Design, Characterization, and Evaluation of a Surface Plasmon Resonance Sensor

by

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Department of Mechanical Engineering and Materials Science
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Date:_______________________

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Gabriel Lopez

Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Mechanical Engineering and Materials Science in the Graduate School of Duke University

2012
ABSTRACT

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Abstract

Characterization of thin films, prominently including self-assembled monolayers is important to the understanding of interfacial events in both biological and manufactured systems. To facilitate such work, a surface plasmon resonance device was constructed, and tests were conducted to evaluate the performance of the device relative to current systems and mathematical models. First, relevant analytical equations are introduced to describe the behavior of the system. In subsequent chapters, the design of the device, its calibration, and operating procedure are explained. Finally, the system is tested against samples with known behaviors, and the experimental and analytical results are compared.
Contents

Abstract ........................................................................................................................................iv

List of Tables ................................................................................................................................vii

List of Figures ..............................................................................................................................viii

Acknowledgements .....................................................................................................................xiii

1 Introduction ................................................................................................................................1

  1.1 General History ....................................................................................................................1

  1.2 Capabilities of Kretschmann configuration surface plasmon resonance sensors ..................6

  1.3 Advantages and disadvantages relative to other techniques .............................................7

2 Modeling a Surface Plasmon Resonance device ......................................................................10

  2.1 Plasma oscillations .............................................................................................................10

  2.2 Exciting surface plasmons with wavevector matching ........................................................15

  2.3 Optical properties of dielectric materials .....................................................................17

  2.4 Fresnel coefficients for an n-layer system ....................................................................21

  2.5 Determining field depth and adlayer thickness ..............................................................32

  2.6 Kinetic Measurements .....................................................................................................33

3 Experimental design ..................................................................................................................35

  3.1 Physical Design ...............................................................................................................35

  3.2 Software Design .............................................................................................................39

4 Experimental results ..................................................................................................................47

  4.1 Baseline results ................................................................................................................47
4.2 Angular shift from a self-assembled thiol monolayer .................................. 52
4.3 Possible sources of uncertainty and error .................................................. 55
5 Conclusions .................................................................................................. 58
5.1 Thoughts on the manufacture and testing of a surface plasmon resonance sensor ......................................................................................... 58
6 Future Work .................................................................................................. 59
6.1 Improvements to the sensor ...................................................................... 59
6.2 Improvements addressing error in the sensor .......................................... 59
6.3 Improvements adding further functionality to the sensor ........................ 60
 Appendix A: Aligning a laser-mirror system .................................................. 62
 Appendix B: Cleaning procedure for gold coated slides .............................. 69
 Appendix C: Aligning a prism-detector goniometer system ............................ 72
 Appendix D: Bill of Materials ........................................................................ 86
 Appendix E: Photodetector precision and accuracy ....................................... 90
 Appendix F: Characterization of laser power fluctuations ............................ 96
 Appendix G: Procedure for mounting slides in the sensor ............................ 102
 Appendix H: Photodetector circuit layout ...................................................... 108
 Appendix I: Sensor control and data acquisition code ................................... 110
 Bibliography .................................................................................................. 135
List of Tables

Table 1: Optical properties of the Kretschmann system presented in Figure 9. .......... 23
Table 2: Optical properties of a thiol monolayer. ........................................... 24
Table 3: Indices of refraction at 632.8 nm for common surface plasmon resonance sensor substrates............................................................... 29
Table 4: Required software parameters for running the test procedures. ................. 42
Table 5: Optical properties of the Kretschmann system presented in figure 26 ............ 48
Table 6: Properties of the layer model after fitting to experimental results. ............... 50
Table 7: Commercial of the shelf items used in physical system. ............................ 86
Table 8: Raw materials used in manufacture of parts for the physical system............. 88
Table 9: Auxiliary and specialty items used in physical system................................. 88
Table 10: Photodetector internal components...................................................... 89
List of Figures

Figure 1: Sketches of three optical plasmon coupling methods, after [19]. .................................2

Figure 2: Sample configurations of three prism-based designs, after [53]. ........................................3

Figure 3: Sketch of two techniques to measure surface plasmon resonance response. ..........5

Figure 4: A surface plasmon oscillation, after [17]. ........................................................................13

Figure 5: Dielectric function versus frequency, after [26]. ..............................................................15

Figure 6: Internal and External Reflection of a Parallel Polarized Wave, after [17]. ........19

