the instrumentation used to perform the analysis. All components are mounted onto a Newport model XSN-46 optical bench with 1/4-20 mounting holes on 1 inch centers. This table is equipped with air spring vertical isolators to dampen vibrations during analysis. A Spectra-Physics model 2030 Argon Ion laser (Argon Ion) operating at 514.5 nm with an exit beam diameter of 1.58 mm serves as the pump beam. Polarization of the light is vertically 100:1. Laser power measurements are made with a power meter located in the laser head. The irradiance of the radiation is controlled by varying the current to the laser tube with a Spectra-Physics Exciter model 210 power supply. The emerging pump beam is modulated with a Stanford Research System mechanical chopper (model SR540, (C)) and steered toward a dichroic beam splitter (DBS, center wavelength of 550 nm) by a wedge prism (WP). This prism is used to attenuate the beam (the Argon Ion laser cannot operate at the low irradiance required for analysis). The geometry of the prism prevents a ghost image from the second prism face. At the dichroic beam splitter the light is reflected towards mirror 2 (M2).

The probe laser is an Ealing Electro-Optics model 21-5038 Helium Neon laser ((HeNe), 632.8 nm). It has a power output of 15 milliwatts and an exit beam diameter of 0.8 millimeter. The radiation is randomly polarized.
Figure 9. Instrumentation configuration used for Photothermal Interferometer
Utilizing a broad band Glan-Thompson polarizer (GTP), the horizontal polarization is removed from the probe beam and the vertical polarization is further enhanced with a sheet polarizer (SP). The sheet polarizer is also used to attenuate the beam by rotation of the sheet perpendicular to the optical axis. Mirror 1 (M1) reflects the probe beam towards the dichroic beam splitter, which transmits the light and combines both beams.

Mirror 2 directs the radiation to a beam splitter (BS) and samples a portion of the incident energies (~8%). A 100 mm focal length achromatic lens (L3) is used to focus the beams onto a reference photodiode (RP). The voltage output from the photodiode is directly monitored at a Techtronics model 2430A digital oscilloscope and is used to trigger the oscilloscope. Probe beam irradiance variations are evaluated during the dark cycles of modulation while the pump beam intensity changes are determined from the peak-to-peak voltage of the modulated square wave.

A broad band polarizing cube reflects the light from the beam splitter towards the sample (POC), reflects vertical polarization and transmits horizontal polarization). A quarter wave plate (QWP) is used to circularly polarize the two beams by shifting the electric field vector of the incident radiation 45°. Again, the quarter wave plate is broad band and therefore not specific to either wavelength. The light is then brought to a waist (focus) onto the sample with a 35 mm plano-convex objective lens (L1). Sample surfaces are mounted onto a single axis translation slide with a side drive micrometer to adjust the sample to lens distance parallel to the optical axis. A change in this dimension provides a mechanism to vary the incident spot size of the radiation at either surface and the imaging of the reflected light. The radiation transmitted through the sample is focused onto a photodiode (AP) with a 100 mm achromatic lens (L2). In practice, this aspect of the design is used solely for alignment of the beams (as discussed later).
The reflected light from the surfaces, on a parallel yet backward track, impinges on the objective lens (L1). Depending upon the surface to lens distance, three scenarios exist: 1) if the surface to lens distance is shorter than the focal length of the lens, the light after the lens will be diverging; 2) if the surface to lens distance is longer than the focal length of the lens, the light refracted through the lens will be converging; and 3) if the surface to lens distance is exactly at the focal length of the lens; the light transmitted through the lens will re-collimate to the original laser beam parameters (as if no optical element were in the path). It should be noted that this is a paraxial approximation of the events. However, the intuition of the approach leads to a realization that the absolute distance between reflected light and the objective lens can easily be determined by examination of the irradiance in the far field of the reflected power.

Light passing through the objective lens then re-encounters the quarter wave plate. Once again, the electric field vector of the radiation is shifted 45° and upon two passages through this element, the initial vertical polarization is changed to horizontally polarized light (i.e., 2*45°=90°). The reflected light will now be transmitted through the polarizing cube. Mirrors 3 and 4 (M3, M4) direct the light toward the signal photodiode (SP, United Detector Technology). A 120 mm achromatic lens (L4) is used to focus the reflected radiation onto the signal photodiode. An interference filter (IF), 632.8 nm with a half wave full maximum equaling 10 nm and a red colored glass filter are placed between the lens and photodiode in that order. These elements serve to reject the irradiance from the pump. Kinematic mounts are used for all mirrors, lenses, and the dichroic beam splitter (two axis adjustments).

The photo-voltage from the signal photodiode is variably amplified using a United Detector Technology single and double axis signal conditioner model 301-DIV. The output voltage from the amplifier is directed to a Techtronics digital oscilloscope in the AC coupled mode. The change in voltage versus time of the reference photodiode and the
signal photodiode are averaged over a variable number of screen acquisitions by the oscilloscope. Voltage traces are then saved at the oscilloscope and transported to a Packard Bell model PB8810 computer by way of an IEEE general purpose interface bus. A BASIC program has been written with menu option to perform this task. The binary data are then converted to an ASCII serial representation and saved to a floppy disk. Data manipulations can then be performed using various commercial software packages (Lotus 123 TR, Microsoft Excel TR, Mathematica TR & Mathcad TR) and custom programmed software.

Alignment of the system is critical due to the interferometric nature of the phenomenon. The incident light must strike the sample normal to its surface in order to assure that the back reflected beams from both sides are collinear. Any angular displacement of the radiation as it strikes the surface will cause the light to refract at an angle within the support. This angular displacement is compounded upon reflection from the second surface resulting in only partial overlap of the two beams. Initially, the instrument is set up by removing the sample and both lenses 1 and 2. An alignment photodiode (AP) with a large active surface (1 cm²) is placed on the optical bench and centered with the mounting hole. A 0.75 mm pinhole is mounted in a z axis coarse translational mounting post holder.

The radiation from the Helium Neon laser is iteratively adjusted for height (z axis) by moving this aperture between the A1 and A2 locations. Mirrors 1 and 2 are tilted in the Z dimension until no adjustment of either the photodiode or the aperture are necessary and a maximum voltage is observed on a digital volt meter (DVM) connected to the photodiode. Two apertures are then fixed in the A1 and A2 positions, centered at the mounting holes of the table, using a z axis translational mounting post holder. Positioning of the beam in the y axis is performed by tilting mirrors 2 and 5 left and right. When a
maximum voltage is observed at the digital voltmeter, the light has been aligned parallel to
the mounting holes.

The Argon Ion laser is then aligned with the Helium Neon laser. Again, it is
important that these two beams be coincident. The wedge prism and the dichroic beam
splitter are tilted for height and left or right adjustment with the kinematic mount. The
voltage at the DVM is monitored until a maximum signal is observed. The light from both
lasers is now collinear and normally incident (between POC and the sample).

The primary function of lens 2 is to serve as an alignment fixture for the objective
lens. Using only the light from the Helium Neon laser, the lens is returned to the system
and an additional aperture placed at the A3 location. It is positioned for height, left or
right adjustment, and skew. Two criteria are used to ensure that the lens is properly
placed. The first criterion is that the maximum amount of irradiance is focused through
the pinhole (A2). The second is that the reflection from the lens is back directed, centered
with aperture 3 (visual inspection). With these criteria met, it can be concluded that the
light is centered within the lens and the lens is not skewed in any direction. Tilt in the lens
would lead to off axis focusing (i.e., light focused within focal plane however, not on
optical axis).

Proper positioning of L2, as alluded to above, serves as a valuable alignment
fixture for the objective lens. Should L1 be positioned in a manner such that the laser
beam is not refracted through the center of the lens, then the beam emerging from L2 will
be eclipsed by aperture 2 and the maximum voltage at the DVM will not be observed.
Therefore, the lens is easily positioned. Again, to further insure proper placement, the
reflected light from the lens is visually observed at pinhole A1 to verify that no tilt exists
within the lens mounting.
With the excitation path aligned, the reflection path can be established (POC to SP). A Shott's glass NG-3 neutral density filter (O.D. of 2.5) is used for the sample. Due to the high optical density, little reflection from the second surface is observed (note, there is no interference pattern either). The light reflected from the sample is aligned such that the maximum radiation traverses back through pinhole 1 (A1). Apertures are established at the same height as the incident beam at position A4 and A5. Lens 4 (120 mm achromatic doublet) is removed from the system. Mirrors 3 and 4 are adjusted by the kinematic mount to provide the maximum voltage output at the signal photodiode. The lens is replaced and positioned in two dimensions (z and y) to focus the reflected light onto the active area of the photodiode.

In summary, the Glan-Thompson and sheet polarizer produce a probe beam that is vertically polarized with variable attenuation. Mirrors 1 and 2 are used to align the Helium Neon laser with the sample. The dichroic beam splitter combines the radiation from both lasers and, in conjunction with the wedge prism, steers the pump beam to be collinear with the probe. The polarizing cube and quarter wave plate combine to separate the incident from reflected optical paths. The objective lens brings the beams to a waist on the sample surfaces. The collection of the reflected light is controlled by adjusting the sample to lens distance with the translation slide. Mirrors 3 and 4 serve to align the reflected irradiance toward the signal photodiode. Lens 4 brings the light down to a focus on the photodiode with a spot size much smaller than the active area of the diode itself. A reference signal path has been constructed utilizing a beam splitter, a focusing lens, and photodiode. This aspect of the design provides a method to monitor variation in the intensity of the laser beams as well as a time alternating signal to trigger the oscilloscope. An alignment optical path exists after the sample using aperture 2, a focusing lens, and a photodiode.
With the system constructed and the beams aligned, real sample analysis can be performed. Procedurally, samples are placed onto a kinematic mount. With the pump beam blocked and aperture 1 and 2 in position (all others are now removed), samples are aligned such that they are normal to the beam. This is done by tilting the sample via the kinematic mount mechanism until a maximum signal is measured at the signal photodiode. Aperture 1 is removed from the system and the pump beam un-blocked for analysis. When samples are translated or interchanged, the pump beam is again blocked. Pinhole 1 is returned to the system and adjusted in the $z$ axis until the maximum signal is observed at the alignment photodiode. The tilt of the sample is checked to verify normal incident behavior and aperture 1 is again removed.
CHAPTER 5
SYSTEM EVALUATION

The following section describes the procedures used to evaluate the optical and instrumental characteristics of the above design. These experiments serve to verify that the components perform as intended. In addition, a physical understanding of the properties of the radiation will help to gain some insight about the nature of the observed phenomenon.

CALIBRATION OF INCIDENT PUMP RADIATION

As described in the instrumental design section of this document, the only means to determine the output power of the Argon Ion laser occurs at the laser head itself with a built in power meter. However, there are a number of optical elements in the system including a wedge prism (which only reflects a portion of the light) that suffer from reflective losses of the incident radiation. Therefore, a correlation of the power meter intensity measurement with the irradiance occurring at the sample must be measured with reasonable precision.
The correlation is performed by calibrating a given photodiode signal voltage power measured at the power meter. The photodiode is placed in the position normally used for the sample with lens 1 removed. It should be noted that the beam size from the laser at this distance is still smaller than the active area of the photodiode. Thus, all the irradiance is on the diode face. The incorporation of a mirror (Newport catalog number AL.2) and a right angle prism (Rolyne catalog number 40.0035) is necessary to both steer the beam towards the sample position and attenuate the beam by reflection from one of the surface faces of the prism. These elements are necessary because at the lowest safe operating power of the Argon Ion laser (50 milliwatts) the photodiode will be saturated.

With the optical components positioned, the current to the Argon Ion laser is varied to provide different output intensities (50, 80, 100, 130, 150, and 170 milliwatts). The photo-voltage from the diode is measured directly at the oscilloscope. A frequency generator connected to the oscilloscope is used as a trigger. The DC voltage for each output power is signal averaged approximately 50 times. The mean value is then recorded directly from the oscilloscope. The output irradiance is corrected for power loss at the mirror and the reflective intensity from the prism (calculations are discussed in the section on results). The voltage response versus corrected laser intensity is correlated using linear regression and a best fit line established.

Having a correlation of the output irradiance versus the photo-voltage from the diode, the mirror and prism are removed. Lens 1 is returned to the configuration and aligned. The light from the Argon Ion laser passing through the optical system can now be measured by the same procedure discussed above. The true irradiance incident at the sample is then determined using the regression analysis.
DETERMINATION OF FUNDAMENTAL FOCUSED LASER PARAMETERS

The propagation of a laser beam is unlike light emitted from any other source. Due to the high spatial coherence and extremely low divergence angle, unique properties of the radiation can be defined with extraordinary accuracy. In order to understand the nature of the photothermal interferometric phenomenon observed, two characteristics of the incident focused laser light, from both the Argon Ion and Helium Neon lasers, need to be established. The first property to be considered is the convergence and divergence angle of the beam after passage through the focusing lens. These angles define how the beam diameter varies along the optical axis as it converges to its minimum spot size (waist) and then diverges away.

The next characteristic to be determined is the actual focal point of the light. That is, where along the optical axis is the beam waist? Knowing how the spot sizes change, where the position of maximum focus lie, and the power (watts) incident at the sample (from the previous experiment); the irradiance (watts/meter^2) through the focused path can be mapped. With these parameters established, several physical attributes of the system are expressed. For example, knowing the spot size of the pump beam at the absorbing surface, the absorbance of the surface, and the illumination time during modulation; the amount of energy in joules that is released into the medium can be calculated. The relationship of the pump and probe beam spot size (aspect ratio) at different sample to lens distances can be evaluated and its effects on signal can be established. Lastly, the reflected probe beam spot sizes from both surfaces of the sample are established as well as how their irradiance causes the interference.