Figure 7: Reflectivity over 0 < N<sub>2</sub> < 10, and 0° < Θ < 90° at constant N<sub>1</sub> = 1. .............20

Figure 8: Reflectivity over 0 < N<sub>2</sub> < 100 at constant N<sub>1</sub> = 1, and Θ = 45°, after [17]. ........20

Figure 9: Simple prism-metal-air Kretschmann SPR layer system. ............................................22

Figure 10: Predicted reflectivity for a simple Kretschmann system excited by a 632.8 nm parallel polarized laser source. ..............................................................................23

Figure 11: Prism-gold model from figure 9 exhibiting a shift in plasmon resonance angle when including a 2.5 nm and 5 nm thiolate layer. .........................................................24

Figure 12: Prism-gold-organic model with prism-air reflectivity curve overlay. ..................25

Figure 13: Prism-gold model exhibiting a shift in plasmon resonance angle when including a 12 nm n = 1.4 and 12 nm n = 1.8 layer .................................................................26

Figure 14: Prism-gold model with increasing dielectric extinction coefficients in Δκ 0.5 increments. ..............................................................................................................27

Figure 15: Prism-gold model with decreasing dielectric extinction coefficients in Δκ 0.5 increments. ..............................................................................................................27

Figure 16: Reflectivities of 50nm thick substrate media from table 3 replacing the metal layer of table 1 with a laser source of a wavelength of 632.8 nm. .................................29
Figure 17: Effect of layer thickness of a gold substrate on resultant surface plasmon resonance curve. ................................................................. 30

Figure 18: Prism-gold model exhibiting waveguide behavior from a 900 nm thick layer of index of refraction $n = 1.4$. .......................................................... 31

Figure 19: Typical kinetic surface plasmon resonance curve. (1) Initial baseline, (2) binding to the surface until equilibrium is reached, (3) disassociation from the surface as a buffer wash is introduced, and (4) reestablishment of the baseline value Signal vs. Time. ........................................................................................................... 34

Figure 20: Layout of experimental system. (1) Laser mount, (2) Helium-Neon Laser, (3) Steering mirrors, (4) Polarizing cube, (5) Iris, (6) Photo-detector, (7) Prism and mounting hardware, (8) Goniometers ................................................................................. 35

Figure 21: Top and side views of the prism and detector setup. (1) Photodetector, (2) Prism clamp mounting bracket, (3) Prism and clamp, (4) Upper and lower goniometers, (5) X-Y Translational stage for photodetector, (6) Photodetector swingarm, (7) Drip pan, (8) X-Y translational stage for prism, (9) Yaw-Tilt stage for prism ................................................................................... 37

Figure 22: Software user interface front panel. ......................................................................................................................... 40

Figure 23: Reflectance versus angle tab. ......................................................................................................................... 40

Figure 24: Reflectance versus time tab. ......................................................................................................................... 41

Figure 25: Curve minima versus angle tab. ......................................................................................................................... 41

Figure 26: Flowchart for software behavior during an angular sweep. ........................................................................... 43

Figure 27: Flowchart for software behavior during a time sweep. ........................................................................... 44

Figure 28: Flowchart for software behavior while minima tracking. ........................................................................... 45

Figure 29: Full experimental Kretschmann SPR layer system. ......................................................................................... 47

Figure 30: Predicted response reflectivity for the modified layer system detailed in table 5, excited by a 632.8 nm parallel polarized laser. ........................................................................................................... 48

Figure 31: Clean slide baseline measurements. ........................................................................................................... 49

Figure 32: Clean slide measurements fit to the full layer model. ......................................................................................... 51
Figure 33: Baseline and Gold with thiol monolayer experimental results..................53

Figure 34: Detail of baseline and gold with thiol experimental results near resonance (Δθ = 0.45°)..............................................................................................................................53

Figure 35: Gold with thiol monolayer measurements fit to the full layer model. ........54

Figure 36: Sampled ambient temperature near sensor over a 4 month period, plotted by time of day the sample was taken. ..................................................................................................................56

Figure 37: Detail of the curve minimum of a slide left in the environment relative to a recently cleaned and recently cleaned and thiolated slide. .........................................................57

Figure 38: Measured response when powering the photodetector op-amp.................60

Figure 39: Laser-mirror layout for the SPR system, also showing two irises to be used in the beam alignment procedure, and a misaligned second mirror. ...............................63

Figure 40: Simplified one-mirror system....................................................................63