The variation in the diameter of the beam with respect to distance as well as the focal point of the waist can be determined by various calculations based upon the manufacturer's specification. However, these specifications are typically quoted as either
minimum or maximum values (e.g., the beam divergence angle is not more than some value). In addition, all the calculations are initiated on the basis of the original beam waist diameter. Normally, manufactures will cite the exit beam diameter at the output aperture of the laser head. This is not necessarily the true beam waist. The true beam waist will typically occur within the laser cavity itself. Calculations based upon the exit diameter will, therefore, exhibit some degree of error.

Because of the ambiguity involved in using the manufacturers' specifications to perform the optical calculations, it was decided to determine the above properties experimentally. Referring to the system configuration (Figure 9) a 0.75 mm pinhole is mounted onto the translational stage normally used for the sample. The pinhole is aligned in such a manner that the maximum signal can be observed at the alignment photodiode. The oscilloscope is used to perform the measurements and is triggered with a function generator. A reference signal is measured at the same time (at the reference photodiode) to compensate for intensity fluctuations. The pinhole is then translated (1 mm increments) to a new location along the path, re-aligned, and the measurements repeated. Each laser beam is done separately and calculations based upon the results are performed.

**PROBE BEAM REFLECTION PARAMETERS**

As the characteristics of the incident light were evaluated in the previous experiment, the nature of the reflected radiation is now considered. Some understanding of the signal origin and, therefore, the interferometric phenomenon can be determined by evaluating the power distribution from these reflections. From a geometric optical perspective three basic scenarios exist to describe the reflected irradiance. The first is if the distance between the lens and the reflecting surface is within the focal length of the
lens, then the lens will project a virtual image of the reflection. This arises due to the new waist position reflected back before the focal point of the lens and the light emerging from the lens will be completely diverging. The next scenario is if the distance between the two is exactly the focal length of the lens. Here, the lens will re-collimate the light back to the original beam size that was incident at the lens. The last situation is if the reflecting surface is beyond the focal point of the lens. In this case, the lens will cause the light to converge to a new focus at some distance back along the incident path.

The irradiance of the reflected probe beam is evaluated by measuring its intensity upon reflection from a neutral density filter at the signal photodiode. A NG-3 shot glass filter (OD equals 2.5) is used to minimize the interference caused by the second reflection. The filter is mounted on a single axis translational slide in the location normally reserved for the sample. A 1 millimeter aperture is placed 22.9 centimeters before the signal photodiode. The pinhole is mounted on a two axis translational assembly to allow minor orthogonal adjustments of the absolute location of the aperture about the optical axis.

With the experimental setup in place, the filter is moved through the focused path of the lens in 100 micrometer increments. At each filter location, minor adjustments are made at the pinhole to assure that the center of the beam is being sampled. Using a function generator at the external trigger of the oscilloscope, both the DC photo-voltage from the reference and signal photodiodes are averaged approximately 50 times.

**SIGNAL CONDITIONING: EVALUATION OF AC COUPLING**

It was mentioned in the instrument design section of this document that the standard protocol used to measure the signal response involved AC coupling at the oscilloscope. This was done in order to resolve small AC signal variations on a relatively
large DC background. Because the modulation frequencies used for these experiments are small (< 30 hertz) it was observed that the electronics (oscilloscope) distorted the waveforms generated during analysis. At these low frequencies the oscilloscope acted as a high pass filter, and convoluted the signal waveforms.

Convolution of a signal waveform corresponds to an integral relating the input and output of the system (70). Mathematically, it involves the multiplication of the input signal by a displaced impulse response function inherent to the system. Therefore, it can be expressed as

\[ S(t) = \int x(t) \xi(t-t) dt \]

Where \( S(t) \) is the output signal waveform, \( x(t) \) is the true signal waveform, \( \xi(t-t) \) is the convolution function, and \( t \) is the convolution time. In the case of a high pass filter, three equations can represent the output voltage. These are Kirchoff's Laws of current, voltage, and the equation governing a capacitor. Respectively they are expressed as follows:

\[ (5.1) \quad i(t) = \frac{V_o}{R} \]
\[ (5.2) \quad V_i = V_c(t) + V_o(t) - \text{Kirchoff's Voltage Law} \]
\[ i(t) = C \frac{dV}{dt} - \text{Equation governing a capacitor} \]
\[ (5.3) \quad V_c = V_{\text{in}} + \frac{1}{C} \int_0^t i(t) dt - \text{Equation for a capacitor in integral form} \]

Substitution of equations 5.1-5.3 leads to a general expression:

\[ V_1(t) = V_{\text{in}}(t=0) + \frac{1}{RC} \int_0^t V_o(t) dt + V_o(t) \]
Verification of the above expression can be shown if one considers a step function (70) which is known to exponentially decay (as if the voltage output is from an RC circuit). Assuming the initial voltage across the capacitor is zero, then the following expression is valid.

\[
V_i(t) = 0 + \frac{1}{RC} \left\{ \int_0^1 \exp(-t/RC) \, dt \right\} + \exp(-t/RC)
\]

Evaluation of the integral yields:

\[
V_i(t) = \frac{1}{RC}(RC)\exp(-t/RC) \bigg|_0^1 + \exp(-t/RC)
\]

And:

\[
V_i(t) = -\exp(-t/RC) \bigg|_0^1 + \exp(-t/RC)
\]

\[
= -\exp(-t/RC) + \exp(0) + \exp(-t/RC) = 0 + 1 = 1
\]

Therefore, in order for the output voltage to be decaying exponentially, the input must be a step of zero to one volts.

The above exercise is derived to demonstrate that the algorithm provides the means to de-convolute the AC waveforms acquired during analysis. This is empirically demonstrated by using a function generator to produce square waves at the oscilloscope at a frequency of approximately 10 hertz. At the oscilloscope, the AC coupled and DC signals are acquired. The AC coupled signal is then de-convoluted using the above mathematics in a simple numerical integration. The analysis is performed for four different peak-to-peak DC signal intensities.
SYSTEM NOISE CHARACTERISTICS

In order to address the sensitivity of the system, the noise inherent in the design must be evaluated. Here, two aspects of noise are considered. First, the magnitude and source of the electronic noise are determined. This is accomplished by blocking all the light incident upon the signal photodiode (i.e., dark noise) and measuring the AC signal fluctuation. A special envelope function built into the oscilloscope is used to acquire the data. This function allows multiple traces to be stored and the total peak-to-peak voltage variation displayed on the screen. A frequency generator operating at 10 hertz is used to trigger the oscilloscope and 20 oscilloscope traces are used to determine the voltage variation. The experiment is performed at four different gains on the signal amplifier.

During photothermal data acquisitions, the oscilloscope is operated in its averaging mode. This mode continually averages the traces and displays the overall average waveform onto the screen. As this is the normal operating condition, the above experiment is repeated with the exception that the averaging mode is used. Again, 20 traces are used to average the dark voltage and the peak-to-peak voltage variation is reported.

The next aspect of noise considered is the short term intensity fluctuation of the probe laser beam. For this analysis, the reflected probe light from a microscope slide is incident on the signal photodiode. The AC voltage fluctuation is measured in the same manner described above. Again, 20 traces are used for both the envelope and averaging mode on the oscilloscope.
CHAPTER 6
RESULTS OF THE SYSTEM EVALUATION

CALIBRATION OF INCIDENT PUMP RADIATION

The data used for the regression analysis are provided in Table 1. Initially, the reflected power loss due to the mirror is taken into account. From the manufacturer’s specifications for this mirror, the reflective intensity at an incidence angle of 45° and wavelength of 514.5 nm is approximately 91%.

<table>
<thead>
<tr>
<th>Intensity at Power Meter (milliwatts)</th>
<th>Corrected Intensity (milliwatts)</th>
<th>Signal millivolts CV&lt;1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>4.40</td>
<td>612.70</td>
</tr>
<tr>
<td>80</td>
<td>7.04</td>
<td>1005.14</td>
</tr>
<tr>
<td>100</td>
<td>8.80</td>
<td>1293.80</td>
</tr>
<tr>
<td>130</td>
<td>11.44</td>
<td>1696.63</td>
</tr>
<tr>
<td>150</td>
<td>13.21</td>
<td>1972.70</td>
</tr>
<tr>
<td>170</td>
<td>14.97</td>
<td>2245.26</td>
</tr>
</tbody>
</table>
Therefore, the intensity values observed at the power meter are multiplied by 0.91. The power reflected from the right angled prism is calculated based on Fresnel's Equation (66). The substrate used for the right angle prism is BK7 and has a refractive index ($\eta_2$) at 514 nm of 1.52049. The refractive index of air ($\eta_1$) at room temperature is 1.0003.

The light from the Argon Ion laser is vertically polarized and perpendicular to the optical axis of the prism. Given the refractive index of the prism, a $45^\circ$ incident angle of the light, and the vertical polarization of the laser beam, the equation needed to calculate the reflectivity is:

$$r_\perp = \frac{E_r}{E_0} = \frac{\eta_1 \cos \theta_1 - \eta_2 \cos \theta_2}{\eta_1 \cos \theta_1 + \eta_2 \cos \theta_2}$$

Where:

- $r_\perp$ - Reflectivity of Light Polarized Perpendicular to Optical Axis
- $E_r$ - Electric Field Amplitude of Reflected Radiation
- $E_0$ - Electric Field Amplitude of Incident Radiation
- $\eta_1$ - Refractive Index of Incident Medium (air)
- $\eta_2$ - Refractive Index of Reflected Medium
- $\theta_1$ - Incident Angle ($45^\circ$) in Radians
- $\theta_2$ - Refracted Angle in Radians

The refracted angle is determined from Snell's law with the following expression:

$$\eta_1 \sin \theta_1 = \eta_2 \sin \theta_2$$

$$\theta_2 = \sin^{-1} \left( \frac{\eta_1 \sin \theta_1}{\eta_2} \right)$$
Once the reflectivity is determined for the configuration, the reflectance is found by squaring the reflectivity. The irradiance values already corrected for power loss at the mirror are then multiplied by the reflectance giving the approximate irradiance measured at the photodiode (note: approximate because the scatter from air is not taken into account).

The regression analysis for the output irradiance of the Argon Ion laser was used to correct the voltage observed at the photodiode using a built-in algorithm provided with Microsoft Excel™. Figure 10 shows the resulting graph of the linear intensity profile. The algorithm used in the spreadsheet also outputs various parameters of the regression along with some statistical analysis and is given in table 2. The t-test value is calculated from a t-distribution at a 95% confidence level with 6 observations and 4 degrees of freedom and is multiplied by the standard error. The upper and lower confidence limit is the sum or subtraction of this value respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Calculated</th>
<th>Standard</th>
<th>t-test</th>
<th>Upper</th>
<th>Lower</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y Intercept</td>
<td>-74.429</td>
<td>8.005</td>
<td>22.222</td>
<td>-52.207</td>
<td>-96.651</td>
</tr>
<tr>
<td>Slope</td>
<td>154.908</td>
<td>0.754</td>
<td>2.093</td>
<td>157.001</td>
<td>152.815</td>
</tr>
</tbody>
</table>

The correlation coefficient ($R^2$) is 0.9998 suggesting that 99.98% of the variation in the dependent variable (signal measured) can be explained by the change in the independent variable (output power). The significance of the this value is tested using a t-test distribution for correlation coefficients (71 p., 425).
Regression Analysis of Output Intensity @ Sample

Figure 10. Regression analysis for measured irradiance
With four degrees of freedom and a confidence level of .1%, the correlation coefficient must be larger than .974. Therefore, it can be concluded (based upon $R^2$ and the t-test statistical value) that the regression is reliable.

With the system returned to its normal state and the photodiode placed in the sample position, the irradiance is again measured. Table 3 provides the data determined during the analysis and the power values calculated from the regression analysis.

**TABLE 3**

**INCIDENT POWER DETERMINED FROM REGRESSION**

<table>
<thead>
<tr>
<th>Intensity (milliwatts)</th>
<th>Signal at Power Meter (millivolts)</th>
<th>CV&lt;1% (milliwatts)</th>
<th>Intensity from Regression (milliwatts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>472.46</td>
<td>3.88</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>775.27</td>
<td>6.03</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>997.98</td>
<td>7.61</td>
<td></td>
</tr>
<tr>
<td>130</td>
<td>1309.87</td>
<td>9.82</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>1521.00</td>
<td>11.32</td>
<td></td>
</tr>
<tr>
<td>170</td>
<td>1730.66</td>
<td>12.81</td>
<td></td>
</tr>
</tbody>
</table>
DETERMINATION OF FUNDAMENTAL FOCUSED LASER PARAMETERS

The energy distribution for a TEM$_{00}$ laser beam (gaussian energy distribution) can be expressed by the following equation (64, p., 665):

$$I(r) = \frac{2P}{\pi w^2} \exp\left\{-\frac{2r^2}{w^2}\right\}$$

$I(r)$ is the irradiance as a function of the radial position, $P$ is the incident power, and $w$ is the spot size (radius) at 1/e$^2$ of the intensity. The power transmitted through a centered circular pinhole is derived as the following integral where "a" is the radius of the pinhole (64 p., 666):

$$\int_0^a I(r) = \int_0^a [(2\pi r)\left\{2P/\pi w^2\right\} \exp\left\{-2r^2/w^2\right\}] dr$$

The fractional power transmitted ($F_{P_t}$) due to the aperture is determined by evaluation of the integral:

$$\frac{\Delta P}{P} = \frac{2}{\pi w^2} \int_0^a [(2\pi r)\exp\left\{-2r^2/w^2\right\}] dr = 1-\exp\{-2a^2/w^2\} = F_{P_t}$$

By rearranging the above equation, the data obtained from the experiment can now be expressed in a form that calculates the 1/e$^2$ irradiance for each pinhole position. This equation is expressed as:

$$w = \sqrt{-2a^2/\ln(1-F_{P_t})}$$
A plot of \( w \) versus the distance (\( Z \)) shows the change in the \( 1/e^2 \) radius (\( w(Z) \)) of the propagating beam from a geometric optical representation. The variables \( w_i \) being the initial \( 1/e^2 \) radius and \( \theta/2 \) the half beam divergence angle. Therefore:

\[
w(Z) = w_i + \{\tan(\theta/2)\}Z
\]

Figures 11-a and 11-b shows the regression analysis of the spot sizes versus lens distance for Helium Neon and Argon Ion lasers respectively. From the regression output (table 4 and 5), the half beam divergence angle is calculated from the arc tangent of the slope. The Y-intercept gives the beam radius at the lens (\( w_i \)) and the X-intercept establishes where the beams come to a geometric focus.

**TABLE 4**

**REGRESSION PARAMETERS FOR HELIUM NEON LASER**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Calculated Value</th>
<th>Standard Error</th>
<th>t-test Value</th>
<th>Upper 95%</th>
<th>Lower 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y Intercept</td>
<td>-0.673</td>
<td>0.0133</td>
<td>0.0315</td>
<td>-0.704</td>
<td>-0.641</td>
</tr>
<tr>
<td>X-Intercept</td>
<td>31.384</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arctan slope</td>
<td>1.226°</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( R^2 = 0.99907 \)
TABLE 5
REGRESSION PARAMETERS FOR ARGON ION LASER

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Calculated Value</th>
<th>Standard Error</th>
<th>t-test Value</th>
<th>Upper 95%</th>
<th>Lower 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y Intercept</td>
<td>-1.159</td>
<td>0.0156</td>
<td>0.0315</td>
<td>-0.704</td>
<td>-0.641</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0343</td>
<td>0.000293</td>
<td>0.000693</td>
<td>0.0366</td>
<td>0.0350</td>
</tr>
<tr>
<td>R²</td>
<td>0.99942</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-Intercept</td>
<td>33.811</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arctan slope</td>
<td>1.964°</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Based on the above analysis for both lasers, the position of maximum focus and the divergence angle is determined. Calculation of the beam waist for the beams is performed using the following equation (66 p., 232):

\[ w_0 = \frac{2\lambda_0}{\pi\theta} \]

Where:

\[ w_0 \] - Beam waist

\[ \lambda_0 \] - Wavelength of light in vacou

\[ \theta \] - Full beam divergence angle (2*Arctan(slope))

From the values stated above, the minimum beam radius for the Helium Neon laser is 9.412 micrometers and for the Argon Ion laser is 4.775 micrometers.
Figure 11-a. Regression values for Helium Neon beam radius at various distances
Radial Spot Size versus Distance for Argon Ion Laser

Figure 11-b. Regression values for Argon Ion beam radius at various distances
Using the divergence angle, the beam waist diameter \((d_0 = 2w_0)\), and the focal point (determined from the geometric analysis), the true laser beam profile is calculated with the following equation (66 p., 232):

\[
d^2 = d_0^2 + \theta^2 z^2
\]

In Figures 12-a and 12-b, a plot of the geometric (solid lines) and gaussian (solid squares) beam propagation for both lasers is given within the vicinity of the waist. The geometric analysis of the beam agrees with the real gaussian propagation at distances far from the beam waist. This region is referred to as the far field in which the laser light can be treated by paraxial calculations. However, as the beam asymptotically approaches the maximum focus, the geometric optical calculations are no longer valid. This section of the beam is described as the collimation region in which the wave front of the beam is planer (i.e., infinity radius of curvature). An additional parameter, the confocal length \((Z_r)\), describes the distance prior to and after the waist in which the light can be treated as a collimated beam. From the data, this length is determined from the minimum beam diameter \((d_0)\) and the full beam divergence angle with the following expression (66 Chap., 7):

\[
Z_r = d_0 / \theta
\]

The values are 219.9 and 69.61 micrometers for the Helium Neon and Argon Ion respectively.
Geometric and Gaussian Beam Propagation in Vincinity of Waist for the Helium Neon Laser

Position from Lens (mm)

Figure 12-a. Raytrace for the Helium Neon laser beam propagation
Geometric and Gaussian Beam Propagation for the Argon Ion Laser

Figure 12-b. Raytrace for the Argon Ion laser beam propagation
PROBE BEAM REFLECTION

The data obtained from the experiment is corrected for fluctuation in the probe beam intensity by determining the average irradiance measured at the reference photodiode over the course of the experiment. The absolute value in the series is determined and all values obtained are adjusted by this number. Therefore, a relative change in signal is determined as the filter is translated to different locations. The data are then fit to a gaussian function utilizing the following formula:

\[ y = \exp \left( \frac{-2(x-u)^2}{C^2} \right) \]

Where:

- **y** - Response
- **x** - Distance from lens at each interval
- **u** - Distance of maximum signal (i.e., average for gaussian)
- **C** - An arbitrary constant

A BASIC program has been written to perform this fit in which \( u \) and \( C \) are iteratively varied. For each combination, the sum of the squares of the differences is calculated and the program continues until this value has been minimized. Figure 13-a provides the plot of both the experimental values as well as the gaussian fit. This figure shows that at the wings of the gaussian distribution the experimental values deviate from the fit. Two optical phenomena may explain this results. The first involves a deviation from the paraxial approximation as the reflected beam spot size becomes large with
respect to the clear aperture of the lens. This occurs at relatively long distances either before or beyond the focal point of the lens. In addition, as the beam size at the pinhole becomes bigger than the aperture diameter, diffraction will occur producing an Airy power distribution at the signal photodiode. It should be noted that in the fitting of the experimental values only the center 10 points were used.

Despite the lack of fit at the edges of the gaussian profile, several parameters relating to the reflecting beam can be determined. First, by taking the numerical derivative of the gaussian profile, the distance at which the maximum irradiance passing through the pinhole can be found. Figure 13-b shows the graph of the numerical derivative at different distances. A value of 30.83 millimeters was determined from this plot. This number falls within the confidence limit for the focal length calculated in the previous section.

Another optical parameter can be established from these results. Recall that the power distribution for a laser operating in the TEM\(_{00}\) mode can be expressed as:

\[
I(d) = I_0 \exp\left(-2d^2/d_0^2\right)
\]

Where:

- \(I(d)\) - Irradiance within a given diameter
- \(I_0\) - Total Irradiance of beam
- \(d\) - Diameter at any distance
- \(d_0\) - Minimum waist size

The ratio of the diameter of the beam at some distance to the minimum beam size is equal to the ratio of the position of maximum light throughput divided by the arbitrary constant (C).
Irradiance Through Pinhole at Different Distances

Figure 13-a. Plot of irradiance versus lens to sample distance
Figure 13-b. Numerical differentiation of Gaussian response
This quantity was determined during the iterative fit of the experimental values and found to be 0.61. Using the above equation, and setting $d=1$ millimeter (pinhole diameter) for a 100% throughput of light (i.e., $I(d)/I_0 = 1$), the following equation results:

$$30.83 \text{ mm}/0.61 = 1 \text{ mm}/d_0$$

The minimum beam diameter is determined to be 19.4 micrometers which is in close agreement to the 18.83 micrometers calculated in the previous experiment.

Finally, the most important aspect of these results is that a means has been established to determine the irradiance of either reflection at different sample to lens distances. Utilizing these results, accurate raytraces for the reflected probe light can be performed. The amount of overlap of the two reflections throughout the reflected optical path can be established and should help to elicit some of the fundamental principles involved in this interferometric phenomenon.

**SIGNAL CONDITIONING: EVALUATION OF AC COUPLING**

Figure 14 is an example of the DC versus AC signal acquired at the oscilloscope from a function generator. This plot shows the electronic convolution of the DC square wave and reveals two aspects of the AC waveform. First, the maximum and minimum voltages obtained in the DC square wave are overestimated (electronic over-shoot and under-shoot respectively) in the AC coupled mode. The second aspect is the exponential decay (as the RC circuit discharges) of the signal after a maximum or minimum occurs in the DC signal. Recall that a step function was used to prove that the numerical method derived to de-convolute the AC signal response would perform as required.
Figure 14. AC and DC signal response from function generator
Figure 15 is provided as an example of the output from the numerical de-convolution of the AC waveform and compared to the original DC square wave. From this graph it can be concluded that the numerical algorithm has successfully removed the exponential decay, overshoot, and undershoot. The correlation coefficient (goodness of fit) for this data and the other three DC power levels used during analysis are provided in Table 6.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>DC Amplitude (volts)</th>
<th>$\Sigma(d^2)$</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.550</td>
<td>1.566</td>
<td>0.99893</td>
</tr>
<tr>
<td>2</td>
<td>1.403</td>
<td>1.024</td>
<td>0.99756</td>
</tr>
<tr>
<td>3</td>
<td>0.6016</td>
<td>0.2400</td>
<td>0.99696</td>
</tr>
<tr>
<td>4</td>
<td>0.3654</td>
<td>0.02175</td>
<td>0.99929</td>
</tr>
</tbody>
</table>

The coefficient is calculated by first determining an estimate of the variance between the DC square wave ($y'$) and the de-convoluted square wave ($y$) at each point in the time series ($N$). This is done by determining the difference at each time point ($d$) and using the following expression (72 p., 102):

$$ S_y^2 = \frac{\sum [(y-y')^2]}{N} = \frac{\sum (d^2)}{N} $$
Comparison of De-Convoluted Wave to Original

Figure 15. Results from the de-convolution of the AC signal waveform compared with the original DC square wave
This variance over the time interval for the de-convoluted data is calculated by:

\[ \sigma_y^2 = \frac{1}{N} \left( \frac{\sum y}{N} \right)^2 \]

Having estimates of the variances, the coefficient of correlation \((r)\) is determined by:

\[ r = \sqrt{1 - \frac{\sum y^2}{\sigma_y^2}} \]

The above is a general method of determining the goodness of fit for any type of regression (72 p., 89). Here, the original DC waveform is used for the regression estimate (i.e., the true response value).

SYSTEM NOISE CHARACTERISTICS

Measurements of the dark voltage establish not only the electronic noise in the system but also the absolute limit to both sensitivity and precision. Table 7 (S.D. = standard deviation and CV = coefficient of variance) contains the results of these values for both the raw and signal averaged data. The raw data values represent the fundamental noise of the system. This type of noise is generally associated with random motion of electrons in resistive elements caused by thermal agitation (70, p. 410). The root mean square (rms) deviation can be determined based upon the assumption that the voltage variation is random and normally distributed. Therefore the peak-to-peak voltage measured represents the six standard deviations within a normal curve. Since there are a
large number of data points involved in this measurement (20 oscilloscope traces, 512 data points per scan, and 5 replicates), the rms deviation is equal to the standard deviation of the peak-to-peak voltage (i.e., peak-to-peak voltage divided by 6, approximately 1.4 millivolts).

TABLE 7

DARK VOLTAGE

<table>
<thead>
<tr>
<th></th>
<th>Raw Data in millivolts</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 M</td>
<td>300 K</td>
<td>100 K</td>
<td>30 K</td>
</tr>
<tr>
<td>Average</td>
<td>8.19</td>
<td>8.205</td>
<td>8.08</td>
<td>8.92</td>
</tr>
<tr>
<td>S. D.</td>
<td>2.041</td>
<td>1.426</td>
<td>1.636</td>
<td>2.153</td>
</tr>
<tr>
<td>C.V.</td>
<td>24.91%</td>
<td>17.31%</td>
<td>20.24%</td>
<td>24.14%</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Signal Averaged Data in millivolts</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>0.36</td>
<td>0.4</td>
<td>0.4</td>
<td>0.36</td>
</tr>
<tr>
<td>S. D.</td>
<td>0.0358</td>
<td>0.0506</td>
<td>0.0506</td>
<td>0.0358</td>
</tr>
<tr>
<td>C.V.</td>
<td>9.94%</td>
<td>12.65%</td>
<td>12.65%</td>
<td>9.94%</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Another observation that can be made directly from the raw data results is that the rms noise does not vary when the gain on the amplifier is increased. This should not be the case assuming that the photodiode or the amplifier itself was the main source of noise. For example, if the major source of electronic noise was from the photodiode, then increasing the gain would increase the noise. It appears that either the connection to the
oscilloscope from the amplifier or the oscilloscope itself is the chief source of electronic noise.

Signal averaging provides a 22 fold improvement in the rms noise. The precision appears to improve by a factor of 2-3. However, this is a little deceiving and represents another source of noise in the system. The minimum full screen voltage level of the oscilloscope is 16 millivolts (2 millivolts per division and used for this analysis) and represents 200 digitized points. Each point is then a quantified unit of 0.08 millivolts. As noted in the above chart, the standard deviation for the average voltage variation falls below this level. Therefore, there is a finite resolution during the analog to digital conversion. This is known as quantizing noise and establishes the absolute minimum AC signal deviation that can be resolved during signal averaging.

With the electronic noise aspects determined for the system, the non fundamental (excess) noise is evaluated. The source of this noise is the short term drift of the probe beam. As discussed later, long term drift of either laser beam can be compensated. Table 8 provides the results of measuring the reflected irradiance at the photodiode. Again, the raw signal as well as the voltage obtained during signal averaging are provided.

<table>
<thead>
<tr>
<th>TABLE 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHORT TERM DRIFT OF PROBE BEAM</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Raw Values (mv)</th>
<th>Signal Averaged (mv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>142</td>
<td>12.667</td>
</tr>
<tr>
<td>S.D.</td>
<td>2.450</td>
<td>2.160</td>
</tr>
<tr>
<td>C.V.</td>
<td>1.725%</td>
<td>17.051</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>
The volts per division setting used for the above data was 50 millivolts which gives a full scale voltage of 400 millivolts with each digitized unit being 2 millivolts. Signal averaging again improves the precision of the system.
CHAPTER 7
ANALYSIS OF THE INTERFEROMETRIC PHENOMENON

Optimization of the instrument parameters employed during analysis required the use of various materials that would serve as the sample. The original strategy attempted was modification of a glass support on a single side that allowed for covalent coupling of chromophores to that surface. Although signal could be measured from this system, it was short-lived and not very reproducible. The thermal energy required to perturb the structure of glass (a robust material with a relatively low thermal expansion coefficient) apparently led to the occurrence of thermal reactions which bleached the dye. However, several useful conclusions could be made from this observation.