Figure 41: Simplified two-mirror system.....................................................................64

Figure 42: Laser-iris system with a misaligned laser.....................................................65

Figure 43: Laser-iris system aligned with the first iris....................................................65

Figure 44: Laser-iris system aligned with the second iris. ..........................................66

Figure 45: Laser-iris system aligned with both the first and second irises....................66

Figure 46: Laser-mirror-iris system for aligning the first mirror. ...............................67

Figure 47: Laser-mirror-iris system for aligning the second mirror. ............................68

Figure 48: Simplified goniometer system sketch, showing prim column, detector swing-arm, and potential beam path .........................................................................................72

Figure 49: Initial prism-detector angular relationship...................................................74

Figure 50: Residual error of the initial prism-detector system......................................75

Figure 51: Rough alignment of the prism goniometer system......................................76
Figure 52: Fine alignment of the prism goniometer system.................................77
Figure 53: Adjusted prism-detector angular relationship..............................................78
Figure 54: Residual error of the adjusted prism-detector system.................................79
Figure 55: Ray tracing the hypotenuse of a right-angle prism....................................79
Figure 56: Apparent thickness of a PS911 prism relative to incident beam angle...........80
Figure 57: Comparison of the curvature of the prism and residual data.........................81
Figure 58: Connected scatterplot of the adjusted residuals.........................................82
Figure 59: Relative difference between subsequent residual points.............................83
Figure 60: Prism Angle versus Detector Angle Required to Center Beam....................84
Figure 61: Photodetector in darkness, sampling at 5 Hz for 122 minutes using a floating reference..................................................................................................................90
Figure 62: Histogram of occurrence of photodetector voltages in baseline using a floating reference..................................................................................................................91
Figure 63: Normalized quantile-quantile plot of baseline voltage data for a floating reference..................................................................................................................91
Figure 64: Photodetector in darkness, sampling at 5 Hz for 120 minutes using a grounded reference..................................................................................................................92
Figure 65: Histogram of occurrence of photodetector voltages in baseline using a grounded reference..................................................................................................................93
Figure 66: Normalized quantile-quantile plot of baseline voltage data for a grounded reference source..................................................................................................................93
Figure 67: Histogram of occurrence of photodetector voltages in baseline for both floating and grounded references.................................................................94
Figure 68: Photodetector with the laser on, sampling for 12 hours................................96
Figure 69: First hour of the laser-prism-photodetector baseline...................................97
Figure 70: Change in standard deviation of 1 hour samples with sampling..................98
Figure 71: Rate of change of the sampled data. ..........................................................98
Figure 72: Drift of the measured mean value over twelve hours...............................99
Figure 73: Residual values of a linear best fit line.....................................................99
Figure 74: Residual values of an exponential best fit curve.......................................100
Figure 75: Rescaled residual values of an exponential best fit curve for the data collected between 50 minutes and 4 hours. .................................................................101
Figure 76: Simulated full layer system response using layers listed in table 5.............105
Figure 77: Simulated full layer system with contamination on the gold surface. ......106
Figure 78: Simulated full system with an air bubble in the index matching fluid. .......106
Figure 79: Photodetector circuit diagram.................................................................108
Figure 80: Op-amp pinout, from [49] ....................................................................109
Figure 81: Control and data acquisition code used by the physical system..............134
Acknowledgements

I would like to thank my committee, Dr. Stefan Zauscher, Dr. Robert Kielb, and Dr. Gabriel Lopez. I would particularly like to thank Dr. Zauscher for his guidance as my advisor throughout my time at Duke.

I would also like to thank my lab mates Greg Hardy and Ryan Hill, for their time and advice, my officemates Ted Lyman and Chris George, for their friendship and support, and Patrick McGuire and Nikhil Bumb for their support in the initial hardware manufacture.

Lastly, I would like to thank my parents for their continued support and my other friends and teachers who have helped me throughout my schooling.
1

Introduction

1.1 General History

Surface Plasmon Resonance, or SPR, is a characterization technique that measures collective surface-localized longitudinal standing waves that occur at the boundary of a dielectric media and a metal to measure changes occurring on or near that surface. Surface plasmons are predicted by Maxwell’s equations. The optical excitation of surface plasmons was experimentally demonstrated in 1967 by Heinz Raether and Erich Kretschmann, and independently in 1968 by Andreas Otto [5, 29, 41]. SPR operates on the principle of inducing bulk oscillations of surface plasmons in dielectric materials via excitation from a photon source and measuring near-surface effects by monitoring changes in those oscillations [26].

Three coupling mechanisms exist to optically excite surface plasmons, prisms, waveguides, and grating structures. Optical coupling through prisms, used in the initial experiments of both Kretschmann and Raether, and Otto, matches the momentum of the incident photons with the material plasmon wavevector by testing the system at a number of different source angles. Waveguide coupling uses a similar mechanic to excite the waveguide modes of a material and couples those modes to a metal substrate to excite surface plasmons. Grating couplings are designed with periodic surface
roughness such that some part of the surface is at the correct angle to match the incoming source wavevector [19, 20].

<table>
<thead>
<tr>
<th>Layer</th>
<th>Item</th>
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<th>Item</th>
<th>Layer</th>
<th>Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prism</td>
<td>1</td>
<td>Dielectric</td>
<td>1</td>
<td>Dielectric</td>
</tr>
<tr>
<td>2</td>
<td>Metal</td>
<td>2</td>
<td>Metal</td>
<td>2</td>
<td>Grated Metal</td>
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<tr>
<td>3</td>
<td>Dielectric</td>
<td>3</td>
<td>Waveguide</td>
<td>4</td>
<td>Substrate</td>
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Figure 1: Sketches of three optical plasmon coupling methods, after [19].

The experimental system, presented in Section 3 of this thesis, uses a prism system to couple the source laser to the metal-dielectric boundary. Subsequent discussion will focus on this technique. There are two general families of prism-based surface plasmon resonance devices, named after their respective inventors. Otto configuration devices function through frustrated total internal reflection, the coupling of surface plasmons between two dielectric-metal boundaries if they are sufficiently close. Often, the dielectric between the prism and metal is a small air gap [41]. Kretschmann-Raether configuration devices, more commonly called Kretschmann
devices, function through attenuated total internal reflection, and feature a metal layer bonded directly to the prism surface [29]. A third design is a modified Kretschmann configuration that uses metal backed slides, interfaced to the prism with an index matching fluid or polymer. This third design, the chosen design for this thesis, increases the flexibility of the system because the slide may be removed and exchanged so that different samples may be prepared without individual prisms for each experiment.

<table>
<thead>
<tr>
<th>Otto Configuration</th>
<th>Kretschmann Configuration</th>
<th>Modified Kretschmann</th>
</tr>
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<tbody>
<tr>
<td><img src="image1" alt="Otto Configuration" /></td>
<td><img src="image2" alt="Kretschmann Configuration" /></td>
<td><img src="image3" alt="Modified Kretschmann" /></td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Layer</th>
<th>Height</th>
<th>n</th>
<th>(\kappa)</th>
<th>Layer</th>
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<th>n</th>
<th>(\kappa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prism</td>
<td>Arbitrary</td>
<td>1.7</td>
<td>0</td>
<td>Prism</td>
<td>Arbitrary</td>
<td>1.7</td>
<td>0</td>
<td>Prism</td>
<td>Arbitrary</td>
<td>1.7</td>
<td>0</td>
</tr>
<tr>
<td>Air</td>
<td>320 nm</td>
<td>1.0</td>
<td>0</td>
<td>Gold</td>
<td>50 nm</td>
<td>0.2</td>
<td>3</td>
<td>Slide</td>
<td>1 mm</td>
<td>1.4</td>
<td>0</td>
</tr>
<tr>
<td>Gold</td>
<td>Arbitrary</td>
<td>0.2</td>
<td>3</td>
<td>Air</td>
<td>Arbitrary</td>
<td>1.0</td>
<td>0</td>
<td>Gold</td>
<td>50 nm</td>
<td>0.2</td>
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Sample surface plasmon resonance curves for each configuration (Angle v. Reflectance)

![Sample surface plasmon resonance curves](image4)

Figure 2: Sample configurations of three prism-based designs, after [53].
In optical systems, the resulting reflectance versus angle curve can be related to the optical properties and dielectric function of a material including its thickness by comparing the results to a baseline measurement and a model of the system. The full process to determine the Fresnel coefficients required for the model is explained in Section 2. When entering model parameters, they may either be presented as a complex index of refraction \(N\), or dielectric constants \(\epsilon\) of the material. To convert between the two, we use the following relationships:

\[
N = n + i\kappa, \tag{1.1}
\]

\[
\epsilon = \epsilon_1 + i\epsilon_2, \tag{1.2}
\]

\[
\epsilon_1 = n^2 - \kappa^2, \tag{1.3}
\]

\[
\epsilon_2 = 2n\kappa. \tag{1.4}
\]