The first conclusion was the requirement that the material used to sense temperature changes needed to possess a relatively high thermal expansion coefficient. This would allow lower excitation intensities and minimize undesirable thermal and photochemical reactions. The sample also needed to be mechanically rigid so that no deformation of the first surface would convolute signal variations. Here, the first reflection could serve as a reference beam and the signal measured during modulation would represent how structural changes at the absorbing surface affected this reflection. It was concluded that a two layered sample design would be ideal. Glass was chosen as one of the layers because of its mechanical stability and its low thermal expansion coefficient. Polymers were selected for the next layer due to their thermal properties and the ability to select various plastics with refractive indices close to that of glass.
The most important conclusion drawn from the early experiments was the need for a chemically stable standard. This standard would need to be light fast so that extended use would not deteriorate its absorbance. It would have to be easy to construct in order to minimize variations that occurred during fabrication and, therefore, improve reproducibility. Various procedures were attempted to produce this standard. The majority of these methods involved procedures to bond dye doped polymers onto glass slides. The most successful and the simplest method was bonding colored films (commercially available) onto glass using optical adhesives.

STANDARD FABRICATION

A UV curable optical adhesive (Norland Optical, catalog number NOA-68) is used to chemically attach red transparencies (Apollo, catalog number PP100RB-20) to borosilicate glass microscope slides (1 mm thick, refractive index -1.528). The thickness of the plastic film is approximately 130 micrometers with a refractive index between 1.48 and 1.52. The absorbance spectrum for the film is provided in Figure 16. The adhesive is a thioester cross-linked siloxane that can covalently bind to silanols on the glass surface. The material is formulated to wet-out to an average thickness of 5 micrometers and to withstand thermal shocks (i.e., no loss of adhesion) up to 60° C at a plastic interface and 90° C at a glass interface. It has a refractive index of 1.54 and transmits >99% within the visible range at normal incidence.

Procedurally, the glass slides are first washed by immersing them in boiling 0.05 molar nitric acid for 45 minutes. The slides are rinsed with de-ionized water and placed into a 150° C oven overnight (~12 hours). The slides are stored in a desiccator.
Absorbance Spectrum of Polymer Film

Figure 16. Absorbance spectrum polymer film
The film is cut to the appropriate size, soaked overnight in spectroscopic grade methanol, and placed into a desiccator to allow the excess alcohol to evaporate off. Lamination of the film to the glass continues by dispensing an appropriate amount of the NOA-68 adhesive on one side of the film. The glass slide is placed onto the film and care is taken to remove any air bubbles that might have formed. The entire assembly is illuminated with a 5 watt fluorescent black light through the glass side for one hour. The lamination is fully cured by placing the assembly into a 60° C oven for two to three days.

PUMP DUTY CYCLE

Intuitively, the thermal characteristics of the polymer film are most affected by the modulation duty cycle. During the illumination segment of time, energy is deposited into the system and thermal expansion of the film is expected to dominate. When the pump light is eclipsed by the chopper, only thermal diffusivity contributes to signal. The amount of time allowed for the energy to dissipate determines the thermal state of the system immediately prior to the next illumination cycle. Therefore, subsequent waveforms are a contribution of the absorbed energy and the amount of heat still within the system from the prior excitation pulse.

To test this expectation, an experiment was performed in which the duty cycle was varied with the illumination time held constant. The standard is placed into the instrument in such a manner that the glass interface is the first through which the light passes. To simplify the analysis, the polymer is adjacent to the ambient air. As a result the refractive index and temperature of both sides of the sample are the same.
The instrument was used in its normal operating mode (discussed in section 4). The Argon Ion laser was modulated at 30 Hz with an irradiance (measured at the laser head) of 0.1 watts. The photo-voltage measured at the signal photodiode is amplified with a gain of 300K at the signal conditioner. The sample is aligned and translated axially until the maximum signal is obtained. Iteratively individual open slits of the chopper are masked to produce duty cycles of 50, 25, 16.67, and 8.33 percent. For each duty cycle, the waveforms are averaged approximately 25 times and the traces captured by the computer. The experiment is repeated four additional times with no adjustment of sample.

PUMP INTENSITY & ILLUMINATION TIME

It has been demonstrated by other photothermal techniques that signal to noise enhancement (to certain practical limits) can be achieved by increasing the power of the pump beam. The additional pump light absorbed by the sample leads to more energy deposited within the sample matrix and a corresponding increase in thermal expansion. The increased thermal expansion increases the probe beam displacement from its ambient position. Assuming that the primary source of noise in these systems is the intensity fluctuations of the probe laser (a random AC signal), and that the elevation of the pump intensity has little effect on this noise, the overall signal to noise will improve. By signal averaging over a number of waveforms, the random AC noise in the probe beam can be reduced. Thermal expansion is not only dependent upon the power of the pump laser but also the time integral of illumination. The amount of energy released into the system depends upon the duration of time in which the sample is illuminated.

The contribution of pump intensity and illumination time was investigated for this technique. Again, the standard was used for analysis and positioned in such a manner to
obtain the maximum peak-to-peak wave form. An 8.33% duty cycle was employed to allow complete thermal equilibration to ambient temperature. Pump power was set by adjusting the current to the laser tube. Values of 50 to 150 milliwatts were used and measured with the power meter mounted in the laser head. Variations in illumination time were obtained by changing the modulation frequency at the chopper. Times ranging from 12 to 30 milliseconds were used.

**PROBE BEAM INTENSITY**

It has been observed that the intensity of the probe beam affects the peak-to-peak signal response obtained during sample analysis. Precision of repeat measurements is improved by adjusting the signal for variations in the probe beam intensity. During the previous experiments it was assumed that a linear relationship existed between the amplitude of the AC waveform and the intensity of the probe laser. This assumption is tested by varying the power of the probe beam and observing the effect on the peak-to-peak signal. A sheet polarizer (see Section 4) is used to vary the power incident upon the sample. Initially, the polarizer is aligned with the vertical polarization of the Helium Neon laser in order to obtain the maximum power. Rotation of the polarizer about the optical axis blocks the light in a continuously varying manner. At 90° the light is completely blocked. The standard is used as the sample and aligned to obtain the maximum signal. All peak-to-peak values are adjusted for pump intensity by dividing the signal and reference. The values are then plotted against the photo-voltage for the probe beam determined during the dark cycles. An 8.33% modulation duty cycle is used for this analysis with the pump laser operating at 50 milliwatts measured at the power meter. Signal waveforms are averaged approximately 25 times.
STABILITY OF THE STANDARD

With the operating parameters established, the stability of the standard can now be verified. The instrument settings are the same as described above. A special data acquisition program was written to obtain both the signal and reference oscilloscope traces on five minute intervals over the course of one hour. To avert skewing of the results, the waveform data were not averaged by the oscilloscope during this analysis. The pump intensity was set at 50 milliwatts and the 8.33% duty cycle was used.

SIGNAL REPRODUCIBILITY

The precision of the signal obtained from this approach is considered in this section. Three aspects of reproducibility are determined using the standard as the sample. The first aspect is the precision of the signal while the sample is held fixed in position. Over the course of an eight hour period, measurements are made at one hour intervals. For this experiment, the signal waveforms are averaged approximately 25 times at the oscilloscope. The pump light is blocked during the intervening time. The peak-to-peak voltage for each waveform is calculated. The percentage difference between the average value and the intensity measured for the individual scans are determined. The AC signal from the sample is then adjusted by these percentages and the values reported reflect the actual peak-to-peak voltage response.

The next aspect is the signal repeatability over the cross section of the sample surface. For this analysis, the sample is moved to different locations perpendicular to the optical axis and adjusted to obtain the maximum signal. Compensations for variations in
the pump and probe intensities are made as described above. The overall average signal amplitude is determined and statistically compared.

Finally, the precision of a single scan is determined. These values are reported as part of the above two experiments. For all these analyses of precision, the pump power is set to 50 milliwatts to assure that the response is in the linear region. The duty cycle used is 8.33% with a 12 millisecond illumination time employed.

**AFFECT OF THE LENS TO SAMPLE DISTANCE**

As discussed in the historical section of this document, the amplitude and sign of the peak-to-peak response during photothermal analysis is dependent upon the distance between the sample and objective lens. This occurrence arises because the sample under periodic heating acts as an optical element. The combination of the two elements changes the nature of the observed waveform and establishes a basis in which the photothermal phenomenon can be expressed in an optical manner. Therefore, the change in the signal response upon varying the position of the sample is an important parameter for investigation.

The signal change that occurs when the sample is placed at different distances from the objective lens is considered in this section. The experiment is performed by mounting the standard on a single axis translational slide equipped with a venier scale. The maximum motion of the mount is 2.5 centimeters parallel to the incident axis. Waveform data are collected after signal averaging at distance intervals of 0.5 millimeters. System parameters used during the analysis are those already described above (i.e., 50 milliwatt pump intensity, 8.33% duty cycle, 12 millisecond illumination time, ect.). The experiment was done five times.
Two aspects of the system must be defined before the experiment can be performed. First, the actual distances between the polymer side of the sample, lens, and the vernier scale setting are calibrated. This is done by using different reference points on the respective mounts and measuring the true distance with a caliper at different translation locations. The other aspect that needs to be evaluated is the optical free space of the reflection path. This is done by observing only the probe beam irradiance at different translational distances with a field stop. Therefore, it can be determined at what distances no element in the path acts as a limiting aperture.
CHAPTER 8
RESULTS AND DISCUSSION

VARIATION OF PUMP DUTY CYCLE

Waveform data obtained from the standard at different duty cycles and pump powers are presented in Figures 17-a through 17-d. The solid squares represents the pump reference and the solid line is the signal. Within each scan, five pump cycles occur. Therefore, five individual wavelengths exist within each scan. Data analysis of the waveforms consists of determining the average peak-to-peak (P/P, first 5 complete wavelengths) AC signal for each oscilloscope trace. The average for each scan is then determined over the five repetitions. Table 9 contains the values obtained.

<table>
<thead>
<tr>
<th>Duty Cycle</th>
<th>Average P/P</th>
<th>Standard Deviation</th>
<th>Coefficient of Variance</th>
<th>95% Confidence</th>
<th>Number of Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>1.669</td>
<td>0.0616</td>
<td>3.69%</td>
<td>+/- 0.171</td>
<td>5</td>
</tr>
<tr>
<td>25%</td>
<td>1.446</td>
<td>0.114</td>
<td>7.86%</td>
<td>+/- 0.316</td>
<td>5</td>
</tr>
<tr>
<td>16.67%</td>
<td>1.513</td>
<td>0.115</td>
<td>7.61%</td>
<td>+/- 0.319</td>
<td>5</td>
</tr>
<tr>
<td>8.33%</td>
<td>1.559</td>
<td>0.0473</td>
<td>3.03%</td>
<td>+/- 0.131</td>
<td>5</td>
</tr>
</tbody>
</table>
Figure 17-a. Average waveforms at a 50% duty cycle
Figure 17-b. Average waveforms at a 25% duty cycle
Waveform Raw Data 16.67% Duty Cycle

Figure 17-c. Average waveforms at a 16.67% duty cycle
Figure 17-d. Average waveforms at a 8.33% duty cycle
The standard deviation and coefficient of variance are determined. A 95% confidence interval is calculated from a student t-test for 4 degrees of freedom. From previous observations (verified later), it has been noted that the precision of the measurements is improved when the AC signal is corrected for the pump and probe intensity. Therefore, the above values are divided by the peak-to-peak response of the pump beam (pump intensity) and the average minimum value of the reference waveform (probe intensity). Table 10 contains the new values.

**TABLE 10**

**STATISTICAL ANALYSIS OF THE ADJUSTED WAVEFORM DATA**

<table>
<thead>
<tr>
<th>Duty Cycle</th>
<th>Average P/P</th>
<th>Standard Deviation</th>
<th>Coefficient of Variance</th>
<th>95% Confidence</th>
<th>Number of Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>186.535</td>
<td>4.235</td>
<td>2.27%</td>
<td>+/- 11.757</td>
<td>5</td>
</tr>
<tr>
<td>25%</td>
<td>181.137</td>
<td>2.071</td>
<td>1.14%</td>
<td>+/- 5.750</td>
<td>5</td>
</tr>
<tr>
<td>16.67%</td>
<td>181.526</td>
<td>1.097</td>
<td>0.600%</td>
<td>+/- 3.045</td>
<td>5</td>
</tr>
<tr>
<td>8.33%</td>
<td>174.580</td>
<td>4.291</td>
<td>2.46%</td>
<td>+/- 11.912</td>
<td>5</td>
</tr>
</tbody>
</table>

An ANOVA (analysis of variance) significance test is performed to determine whether the effect of duty cycle changes the peak-to-peak response. The principle of this statistical technique is to provide a method of testing the difference between means of several populations (72, p. 163). The null hypothesis states that the mean values calculated from several groups of data represent the population mean. The test is performed by comparing the estimated population variance with the variance determined for each group. These variances are compared through an F test. "F" for the test is
determined by the ratio of the variance within each data group and the variance among the
groups such that:

\[
F = \frac{\sigma^2_{\text{among}}}{\sigma^2_{\text{within}}}
\]

with:

\[
\sigma^2_{\text{among}} = \frac{1}{(c-1)} \sum_{i=1}^{c} \left( n_i (X_i - \bar{X})^2 \right)
\]

\[
\sigma^2_{\text{within}} = \frac{1}{\sum (n_i - 1)} \sum_{i=1}^{c} \sum_{j=1}^{n_i} (X_{ij} - X_i)^2
\]

Where:

\(\bar{X}\) = Overall Average for All Duty Cycles
\(n\) = Total Number of Observations
\(n_i\) = Number of Observations for Each Duty Cycle
\(c\) = Number of Different Duty Cycles
\(X_i\) = P/P Voltage for Each Scan
\(\bar{X}_i\) = Average P/P for each Duty Cycle
The value determined for the variance among the data is 120.222 and the value within the data is 13.078. An F value of 9.193 is calculated from the ratio of these numbers. The F distribution table gives a value of 6.59 (73, p., 968) at the 0.05 level with three degrees of freedom for the large variance and four degrees of freedom for the small variance. As the calculated F value is larger than that provided in the table, it can be concluded that there is a 95% certainty that the null hypothesis can be rejected. Therefore, there is a statistically significant difference between the mean values of each duty cycle.

Qualitative analysis of the profiles of the different duty cycle waveforms provides some insight into the physical nature of the photothermal response. For the 50% duty cycle trace, the signal waveform is phase shifted with respect to the pump modulation. Although the AC signal ascends towards positive values when the radiation is first absorbed by the sample, several milliseconds elapse before the signal begins to descend when the light is blocked. Additionally, even though the peak-to-peak signal response for this duty cycle is similar to the other duty cycles, it is displaced toward negative values (i.e., average value of waveform is a negative voltage).

By simply reducing the duty cycle by a factor of two, the interferometric nature of the phenomenon becomes apparent. Here, the signal waveforms are in phase in that the inflection points for both waveforms occur at the same time. This is further resolved when an 8.33% duty cycle is employed. Qualitatively, the optical path length difference of the standard changes in such a manner that the two reflected beams constructively interfere (i.e., irradiance is increasing) as the pump illumination is incident on the sample. When the light is eclipsed, the irradiance and the optical path length difference re-equilibrate to their initial position. A sufficient dark cycle now exists for the signal to plateau to the AC offset voltage before the next pump irradiation.
The undershoot observed in the 8.33% duty cycle waveform is attributed to the electronic convolution from the AC coupled mode of the oscilloscope as described in the instrumentation section. The same algorithm employed to de-convolute and regenerate a DC signal from the AC response of a square wave was used on this oscilloscope trace. Figure 18 shows the outcome of this transformation. The plot confirms that the undershoot is an artifact of the electronics and that the signal asymptotically approaches the initial value. The time required for the signal to return to the initial baseline provides information (based upon other parameters) about the thermal diffusivity of the material.

Overall, the combination of electronic effects and a steady state temperature that is different from the ambient temperature convolutes the photothermal interferometric signal response at relatively high duty cycles. Although some signal loss occurs at the lower duty cycles, the waveform data become simplified and better represent the physical changes occurring within the absorbing film. Analytically, by keeping the system as simple as possible, superior performance should result. Finally, with the waveform data expressing the thermal characteristics of the material, other applications may be possible.

**PUMP INTENSITY & ILLUMINATION TIME**

Various conclusions can be drawn from this experiment. Before any detailed analysis several data manipulations are performed on the waveforms. First, for each of the individual scans, the traces are averaged by the oscilloscope approximately 35 times. The scans are then de-convoluted from the electronic effect of AC coupling (as described earlier). Compensation for variations in the probe beam intensity is performed by determining the average photo-voltage of the probe beam (taken during the dark cycle of modulation) and dividing each point in the waveform by this value.
De-convolution of Photothermal Waveform

Figure 18. De-convolution of photothermal waveform for 8.33% duty cycle
All the individual wavelengths (five pump cycles) in the scans are averaged to provide a single AC response. Finally, the five replicates for each data set are averaged to give a single representative waveform to characterize the various power and illumination time combinations.

The average single AC responses for the different illumination times are illustrated in Figures 19-a through 19-d. The graphs are constructed in such a manner that the different pump powers are plotted successively. The reference signals are arbitrarily scaled relative to the response waveforms within the plots.

These figures demonstrate that the profiles of the waveforms change as power and/or illumination time are varied. Increasing the incident power or illuminating the sample for longer intervals changes the direction of the signal deviation from the baseline during the pump pulse. This indicates that, at first, the irradiance of the probe beam is decreasing to a point and then begins to increase again. Presumably, this is a fringe shift; an interferometric effect. The distance between the two surfaces has been displaced to a position of maximum destructive interference and then toward an optical path length distance of constructive interference.

Another observation that can be made from these data is that fringes appear earlier in the progression of pump powers when the illumination time is increased. To further demonstrate this phenomenon, Figure 20 is provided in which the maximum negative signal response at each illumination time is plotted for the different powers. Table 11 presents the data and the statistical analysis used to produce this graph. The 95% confidence interval presented in the chart is determined by taking the standard deviation of the five replicates and multiplying this value by the number given for four degrees of freedom in a t-distribution (student t-test).
Figure 19-a. Average waveform data plotted in order of pump power at a 12 millisecond illumination time
Figure 19-b. Average waveform data plotted in order of pump power at a 16 millisecond illumination time.
Figure 19-c. Average waveform data plotted in order of pump power at a 20 millisecond illumination time
Figure 19-d. Average waveform data plotted in order of pump power at a 30 millisecond illumination time
Figure 20 shows that the AC response from the film is fairly linear at the lower power levels and plateaus at higher incident energies. The fact that the plateau region diminishes in the signal intensity as illumination time is increased may be an artifact of the electronics. Either the acquisition speed of the oscilloscope or the response time of the signal photodiode might produce this effect.

### Table 11

**Signal Response: Different Power Levels and Illumination Times**

<table>
<thead>
<tr>
<th>Power (in milliwatts)</th>
<th>AC Signal Adjusted for HeNe Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 ms</td>
</tr>
<tr>
<td>0.05</td>
<td>10.21+/-.298</td>
</tr>
<tr>
<td>0.06</td>
<td>13.33+/-.393</td>
</tr>
<tr>
<td>0.07</td>
<td>16.44+/-.458</td>
</tr>
<tr>
<td>0.08</td>
<td>18.33+/-.537</td>
</tr>
<tr>
<td>0.09</td>
<td>20.34+/-.310</td>
</tr>
<tr>
<td>0.100</td>
<td>20.84+/-.919</td>
</tr>
<tr>
<td>0.110</td>
<td>20.80+/-.143</td>
</tr>
<tr>
<td>0.130</td>
<td>21.20+/-.829</td>
</tr>
<tr>
<td>0.150</td>
<td>20.48+/-.295</td>
</tr>
</tbody>
</table>
Absolute Maximum Deviation from Baseline at Different Incident Powers

Figure 20. Photothermal response at different power and illumination times
PROBE BEAM INTENSITY

The effect of the probe beam intensity on the photothermal signal can be seen in Figure 21. The experimental values plotted are adjusted for pump intensity variations by taking the average value of the peak-to-peak reference signal for all acquired scans. For each scan, the percentage difference from the average is determined and the AC signal from the polymer film is corrected by this percentage. An overall average AC signal response is determined for the five replicates at each probe power. The 95% confidence interval is determined from a t-distribution. Table 12 provides the values used for the graph.

A regression analysis on the above data was performed to determine if a correlation exists between the probe intensity and the AC signal. The values calculated from the analysis are presented in Table 13. Using these values, a t significance test was performed on the regression output to determine the confidence that the slope accurately predicts the association between the variables (74). The null hypothesis is stated such that the slope from the regression is zero \((m_0)\) and no correlation exists. The alternative hypothesis states that the slope is not zero and is determined from the regression \((m)\). A critical region (CR) is found with the following expression:

\[
CR = (m-m_0)/s_b
\]

and

\[
s_b = \frac{s_{yx}}{\sqrt{\sum(x^2)-\frac{(\sum x)^2}{n}}}
\]
AC Response versus Probe Intensity

Figure 21. AC signal response at different probe beam intensities
Where \( n \) is the number of observation pairs, \( s_b \) is an estimate of the sampling error, and \( s_{yx} \) is the standard error of the slope. Performing the above calculation, a value of 4.923 is determined. From a t-table, a value at the 95% confidence limit is given as 2.776.

### TABLE 12

**SIGNAL VERSUS PROBE BEAM INTENSITY**

<table>
<thead>
<tr>
<th>Probe Intensity in volts</th>
<th>Average Response in volts</th>
<th>Standard Deviation</th>
<th>95% Confidence Interval</th>
<th>Number of Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0215</td>
<td>0.2465</td>
<td>0.00982</td>
<td>+/- 0.02726</td>
<td>5</td>
</tr>
<tr>
<td>0.0432</td>
<td>0.4550</td>
<td>0.00956</td>
<td>+/- 0.02654</td>
<td>5</td>
</tr>
<tr>
<td>0.0618</td>
<td>0.6643</td>
<td>0.00698</td>
<td>+/- 0.01938</td>
<td>5</td>
</tr>
<tr>
<td>0.0831</td>
<td>0.8601</td>
<td>0.01472</td>
<td>+/- 0.04081</td>
<td>5</td>
</tr>
<tr>
<td>0.1042</td>
<td>1.1106</td>
<td>0.00851</td>
<td>+/- 0.02362</td>
<td>5</td>
</tr>
<tr>
<td>0.1259</td>
<td>1.2987</td>
<td>0.01340</td>
<td>+/- 0.03720</td>
<td>5</td>
</tr>
</tbody>
</table>

As the calculated value is larger, the null hypothesis can be rejected thus indicating that a meaningful regression relationship exists between probe intensity and signal output. The linearity of signal versus probe irradiance can be explained by considering two aspects of the system. First, the interferometric nature of the response, suggests that the signal change during modulation is a percentage of the reflected irradiance from surfaces. Second, the signal is measured by stripping the DC from the experimental waveform.
Using the AC coupling mode, the signal is easily resolved even though the DC level increases with increasing probe power. The limit, in the resolution, is dependent on the electronics.

**STABILITY OF THE STANDARD**

With the operating parameters established, the stability of the sample was verified to assure that the results obtained above were not artifacts of sample deterioration. The maximum deviation of the signal was adjusted for intensity variations of both the pump and probe beams. This was accomplished by taking the average voltage values for both beams and by determining the percentage difference at each acquisition interval. The photothermal signal change from the baseline was evaluated and adjusted by the overall percentage change. Table 14 contains the values determined from this analysis.

A linear regression was performed on the time series data above with a correlation coefficient calculated to be 0.002557. The time between pump pulse for the above experiment was 144 milliseconds. After the course of one hour this represented approximately 25,000 heating cycles. With the correlation coefficient extremely small and
the number of heating cycles relatively large, no further significance tests were employed.
It was concluded that no sample deterioration was occurring over time. Therefore, there
is a high level of confidence that using the standard to evaluate various photothermal
parameters will provide meaningful results.

**TABLE 14**

RESULTS FROM STABILITY STUDY

<table>
<thead>
<tr>
<th>Statistics</th>
<th>Probe</th>
<th>Pump</th>
<th>Raw</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>0.13800</td>
<td>0.1105</td>
<td>1.5835</td>
<td>1.5806</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.00153</td>
<td>0.003575</td>
<td>0.09604</td>
<td>0.05582</td>
</tr>
<tr>
<td>Coefficient of Variance</td>
<td>1.109%</td>
<td>3.235%</td>
<td>6.065%</td>
<td>3.531%</td>
</tr>
<tr>
<td>Observations</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

Additionally, this experiment also establishes a base precision for the technique. A
coefficient of variance of 3.5% was determined over the course of the experiment. This
value is a worst case scenario. As indicated in the experimental section, no signal
averaging was done at the oscilloscope. Therefore, the overall precision will improve
when the waveforms are averaged at the oscilloscope.
SIGNAL REPRODUCIBILITY

Although a baseline precision was determined during the stability study, the overall reproducibility is now discussed. The first analysis of precision involved holding the sample fixed in position and periodically (once an hour) taking measurements from the sample. Table 15 contains the values associated with this experiment. The average signal amplitude of the five wavelengths within each scan is reported. The standard deviation and the coefficient of variance (S. D. and CV respectively) are also shown. The 95% confidence interval is determined from a t-table at four degrees of freedom. Additionally, an overall statistical analysis is given in which the precision of repeat measurements is defined.

The variance within the single scans establishes the minimum precision that can be obtained. The errors contained within this evaluation reflect those of the electronics and presumably minor optical error. The previous experiment established that the standard does not deteriorate over time, so sample degradation can be removed as a contributing source of error. The significant decrease in precision when the repeatability is evaluated over time suggests a larger error source. Environmental changes (temperature, humidity, etc.) may contribute to signal variation.

Hypothetically, there are two system aspects that may increase the error associated with long term reproducibility. The first potential source of error is long term optical drift. Considering the extremely small spot sizes for the two laser beams, any variation in the optical axis of the beams can cause changes in the coincidence of the beams at the sample surfaces. This will produce an effect in which the probe beam senses different regions of the temperature gradient at the sample and therefore different areas of thermal expansion. The nature of the interference between the overlap of the two probe beam reflections also causes variations in the signal obtained. If there is a change in the incident
angle of the probe beam away from the normal optical axis then the two reflections are no longer collinear. The signal amplitude will then represent only partial overlap of the reflections.