Of prism-based solutions, Kretschmann and Otto configuration surface plasmon resonance sensors have seen somewhat different applications. Inkless fingerprint scanners and some new multi-touch computer systems use Otto configuration frustrated total internal reflection systems to image and locate a finger respectively. Kretschmann, or modified Kretschmann style sensors are more often used in materials science and chemistry laboratories because their arrangement allows for more robust measurement of interactions at the metal-environment interface. For these reasons, the experimental setup uses the modified Kretschmann arrangement.
The reflected beam intensity will vary based on the source beam, prism angle and material properties of the system including any additional analyte. To generate an output signal then, a laser can be scanned over an angular range and a detector will measure the intensity at that respective angle. Alternatively, a laser or polychromatic source can be used, and the output can be measured with a spectrophotometer to determine the attenuation at a fixed angle or frequency [19, 20].

![Angular Sweep](image1.png) ![Attenuated Frequency](image2.png)

**Figure 3: Sketch of two techniques to measure surface plasmon resonance response.**

Our setup uses a laser beam and an angular sweep; systems using this method have reported higher accuracies than spectrophotometer-based devices [43]. Some other sensors that use this or a similar approach include the Res-Tec RT-08 sensor, now owned by LOT-Oriel, and the original laboratory device that inspired the Pharmacia Biosensor BIAcore sensor, the most widely used commercial device, now owned by General Electric Healthcare [31, 33, 44].
1.2 Capabilities of Kretschmann configuration surface plasmon resonance sensors

The advent of the surface plasmon resonance sensor expanded the ability of scientists and engineers to study near-surface structures and the formation of those structures. Surface plasmon resonance sensors have seen use in biological applications including environmental testing, toxin profiling, immunoassay tests, DNA, and protein sensing, and chemical applications such as the characterization of phenols, pesticides, and explosives [2, 7, 19, 20, 43]. A variant of surface plasmon resonance that uses silver nanoparticles to further localize the plasmon resonance effect has been used to study the interactions of self-assembled thiol monolayers on those nanoparticles [32].

Surface plasmon resonance can be performed in situ, in either a gas or liquid environment, and can follow reactions in real-time. These techniques include measuring the response over an angular interval, tracking the angular shift of the curve minima over time, and tracking the reflectivity at a fixed angle over a time [16, 44]. Surface plasmon resonance measurements are label-free, which means that everything in the system will contribute to the response, so many otherwise difficult molecules can be studied. At the same time, any imperfections in the system, surface roughness of the gold layer, or contaminants, will also cause changes in the response. With a stable system, these measurement procedures can yield information on layer thickness, equilibrium states and reaction kinetics [43].
Additional capabilities that may be integrated into surface plasmon resonance sensors for specific experiments include use of a compartmented fluid cell, more complex prism geometries, or second metal surface to measure variations in the system baseline. A dynamic baseline can help to correct for temperature variations, power fluctuations of the system, and minor alignment errors. To extend the capabilities of the sensor, surface plasmon resonance sensors can also be paired with heating and cooling devices such as a peltier cell, equipment for electrochemistry, or additional photosensitive equipment for working with fluorescent markers [1, 43]. With a high-resolution CCD detector, 3-D surface images can be made to study patterned surfaces [44].

1.3 Advantages and disadvantages relative to other techniques

Surface plasmon resonance sensors are often used alongside other measurement techniques. Some complimentary methods are Ellipsometry, x-ray photoelectron spectroscopy (XPS), and quartz crystal microbalance (QCM) measurements [2, 25, 35].

Ellipsometry uses a laser in a known polarization state to interrogate a surface. The interaction with the surface will cause some level of elliptical polarization in the beam, which can be measured and related to information about the layer structure. As with surface plasmon resonance, using a baseline or model that satisfactorily corresponds to the physical baseline is important because ellipsometry measures the change in the surface relative to that baseline. However, an ellipsometer does not
require a dedicated reference beam as a part of that baseline [22]. Ellipsometry includes information on the phase-shift of the beam that is not used in surface plasmon resonance, but experimental results generally require more intensive model fitting to determine physical parameters. Ellipsometry and surface plasmon resonance systems are physically similar though, and it is often possible to integrate both onto a single device as in [51].