### TABLE 15

**SIGNAL REPRODUCIBILITY**

<table>
<thead>
<tr>
<th>Interval</th>
<th>Average</th>
<th>S. D.</th>
<th>+/- 95%</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1.3687</td>
<td>0.00931</td>
<td>0.0258</td>
<td>0.68%</td>
</tr>
<tr>
<td>2</td>
<td>-1.3842</td>
<td>0.0141</td>
<td>0.0391</td>
<td>1.02%</td>
</tr>
<tr>
<td>3</td>
<td>-1.4574</td>
<td>0.0105</td>
<td>0.0291</td>
<td>0.72%</td>
</tr>
<tr>
<td>4</td>
<td>-1.3718</td>
<td>0.00672</td>
<td>0.0187</td>
<td>0.49%</td>
</tr>
<tr>
<td>5</td>
<td>-1.3678</td>
<td>0.00725</td>
<td>0.0201</td>
<td>0.53%</td>
</tr>
<tr>
<td>6</td>
<td>-1.3660</td>
<td>0.00833</td>
<td>0.0231</td>
<td>0.61%</td>
</tr>
<tr>
<td>7</td>
<td>-1.4195</td>
<td>0.00838</td>
<td>0.0233</td>
<td>0.59%</td>
</tr>
<tr>
<td>8</td>
<td>-1.3768</td>
<td>0.0112</td>
<td>0.0339</td>
<td>0.81%</td>
</tr>
</tbody>
</table>

**Overall Statistical Analysis**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>-1.389</td>
</tr>
<tr>
<td>S. D.</td>
<td>0.0305</td>
</tr>
<tr>
<td>+/- 95%</td>
<td>0.0721</td>
</tr>
<tr>
<td>CV</td>
<td>2.19%</td>
</tr>
</tbody>
</table>

Changes in the alignment of the beams can arise due to drift of the light propagation in the laser tube. This would be a real source of error, because of the small dimensions employed during analysis. Another potential source of optical drift may
involve the build up of vibrational noise. The change in the absolute location of the optical elements and the sample might randomly vary over time. Variations in the absolute sample location will be discussed more fully in the next few paragraphs. However, considering the statistical evaluation presented above, the precision is reasonable.

Assuming the signal is normally distributed and the coefficient of variance represents this distribution, then 68.26% of the measured values will be approximately +/- 2.2% about the mean. All signal values (from the confidence interval) would be within +/- 5.2% of the measured mean.

The reproducibility over different sample surface locations provides an interesting yet expected aspect of this interferometric phenomenon. The AC amplitude deviation during modulation is dependent upon the initial surface separation. Therefore, the signal amplitude changes at different locations. Figure 22-a through 22-c, shows three waveforms (after de-convolution) as an example. In the case of waveform 1, the original distance between the film and glass surface produces constructive interference. The thermal expansion that occurs during the pump pulse causes increasing destructive interference. Waveform 2 is the exact opposite. Waveform 3 is an example of the situation in which the heat produced at the sample starts to constructively interfere and then destructively interfere.

Waveform data were collected in which the initial signal change was clearly either constructive or destructive interference for the different sample locations. These values were statistically analyzed separately and provided in Table 16. The data obtained demonstrate a drastic loss in precision when the sample is relocated. Human error may play a critical part in the reproducibility of these values. As stated previously, the sample is aligned manually until the maximum signal amplitude is acquired.
Figure 22-a. Waveform for constructive interference
Figure 22-b. Waveform for destructive interference
Figure 22-c. Waveform between constructive and destructive interference between surfaces
There may be some ambiguity in this method and other instrumental approaches might provide improved results (e.g., computer automated with a feedback algorithm). This will be discussed in the improvement section of this document.

Table 16

<table>
<thead>
<tr>
<th>Deviation from Baseline</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Statistics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>1.248</td>
<td>-1.432</td>
</tr>
<tr>
<td>S. D.</td>
<td>0.147</td>
<td>0.108</td>
</tr>
<tr>
<td>+/- 95%</td>
<td>0.348</td>
<td>0.255</td>
</tr>
<tr>
<td>CV</td>
<td>11.81%</td>
<td>7.55%</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Another potential source of error involves the sample itself. The degree to which the two surfaces are parallel with each other determines how well the reflections overlap and the symmetry of the interference pattern. Variations in the observed signal when the sample was relocated might constitute the differences in the degree of parallelism between the two surfaces. With the use of higher quality materials and equipment, the precision can be improved (discussed later).

The average values for the above data were statistically analyzed with a t-test (75) to determine if the difference between the means was due to random scatter in the data. The null hypothesis states that no difference exists between the means of the two data sets ($\Delta \bar{X}$). The alternative hypothesis expresses that there is a significant difference between
the means. A t value is determined based upon the variance of the two data sets ($V_{X1}$ & $V_{X2}$) by:

$$t = \frac{\Delta \bar{X}}{S_{\Delta X}}$$

And:

$$S_{\Delta X} = \left( \frac{V_{X1}}{n_1} + \frac{V_{X2}}{n_2} \right)^{1/2}$$

Using the values in Table 16 a t-value of 2.853 is calculated. Inspection of a t-table at a 95% confidence level and fourteen degrees of freedom (i.e., $n_1 + n_2 - 2$) gives a value of 2.145. Since the calculated value is larger than the number from the table, the null hypothesis can be rejected and there is a statistically significant difference between the average of the two data sets.

**AFFECT OF THE LENS TO SAMPLE DISTANCE**

Observation of the reflected irradiance from the probe beam established that an eight mm translation distance could be used for this experiment. The reflected light imaged by the objective lens at locations outside this range overfills the polarizing cube and the cube acts as a limiting aperture. This range corresponds to a venier scale setting of 14 to 22 mm. The actual sample to lens distance at these settings was determined to be 27.8 to 35.8 mm respectively.
Figure 23 is a plot of the average peak-to-peak amplitude (adjusted for probe and pump variations) at each translation interval. Each data point represents the average of the five replicates at that position as well as the average within the scan. Coefficients of variance averaged 8.7% and varied from 5.2% to 13.7% for the data points. The precision measurements are in agreement with the previous experiment and are consistent with positioning errors that occur during translation of the sample. With the current equipment, there is no guarantee that, upon movement, the same absolute location on the standard is sampled.

The profile of the signal response at different lens to sample distance establishes that this system is unique to the photothermal family of spectroscopic techniques. As discussed in the historical section, the intensity and sign of the signal are dependent upon the location in which the sample is placed with respect to the focal point of the objective lens. As an example, the response function for photothermal displacement at positions within the focal length of the lens has negative signal amplitudes. Beyond the focal point, the time varying signal has positive amplitudes. Therefore (as is the case with other photothermal techniques), two maxima exist in the response profile as samples are translated through the focal point of the lens. As can be seen, only one maximum occurs for this approach.

Another unique aspect evident from the graph is the relatively slow decline of the signal. Using photothermal displacement as an example, after three confocal lengths (see Section 1), the signal amplitudes on either side of the focal point have decayed to zero. In thermal lensing spectroscopy at three confocal units, the signal amplitude has decayed to 20%. Based on the results determined in Section 6 (focal and confocal length determination), Figure 24 has been generated in which the relative signal is plotted against units of confocal length. The negative ordinate values represent positions prior to the focal point of the lens.
Figure 23. Response profile of photothermal signal at various lens to sample distances
The relative signal beyond +/- 10 confocal distances from the maximum is still appreciable (greater than 20%). In addition, the maximum signal obtained occurs very close to the calculated focal point of the lens (0.1 millimeters prior).

The maximum signal occurring within the position of the focal length provides some insight on the nature of the interferometric phenomenon. At this location, the cone of incident light and the cone of reflected light originating from the polymer surface perfectly overlap. In addition, from this location (from a paraxial sense) the objective lens re-images the reflected light from the second surface back to the original dimensions of the incident laser beam.

Another observation that is different for this system than typically experienced for other photothermal techniques is the lack of symmetry in the signal response. At positions past the focal point of the probe beam, the rate of signal change with distance is markedly different than that before this position. This is most likely attributable to the non-chromatic character of the current system design. The focal length for the Argon Ion laser is approximately 1.9 mm beyond that of the Helium Neon laser. Thus the aspect ratio (ratio of both beam sizes relative to each other) changes dramatically within this region.

The use of a plano-convex lens in this application, although chromatically a minor problem, may still be superior from an imaging standpoint. This type of lens is ideal for collecting light at short distances and in this application is relatively free of aberration (66). Highly symmetrical interference patterns have been observed with this type of lens as opposed to others (biconvex, aspherics, and achromatics). The control of the pump beam diameter at the sample should occur before the combination of the two beams with high quality beam expanders. This will be discussed in detail in the instrumental improvement section of this document.
Figure 24. Relative response of photothermal waveforms at various confocal distances about the focal point. Negative values represent positions prior to the waist.
CHAPTER 9

FABRICATION OF ANALYTE SPECIFIC SURFACE SENSORS

Having analyzed the various instrument parameters with the calibration standard, the development of analyte specific surfaces to be used as a photothermal transducer was investigated. The overall strategy was to build a polymer layer with a pH sensitive chromophore covalently bound to the plastic and laminated onto a glass support. The glass is intended to provide mechanical stability and the source of the first reflection for the interferometer. The heat produced upon radiationless de-excitation from the chromophore is then sensed by the polymer as it thermally expands. The reflected irradiance at the interface between the plastic and solution will then vary as either the geometry and/or the refractive index of the film changes. Combination of the two reflections will then have a modulated interferometric signal in a manner similar to the standard discussed previously.

Cellulose was chosen as the thermally sensitive polymer used for the fabrication of the pH sensor. The use of cellulose and its derivatives for the construction of optical sensors is discussed frequently in the literature. As some examples, but not inclusive, these materials have been used to construct sensors for absorbance pH measurements (76), fluorescent pH detection (77), and measurement of Br⁻/I⁻ concentrations by fluorescence (78). The sensors are normally made by either dip or spin coating the dissolved polymer onto glass slides or at the ends of fiber optic wave guides. Good stability of the polymer layer has been noted by the authors.
Two different approaches were used to construct these surfaces. The first method involves a series of chemical reactions on borosilicate glass microscope slides (Edmund Scientific) in which a polymer monolayer is covalently attached to the surface. Utilizing a silanization reagent (aminopropyl-triethyloxy silane), an organic functional group (primary amine) is incorporated onto the glass and is used as an intermediate cross linker. The glass slides are then bonded (Norland Optical Adhesive #61, Edmund Scientific) to a non-silanized glass backing, thereby establishing a single reactive glass surface. Cellulose acetate is covalently attached to the glass with N,N'-carbonyl diimidazole (CDI). With the laminate formed, various chemistries can be performed to bind pH sensitive dyes to the structure. The polymer layer can either be further functionalized to provide attachment sites for the pH sensitive chromophore or the cross linker can be hydrolyzed and directly dyed.

The second approach involves attachment of a pH sensitive chromophore to a polymer film (dialysis tubing). This is done by either functionalizing the film with the same procedures used for the first method or by directly dying the polymer. Upon drying, the films are bonded to glass with an optical adhesive specially designed to adhere plastics to glass (Norland Optical Adhesive #68, Edmund Scientific).

Since the procedures for the various methods are the same, the following section outlines the chemistries used. Compounds other than the dyes employed in this analysis (chromatic ionophores, chelates forming colored complexes, immunoglobulins, biotin, etc.) can be attached with these generic protocols.
A number of protocols have been found in the literature for the covalent attachment of 3-aminopropyltriethyloxy-silane (APES) to glass (79, 80, & 81). These procedures are normally used to couple a stationary phase onto a silica gel chromatographic support. The procedure adapted in our lab is that of Weetal's (82). Figure 25-a shows the reaction pathway. The APES is distilled to remove any polymerized silane prior to use. Borosilicate glass microscope slides (1cm X 2 cm) are first cleaned by immersing them in boiling nitric acid (.5 molar) for 20 minutes. The slides are then rinsed in de-ionized water and placed into an aqueous solution of APES (10% v/v, pH = 3.5). The reaction is performed in a nalgene conical centrifuge tube (HDPP). The tubes are sealed and placed into a 70° C water bath for three hours. Upon removal from the bath, the reaction is allowed to continue overnight at room temperature (~ 12 hours). The slides are then washed with copious amounts of de-ionized water followed by acetone and placed in a 150 degree drying oven for 24 hours. The glass is stored in a desiccator.

Verification that the amino groups are present on the glass is done by reacting the silanated glass with a 1 mg/ml aqueous (50 mM phosphate buffer pH=6, 10 mL volume) solution of fluorescein isothiocyanate. Acid washed slides are reacted concurrently and used as a control. The isothiocyanate group on the fluorescein is extremely reactive toward primary amines (83). Figure 25-b provides the reaction procedure. The slides are removed from the solution after 12 hours and washed with several rinses of acetone, ethanol, and de-ionized water respectively. The fluorescent spectra of the silanized glass slides are compared with that of the acid washed slide in the fluorimeter. With the emission wavelength set at 525 nm, the excitation spectrum is scanned.
Figure 25-a. Incorporation of APES

\[
\text{EtO-Si-} \left( \text{CH}_2 \right)_3 \text{NH}_2 + \text{EtO-Si-OEt} \xrightarrow{125^\circ C \text{, 24 Hrs.}} \text{EtO-Si-} \left( \text{CH}_2 \right)_3 \text{NH}_2 \text{EtO-Si-} \left( \text{CH}_2 \right)_3 \text{NH}_2
\]
Figure 25-b. Reaction with the fluorescent probe
Fluorescent Spectra from Glass Slides

Figure 26. Fluorescent spectra from glass slides
Figure 26 shows the spectra of three silanized glass slides versus an acid washed slide. Ordinate values represent a percentage of full scale for the fluorimeter at a gain of one.

**ACTIVATION OF CELLULOSE ACETATE**

Polymers can be covalently attached to the surface once the amino functionality is bonded to the glass. Several methods have been developed for the attachment of cellulose acetate or for any hydroxyl compounds (e.g., agarose, poly (vinyl) alcohol, etc.). One method, discussed in the literature, is the use of N,N'-carbonyl diimidazole (CDI) which forms a stable carbamate bond with primary amines (84, & 85). CDI proceeds to form an intermediate imidazolyl carbamate with hydroxyl groups. Imidazole functions as the active leaving group when N-nucleophiles are present. Figure 27 outlines the reaction sequence.

Procedurally, a 50 mg/ml solution of cellulose acetate (Aldrich, 38% acylation, average molecular weight of 30,000) is dissolved into dry acetone (total volume 15 mL). Dry organic solvents must be used to avoid hydrolysis of the CDI and/or the intermediate complex. 1.5 grams of CDI (Aldrich) is added to the solution and allowed to react at room temperature for five hours. Silanated glass slides are placed into the solution and the reaction is allowed to continue at room temperature for 48 hours. Upon completion, the slides are washed with copious amounts of dry acetone. The same protocol is used in the case of activating a polymer sheet and bonding it to a glass slide with optical adhesives. Dialysis tubing (Sigma) is used and is cut into 4 cm² sections.
Figure 27-a. Activation of hydroxyl polymer

Figure 27-b. Reaction of activated complex with primary amine
AMINATION OF ACTIVATED CELLULOSE

With the polymer activated, aromatic chromophores can be covalently coupled by means of the Mannich reaction. However, primary amines must be incorporated into the polymer for this reaction. This is accomplished by placing the slides or cut films into a 1 M solution of 3,3'-iminobispropylamine (H₂N-(CH₂)₃-NH-(CH₂)₃NH₂, DADPA, Aldrich) for 24 hours and again forming a carbamate linkage with the DADPA. Since this reagent is a primary diamine, some cross-linking presumably occurs. An approximation of the amount of primary amines available for coupling has been done by reacting the polymers in a 4 mg/mL ninhydrin solution (ethanol with 1.5% v/v pyridine). The following schematic (Figure 28) shows the reaction of ninhydrin with primary amines producing a colored complex in solution (86 p., 282).

The films are placed into 3 mL of the ninhydrin solution and brought to a boil. The boiling is maintained for 45 minutes. Calibration standards consisting of various concentrations of ethyl amine are reacted simultaneously in the same hot water bath. When completed, the supernatant is decanted and allowed to cool. Absorbance measurements are taken in a Ultraviolet/Visible spectrophotometer at 570 nm. Figure 29 is a log/log plot of the concentration (X-axis) of the standards versus the log of the absorbance (Y-axis).

Interpolation of the above graph suggests that the total equivalent molar concentration of free amines on the polymer coated glass slides are on average 4.11 X 10⁻³ molar when compared to the solution standards. This would indicate a surface coating of 6.17 X 10⁻⁶ moles/cm² of the free amines. CDI activated cellulose slides were run as a control and produced no detectable absorbance. In addition, silanated glass was also run and again produced no color.
Figure 28. Reaction of ninhydrin with a primary amine
Figure 29. Calibration curve for the analysis
Therefore, confirmation exists that the reactions leading to the production of a mono-layer aminated cellulose on glass is successful. When the cut sheets are evaluated, the equivalent molar concentration is on average $1.39 \times 10^{-2}$ resulting in $2.17 \times 10^{-5}$ moles/cm$^3$. Again, a piece of the CDI activated polymer and non-modified polymer were run as a control. In this procedure, like the FITC protocol, the film is unavoidably sacrificed.

COVALENT ATTACHMENT OF PHENOL RED

Phenol red has been successfully coupled to cellulose materials after the incorporation of primary amines by use of the Mannich reaction (86). This protocol is particularly useful because there are few procedures that allow covalent coupling of aromatic compounds in an aqueous environment. Figure 30 provides the reaction sequence of the Mannich reaction. The coated slides or polymer films are placed into a tube containing a 1 mg/mL aqueous solution (5 mL, 0.1 M morphilinoethane sulfonic acid pH=4.7 used as buffer) of phenol red. One mL of formaldehyde (37%, Aldrich) is added and the tube is sealed and placed into a 60°C oven. The reaction is allowed to continue for one week. Upon completion, the films were washed with a 0.1 molar saline solution followed by de-ionized water until no dye is visible in the wash. They were dried and stored in a desiccator.
Figure 30. Attachment of Phenol Red by the Mannich reaction.
DIRECT DYING OF CELLULOSE WITH CONGO RED

Dyes, like congo red, are known as direct dyes (87) due to their ability to strongly bind to various types of materials. Congo red binds strongly to cellulose. The polymer films were directly dyed with congo red using a modified procedure by Jones (76). In the case of the covalently bonded cellulose films, the slides were first placed into de-ionized water for one week. This allowed the CDI to completely hydrolyze off the polymer, re-establishing the hydroxyl binding sites. For the polymer films that would be bonded to the glass via the optical adhesives, 2 cm² sections were cut and washed several times before starting the dying procedure.

An aqueous dye bath composed of $3 \times 10^{-4}$ molar congo red, 0.025% w/v Na$_2$SO$_4$, and 0.025% w/v K$_2$CO$_3$ in de-ionized water was prepared. 20 mL of the solution was placed into a 50 mL round bottom flask with a reflux condenser and brought to 85°C with a heating mantle. The individual polymer coated slides or cut films were placed in the bath for 30 minutes. Upon completion of the reaction, the films were removed and washed with copious amounts of 0.1 molar saline solution. This was followed by de-ionized water until the wash had no obvious dye. The films were placed into one liter of de-ionized water overnight to continue to remove any loosely bound dye. Films were then dried and stored in a desiccator for future use.

EVALUATION OF THE DIFFERENT PROCEDURES

From the above procedures, four different types of sensors were constructed in which either the polymer film was covalently attached to the glass or bonded with an adhesive. For each of these sensor types, a pH sensitive dye was incorporated into the
polymer by either physical absorption (direct dying) or by covalent attachment to the polymeric structure (Mannich reaction). It should be noted that all four procedures provided a surface in which the absorbance spectrum was influenced by the pH of a solution. However, there were obvious differences in the integrity of the surfaces by the various protocols.

The first qualitative difference that was observed involved the comparison between covalently attaching the polymer and binding it with the adhesive. Upon incorporation of the dye (either procedure), the glass slides that had the polymer covalently bound to the surface proved to be highly irregular. These surfaces were chromatically uneven. Because it would be difficult to quantitate the absorbance over the cross sectional area of the film, the sensor in which the film was glued to the glass was chosen. This also best resembled the standard discussed previously.

Comparison of the two approaches to incorporate dye into the polymeric structure also had significant differences. The concentration of reagents used in the above procedures were chosen to achieve maximum chromophore binding. A sample cell was assembled by cutting one side off a polystyrene cuvette. The cut was done at a slight angle to assure that any reflected light from the cuvette would not recombine with the reflection from the sensor. The cuvette was glued to the film with an optical adhesive (NOA #68) as previously described. In the case of direct dying, the absorbance of the films was move then an order of magnitude greater. Figure 31 is a plot of the absorbance versus the pH of a solution measured in a UV/Vis spectrophotometer for the directly dyed film. During photothermal investigation of these samples, the signal obtained decayed rapidly and, after a few seconds, the incident spot was completely devoid of dye. Apparently the heat produced caused the chromophore to de-sorb from the polymer film.
Absorbance versus pH at 488 nm

Figure 31. Response curve for directly dyed film
The sensor in which the chromophore was covalently linked did not suffer this problem. The amount of absorbance was too small to establish a calibration curve in the ultraviolet/visible spectrometer. However, there was enough signal to determine the change in absorbance of the film between pH 6.6 and 8.2. Procedurally, this was accomplished by placing identical sensors into the signal and reference paths of a ultraviolet/visible spectrophotometer. A pH 6.6 sodium phosphate buffer solution (.1 molar) was added to both and the signal at 545 nm (maximum absorbance change for dye) was recorded. The sensor in the sample beam was then washed with several changes of pH 8.2 sodium phosphate buffer (.1 molar) and the absorbance measured. The sensor was washed again with the pH 6.6 buffer and the procedure repeated a total of 25 times in order to obtain good statistical results.

The change in absorbance of the film for the two pH values was determined to be 0.0136. Table 17 gives the experimentally determined extinction coefficients for phenol red in pH 6.6 and 8.2 phosphate buffer solution at various wavelengths. Assuming that the extinction coefficient of the chromophore does not drastically change when bound to the cellulose film, the equivalent molar concentration of chromophore on the film is calculated from the change in the absorbance. From Beer's law, the following calculation is performed in which the subscripts "r" and "y" refer to solutions at pH 8.2 and 6.6 respectively.

\[ A_r - A_y = e_r b C - e_y b C = (e_r - e_y) b C \]

Based on the manufacturer's specifications, the hydrated film thickness is approximately 0.0130 cm. From the above equation, an estimate of the equivalent molar concentration of chromophore within the film is $5.66 \times 10^{-5}$ moles/liter or $5.66 \times 10^{-8}$ moles/cm$^3$. 
PHOTOTHERMAL ANALYSIS OF pH SENSOR

From the results obtained in the previous section, 514 nm was used for the excitation of the sensor. In order to resolve the signal, the Argon Ion laser was set at an output power of 1 watt (maximum power output) which corresponds to 88 milliwatts of power at the sample (see instrumental section). The sensor was mounted into the instrument and all of the appropriate alignments made. The sensor was maintained in a fixed position during the experiment.

The photothermal response from the sensor was evaluated using five pH standards ranging from 6.6 to 8.2 in increments of 0.4 pH units. The standards were prepared by adjusting a pH 8.2 sodium phosphate buffer used above with 6 molar HCl solution. During analysis, the standards were placed into the sample cell and allowed to equilibrate for a minute. When successive standards were measured, the film was washed with several exchanges of the solution to assure proper pH. The series of standards were measured in order of highest to lowest pH and the series was repeated a total of five times over the course of four hours.
Data analysis was performed by averaging over approximately 50 oscilloscope scans. The scans were then de-convoluted from the electronic effects of AC coupling as described earlier. A representative waveform response was determined by averaging the individual wavelengths (four per scan). Finally, an overall waveform was determined by averaging the five replicates. Figure 32 is a plot of the five waveforms obtained for the different pH standards. Each successive pH measurement has been offset by 0.025 volts along the Y axis for presentation purposes. In addition, the reference signal has been adjusted to maintain the scale. The photothermal amplitude deviations for the standards along with the appropriate statistics are provided in chart 18. The standard deviation is calculated based upon the five replicates and a 95% confidence interval determined from a t-test with four degrees of freedom.

### TABLE 18

**SIGNAL OBTAINED FOR THE DIFFERENT pH STANDARDS**

<table>
<thead>
<tr>
<th>pH</th>
<th>Average Deviation (volts)</th>
<th>Standard Deviation</th>
<th>t-test 95%</th>
<th>Signal to Noise</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.6</td>
<td>-0.0054</td>
<td>0.000693</td>
<td>0.00193</td>
<td>1.00</td>
</tr>
<tr>
<td>7</td>
<td>-0.0124</td>
<td>0.001179</td>
<td>0.00327</td>
<td>2.30</td>
</tr>
<tr>
<td>7.4</td>
<td>-0.02479</td>
<td>0.001665</td>
<td>0.00462</td>
<td>4.59</td>
</tr>
<tr>
<td>7.8</td>
<td>-0.04562</td>
<td>0.002979</td>
<td>0.00827</td>
<td>8.45</td>
</tr>
<tr>
<td>8.2</td>
<td>-0.05427</td>
<td>0.001324</td>
<td>0.00368</td>
<td>10.05</td>
</tr>
</tbody>
</table>
Average Signal Waveform at Different pH Values

Figure 32. Photothermal waveforms for the different pH standards
Figure 32 clearly shows that, with this particular arrangement, the lower limit of sensitivity has been established. At pH 6.6 some of the pump irradiance is present in the signal waveform and limits any further improvement in the signal to noise by increasing pump power. Additional sensitivity cannot be achieved without further improvements in the instrument design. However, there are many design modifications that can be made to increase the sensitivity of the system. These will be discussed later in the Future Development section of this dissertation.

Figure 33 is provided to show the amplitude deviation in signal versus the pH standards. The solid line represents the best fit line from a linear regression of the signal response. Using the thickness of the hydrated film and the probe beam waist size (determined within the instrumental section), an illumination volume through the film is calculated to be $3.686 \times 10^{-8}$ cm$^3$ (i.e., 0.0130 cm thick and a waist radius of $\sim 9.5$ µm). From this volume and the molar concentration of dye within the film, the amount of chromophore excited at a pH of 8.2 is:

$$5.66 \times 10^{-8} \text{ moles/cm}^3 \times 3.68 \times 10^{-8} \text{ cm}^3 = 2.08 \times 10^{-15} \text{ moles}$$

The amount of chromophore excited at a pH of 8.2 is approximately 2.08 femtomoles with a signal to noise of about 10. Therefore, the detection limit is approximately 624 attomoles (i.e., signal to noise of 3). The minimum detectable change in absorbance at the surface can be evaluated by substituting the extinction coefficient of the dye (at 514 nm for a pH of 8.2), the hydrated film thickness, and the above detection limit into Beer's law. An absorbance of $1.25 \times 10^{-13}$ is determined from this calculation. Again further optimization of the system can be done to improve the sensitivity of this approach and will be discussed later.
Figure 33. Photothermal response for the different pH standards
CHAPTER 10
CONCLUSION

The original purpose of this dissertation was to establish feasibility for the detection of analytes at a surface using photothermal displacement spectroscopy. In attempts to improve the instrument design, a new photothermal spectroscopic approach was developed. The nature of the system involves multiple beam reflections within a supporting surface. When optically heated, the optical path length change produces an interferometric signal. This is due to surface displacement and refractive index variations. This is substantiated from the observations of fringe shifts within the signal waveforms. Unlike other photothermal techniques, no limiting aperture (pinholes, irises, knife edges, etc.) are required in the configuration. The method mimics a low finesse Fabry-Perot interferometer.