X-ray photoelectron spectroscopy uses incident rays to determine the elemental makeup of the surface of a substance by measuring the kinetics of the surface of a test item. As with ellipsometry and surface plasmon resonance, XPS is also capable of measuring the depth of thin layers. It is additionally capable of chemically characterizing those surfaces. However, unlike ellipsometry and surface plasmon resonance, it requires a vacuum, so often it may not be performed in situ. Furthermore, it generally requires much more time to determine layer thickness, cannot measure layers thicker than 10 nm, and can damage some samples through the exposure, particularly biological components [34].

Quartz crystal microbalance relates the change in vibrational modes of an excited crystal to the kinetics of a reaction occurring in the environment above the crystal surface. It can be used to determine reaction kinetics, and layer deposition rates similar to surface plasmon resonance and ellipsometry. It is also capable of the same in situ measurements in both gas and liquids, but because surface plasmon resonance uses
optical rather than acoustic waves, surface plasmon resonance devices are ultimately capable of a higher precision [40].

Surface plasmon resonance sensing is a valuable addition for biological and chemical studies, especially when studying thin films. Since its original experimental demonstration and commercialization, SPR has seen greater use as a tool in academic research almost every year [43]. It has, and will continue to be an important tool to compliment prior methods, and allow for new research.
Modeling a Surface Plasmon Resonance device

2.1 Plasma oscillations

Surface plasmons are the mechanic by which surface plasmon resonance sensors function. A plasmon is a quantum of the collective longitudinal oscillation of a plasma, collection of electrons and positive ions where at least one of the charge carriers is mobile [13]. Plasma oscillations may be excited by the reflection of photons from a conductive film, or by reflecting or passing an electron through that film [26]. From [26], in the case of the long wavelength dielectric response, the motion of a free electron in a plasma can be described by:

\[ m \frac{d^2 x}{dt^2} = -eE \]  \hspace{1cm} (2.1)

In a time varying electric field, oscillations of the field can be represented as:

\[ E(t) = E(\omega)e^{-i\omega t} \]  \hspace{1cm} (2.2)

The polarization of the system is the dipole moment per unit volume, so for an electron with a dipole moment of:

\[ p = -\frac{e^2 E}{m\omega^2} \]  \hspace{1cm} (2.3)

The dielectric function is defined in terms of electric field and polarization as:
\[ D = \varepsilon_0 E = \varepsilon_0 E + p , \]  

(2.4)

Where D is the electric displacement, used later in the wave equation. Substituting (2.2) and (2.3) into (2.4), and re-arranging, allows us to write the frequency dependent dielectric function as:

\[ \varepsilon(\omega,0) = 1 - \frac{ne^2}{\varepsilon_0 m \omega^2} . \]  

(2.5)

To simplify the relationship, we define the electron plasma frequency to be:

\[ \omega_p^2 \equiv \frac{ne^2}{\varepsilon_0 m} . \]  

(2.6)

This definition allows us to re-write the dielectric function in terms of the plasma frequency as:

\[ \varepsilon(\omega,0) = 1 - \frac{\omega_p^2}{\omega^2} . \]  

(2.7)

This is also known as the Drude term because it can also be found from using the Drude model to study AC conductivity in a metal [4]. Some restrictions on this simplification are a long-wavelength and high-frequency requirement. The Drude model assumes that electrons are thermalized; that their average speed is temperature dependent.

\[ \lambda > v_0 \tau , \]  

(2.8)

\[ \omega \tau >> 1 . \]  

(2.9)
Both the wavelength and frequency constraint are relative to the relaxation time of the media (2.10), which is based on the elements’ resistivity, \( \rho_u \) [\( \mu \Omega \text{-cm} \)], and the ratio between the elements’ electronic density and Bohr radius (2.11) [4].

\[
\tau = \left( \frac{0.22}{\rho_u} \right) \left( \frac{r_x}{\alpha_0} \right)^3 \left(10^{-14}\right) \text{ seconds,} \tag{2.10}
\]

\[
\frac{r_x}{\alpha_0} = \left( \frac{1}{\alpha_0} \right) \left( \frac{3}{4m} \right)^{\frac{1}{3}}. \tag{2.11}
\]