Physical insight for the phenomena has been provided by a mathematical derivation. It is clear from the model that the distance between the reflecting surfaces and the refractive index of the medium combine to produce the interference. However, based upon the thermal mirror model, the irradiance from the polymer solution interface is also a function of the surface curvature. This factor needs to be incorporated into the current expression in order to produce an equation that simulates the photothermal waveforms. Efforts continue to incorporate this aspect into the model.
An instrument has been designed to measure the photothermal interferometric signal. The design is an improvement over traditional photothermal spectrometers. This is due to the ease of alignment of the pump and probe beams by the optical configuration employed. Recall that photothermal deflection is very sensitive to the perpendicularity of the probe beam and the beam placement within the thermal gradient. The signal obtained by photothermal displacement, in which the probe beam is incident at small grazing angles, is dependent upon the slope of the surface distortion sampled by the beam. By allowing both beams to impinge the sample normal to the surface, alignment of the beam overlap is easily performed with pinholes and a photodiode. Using the same set of pinholes, the retro-reflected light is also aligned in the same manner and can be performed prior to excitation of the sample. Finally, the absolute waist position from the focusing lens can be determined by evaluating the irradiance of the reflected light.

AC coupling of the photo-voltage provided a method of measuring small signal changes on a relatively high DC background. However, the use of AC coupling convoluted the signal waveform. A mathematical algorithm to de-convolute the signal was derived, and data were presented to verify the calculation. Evaluation of the electronics demonstrated that signal averaging at the oscilloscope minimized the AC noise in the system. Optical noise in the system could not be resolved due to the digitization at the oscilloscope. Therefore, at the probe power levels used for these experiments, the oscilloscope is the major noise component.

A stable standard surface was prepared in order to evaluate various parameters that would have signal contributions. The most significant parameter was the modulation duty cycle. When the surface is evaluated using the typical pump beam approach (i.e., continuous modulation at a 50% duty cycle), the waveform data are convoluted from previous heating cycles. The signal is further complicated when fringe shifts start to appear in the waveform thus making the data difficult to evaluate. Lowering the duty
cycle is similar to the pulsed laser approaches discussed in the historical section and produces a time resolved signal. Clearly, information about the thermal nature of the sample is contained in the waveform's profile when this method is used. Efforts are ongoing to evaluate the different thermal aspects that are present in the waveforms. For example, when the pump illumination is eclipsed by the chopper, the time necessary for the signal to return to background is related to the thermal conductivity of the polymer. With information about the incident pump energy and the absorbance of the substance, a quantitative method to evaluate this value should be possible.

With the modulation duty cycle lowered, other aspects of the system were determined. The AC amplitude deviation was found to be linear with the intensity of the probe beam. The proportion of constructive or destructive interference is the same despite what probe intensity is employed. However, as the power is increased, the AC signal becomes easier for the oscilloscope to resolve. Therefore, sensitivity can be increased by raising the power of the probe beam. The signal was found to be linear with the pump power until a point in which fringe shifts appeared. Additionally as the illumination time was extended, the appearance of fringe shifts occurred at lower excitation powers. This indicates that the optical path length change is dependent upon the time integral of the power deposited into the sample. Increasing the detection limit by raising the power of the pump beam has several practical limits. The first is the ability of the system to reject the pump irradiance from the waveform. This was observed during the evaluation of the pH sensor in which a small amount of pump power was present in the signal. With the current optical configuration, there is no improvement in signal-to-noise by increasing the pump irradiance. Another important limitation is laser ablation which occurs when enough energy is generated to break the covalent attachment of the chromophore.
Evaluation of the precision revealed that when the samples were set in a fixed position, repeat measurements were reproducible (~1% for the coefficient of variance). However, the precision significantly deteriorated when measurements were taken over the cross section of the sample. Presumable variations in the parallelism of the two sides affect the performance. In addition, some AC waveforms increased in intensity and others decreased as the samples were relocated. This appears to represent the initial thickness at each location between the two surfaces. Upon thermal expansion, either constructive or destructive interference will occur. A statistical difference in the magnitude of constructive versus destructive interference was found. This is predicted by the mathematical model derived. Consider the expression presented again as:

\[
I_R = R_1 \left( \frac{w_0^2}{w(z_1)^2} \right) + R'_2 \left( \frac{w_0^2}{w(z_2)^2} \right) + R_{12} \left( \frac{w_0^2}{w(z_1)w(z_2)} \right) \left\{ 2\cos(K\Delta z)\cos(\Delta \phi) - 2\sin(K\Delta z)\sin(\Delta \phi) \right\}
\]

During thermal de-excitation, the refractive index of the polymer decreases. The cross reflectance \((R_{12})\) and the reflectance at the surface of the polymer film \((R'_2)\) also decrease in value. Since the total irradiance is proportional to these terms, the expression predicts that during thermal expansion the total irradiance will decrease despite the direction of the interference. In the situation of destructive interference, the effects of the reflectance and optical path length variations cooperate. The opposite is true in the case of constructive interference. Therefore, the difference in intensity may be attributed to the weight of these two terms on the overall signal. It should be realized that there are two phenomena that contribute to the reflectance term. The first is the change in the refractive index of the film as discussed. In addition, the refractive index of the adjacent solution also decreases as heat is conducted away from the surface (e.g., photothermal deflection).
The pH sensor demonstrated analytical utility for this photothermal spectroscopic approach. The calculation of sensitivity is an estimate based upon two assumptions. The first is that the extinction coefficients for the two dye forms do not significantly vary when covalently bound to the support. The second is that the thickness and/or density of the film does not drastically change for the different pH standards. Despite these assumption, the sensitivity is within a reasonable range. Further improvements, to bring this approach to the next level of sophistication, will be discussed in the next section.

In summary, a new approach to detection of analytes at a surface by a novel photothermal spectroscopic methodology has been developed. A mathematical expression has been derived to explain part of the phenomenon. An instrument has been constructed and its parameters analyzed to perform these measurements. An application to demonstrate feasibility has been established.
CHAPTER 11

FUTURE DEVELOPMENTS

INSTRUMENTAL

There are a number of instrumental improvements that could be made to enhance the performance of the system. The first is an optical re-design of the system in which the pump and probe beams have opposite polarization. Consider Figure 34 as a general block diagram for a new configuration. In this example, the pump beam laser (PL) is horizontally polarized. The probe beam (PR) would be vertically polarized by using a Glan Thompson (GT3) and sheet polarizer (P) similar to the current design. The irradiances from the two beams are combined and directed toward the sample with a polarizing cube (POC). A quarter wave plate (QWP) is used to rotate the electric field vector of both beams by 45°. The light reflected from the sample propagates through the quarter wave plate with a net electric field rotation of 90°. The pump power is now vertically polarized and directed toward the probe laser by the polarizing cube. The combination of a dichroic beam splitter and interference filter (DB1 & IF2) serves to protect the probe laser from the pump light. The probe beam is phase shifted by 180° and is horizontally polarized and incident upon the detector (SP). Again interference filters and dichroic beam splitters are used to protect the pump laser (IF1 & DB2).
Figure 34. Block diagram for new instrument design
This optical arrangement should further improve the pump light rejection at the signal photodiode when compared to the current setup. Three optical elements work together to perform this task. First, the interference filter rejects the pump light as it does in the current system. However, the polarizing beam splitter and the dichroic beam splitter also divert the reflected pump irradiance from the detector. This allows higher pump powers to be used for excitation of the sample. Note also the addition of a high quality beam expander (BE) for both the pump and probe lasers. This element provides the ability to vary the relative beam sizes of the two beams to further optimize the technique.

The chopper has been replaced with two crossed Glan Thompson polarizers (vertical and horizontal respectively, GT2 & GT1) and a pockel cell (PKC). A pockel cell consists of a crystal in which, under an applied potential, birefringence is induced in the crystal. Depending on the voltage, a 180° phase change is induced in the propagating electric field of the pump allowing the radiation to pass through the second polarizer. When no voltage is applied, the crossed polarizers block the pump radiation. Therefore, the pump is electro-optically modulated. The advantage of using a pockel cell is that the pulse width can be as short as a nanoseconds. In addition, the duty cycle is infinitely variable.

Another potential instrumental improvement is the replacement of the current signal photodiode with a two dimensional diode array. This provides a means to image the interferogram produced by the sample. A practical and theoretical advantage is achieved using this detector. Practically, it would be much easier to align the sample in the instrument if immediate feedback was available to process the symmetry of the interference fringes. Motorized adjustments of the sample could then be made with the use of a computer algorithm to assure the position of the sample before optical heating. Theoretically, additional information would exist as to the transient nature of the fringe
pattern during pump modulation. Parameters like the relative parallelism and the degree of flatness of the two surfaces could also be investigated.

Other changes to the optical design to improve the instrument include replacing the Argon Ion laser with a dye laser. Using a dye laser, the range of available pump frequencies is expanded. Therefore, a larger spectrum could be used for analysis. Other optical improvements include replacing the existing broad band polarization elements with those specific for the individual pump and probe frequencies. Lastly, specially machined parts with low mechanical tolerances would aid in sample positioning.

The electronic aspects of the system could be further modified. Currently, the DC photo-voltage from the signal photodiode is amplified. The oscilloscope is AC coupled to reject the high DC background. Ideally, the DC voltage should be rejected prior to amplification. A schematic diagram for an electronic component has been devised (Figure 35, as of yet not constructed or tested) to perform such a task. This filter will effectively block the DC component and will pass very low frequencies at unity gain. The cutoff frequency using values in the schematic is much less than 1 Hz. Therefore, the output voltage from this component is the AC signal, amplified by the signal conditioner and acquired at the oscilloscope.

SENSOR DESIGN

The design of the sensor has many aspects that can be improved. First, consider the equation derived for the interferometric irradiance presented in Section 3. Increasing the reflectance from both sides of the sensor improves the interferometric signal. As the reflectance from the surfaces increase, the instrument begins to resemble a high finesse Fabry-Perot interferometer.
Figure 34. Proposed electronic component to block DC signal prior to amplification
Several materials with higher refractive indexes could be substituted for the borosilicate glass (refractive index of 1.473 @ 632.8 nm) currently used. SF 11 and LaSF N9 are two Schott glasses with refractive indexes of 1.778 and 1.845 respectively at 632.8 nm. Synthetic sapphire has a refractive index of 1.77. These materials can be made into solid etalons with parallelism of 0.02 arc seconds and flat to 1/4 of a wavelength (@ 546 nm) over a clear aperture of 20 mm. The total thickness can be held to a micrometer. With these tolerances tightened, the reproducibility of the signal over the cross section of the sensor should improve.

Many improvements can be made to the coating of the inert support. Ideally, the best results would occur when the film is covalently coupled as opposed to bonded with an adhesive. This is particularly the case when high quality etalons are employed. The choice of the thermally sensitive material that is coated onto the support could also increase sensitivity. This material should have a relatively high refractive index when compared to water, a high thermal expansion coefficient, a low thermal conductivity, a low heat capacity, and provide chemical sites for covalent couplings. This aspect of the system is truly the key to the potential sensitivity as tailor made materials are used for construction of the sensor.

EXPERIMENTS AND APPLICATIONS

With a new instrument design that provides increased flexibility, many experimental aspects of the system could be further deduced. The first relationship to be investigated is the aspect ratio of the pump and probe beam. Somewhere a ratio of the beam sizes within the support is optimum for the signal. It is naive to believe that the current dimensions are serendipitously the best case. With the use of high quality etalons
that provide reproducible parallelism, flatness, and thickness, the precision of replacing samples would significantly improve. This allows investigation on the nature of how the thickness of the support affects signal. This is consistent with the fact that when the samples are held fixed, the reproducibility of the signal is good. When samples are relocated, the precision deteriorates significantly. Investigation of the effect of the thickness of the support depends on precise measurements. Currently these are extremely difficult to analyze because replacing samples provides another source of error.

This technology is by no means limited to pH determination. The current optical geometry is easily adapted to any form of chromatography, flow injection analysis, electrophoresis, and analyte specific sensor. There are two scenarios that exist for analyte evaluation. The first is a surface sensor that needs to be re-used. This would include most chromatography and flow injection analysis. This type of application needs to incorporate low energy excitation in order to maintain the surface structure and avoid laser ablation. Obviously the detection can be direct or indirect. That is, the absorbing chromophore can be contained on the surface (as the example of the pH sensor) or indirectly bound to a non-absorbing matrix (in most chromatography). The other scenario is analytical methods that only use a surface once. This encompasses most electrophoretic applications, some chromatography (e.g., TLC), and a host of bio-diagnostic analyse. In this situation, maximum pump power could be used to define the sensitivity, understanding that the surface, should it be destroyed, will not be needed again.
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In September of 1984, Michael secured a Research Associate position with Pandex Laboratories. His responsibilities, over the course of the next three years, involved the development of analytical assays for the Human Immunodeficiency Virus and the Hepatitis B Virus.

In August of 1987, he was promoted to a Research Scientist position and became responsible for the integration of a novel analytical method for the analysis of infectious diseases onto a fully automated instrument. The intended use of this instrument was to perform high volume rapid testing for blood transfusion centers.

In August of 1991, Michael entered the Graduate Program in Chemistry at Loyola University of Chicago. In September of 1992, he received a two year GAANN Fellowship to aid in his research endeavors. In September of 1994, the author received a Schmidt Fellowship to support the preparation of his dissertation. His research at Loyola involved development of a unique Photothermal Spectrometer. From this work, four papers are currently being prepared for publication.
DISSErTATION APPROVAL SHEET

The dissertation submitted by Michael Henry has been read and approved by the following committee:

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The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the committee with reference to the content and form.

The dissertation is, therefore, accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

7/9/96
Date

C. Keith Jameson
Director's Signature