If those assumptions are met, then we may use the frequency dependent dielectric function, (2.7). Additionally, in cases where the sound velocity is less than the Fermi velocity of the electrons, the wavevector contribution to the dielectric function can be modeled as:

\[
\varepsilon(0,k) = 1 + \frac{k^2}{K^2}. \tag{2.12}
\]

This approximation is known as the Thomas-Fermi dielectric function. From (2.7), and (2.12), an approximate frequency and wavevector dependent dielectric function is [26]:

\[
\varepsilon(\omega,K) = 1 - \frac{\omega_p^2}{\omega^2} + \frac{k^2}{K^2}. \tag{2.13}
\]

With the caveat that the behavior at \( \varepsilon(0,0) \) is not well defined because:

\[
\lim_{\omega_p \rightarrow 0} \varepsilon(\omega,0) \neq \lim_{K \rightarrow 0} \varepsilon(0,K). \tag{2.14}
\]
The wave equation for electromagnetic waves in a nonmagnetic isotropic media, such as the glass and gold layer system used in classic surface plasmon resonance, is:

\[ \mu_0 \frac{\partial^2 D}{\partial t^2} = \nabla^2 E \]  \hspace{1cm} (2.15)

The solution will be of the form:

\[ D \propto \varepsilon(\omega, K) E e^{-i\omega t} e^{iK \cdot r} \] \hspace{1cm} (2.16)

Plotting (2.16), a single oscillation is:

Figure 4: A surface plasmon oscillation, after [17].
Plugging (2.7) into (2.16) yields the dispersion relationship:

\[
K^2 = \varepsilon_0 \mu_0 \omega^2 \varepsilon (\omega, K).
\] (2.17)

For the Drude model, this simplifies to:

\[
K^2 = \omega^2 - \varepsilon_0 \mu_0 \omega_p^2.
\] (2.18)

When \( \varepsilon \) is real and positive, \( (\omega_p > \omega) \), the solutions to the wave equation are oscillatory, radiation can propagate, and the metal is transparent to that frequency of excitation. When \( \varepsilon \) is real and negative, \( (\omega_p < \omega) \), then the solutions decay exponentially with a characteristic length inversely related to the wavevector, and plasmons are created [4, 26].

From [26], we can re-write the dielectric function in terms of the square of the refractive index, the optical dielectric constant \( \varepsilon (\infty) \), the longitudinal optical phonon frequency \( \omega_L \), and transverse optical phonon frequency \( \omega_T \).

\[
\varepsilon (\omega) = \frac{\omega_L^2 \varepsilon (0) - \omega^2 \varepsilon (\infty)}{\omega_T^2 - \omega^2}.
\] (2.19)
Plotting (2.19) illustrates the plasmon formation criteria well:

![Figure 5: Dielectric function versus frequency, after [26].](image)

For all frequencies between $\omega_L$ and $\omega_T$, the dielectric constant is negative, the wavevector K is imaginary for a real frequency, and plasmon formation is possible.

### 2.2 Exciting surface plasmons with wavevector matching

To excite surface plasmons, the incident wavevector should match the intrinsic wavevector of the media. When the wavevector of a source is matched to that of the media, phonon-photon coupling creates a response known as surface plasmon resonance. The wavevector of a metal and sample are [12]:

$$ k_{\text{plasmon}} = \frac{2\pi}{\lambda} \sqrt{\frac{\epsilon_{\text{metal}} \epsilon_{\text{sample}}}{\epsilon_{\text{metal}} + \epsilon_{\text{sample}}}}. $$

(2.20)
And the wavevector for an incident source is:

\[ k_{beam} = \frac{2\pi}{\lambda} n_{prism} \sin(\theta) \]  \hspace{1cm} (2.21)

The process of detecting surface plasmon resonance is equivalent to detecting the conditions where wavevector matching occurs, so by setting \( k_{beam} = k_{plasmon} \), it can be shown that:

\[ \theta_{resonance} = \sin^{-1} \left( \sqrt{\frac{\varepsilon_{metal}\varepsilon_{sample}}{\varepsilon_{metal} + \varepsilon_{sample}}} \right) n_{prism} \]  \hspace{1cm} (2.22)

From (2.22), it can be seen that the angle of the incident source, and the properties of the coupling structure and test media that the source traverses, affects the wavevector relationship. From this, a method to check for surface plasmon resonance vis-à-vis wavevector matching would be to scan through a range of angles, measuring the relative intensity of the reflected beam. This is the principle by which the Kretschmann sensor, described in Section 3, operates. An alternative process is to utilize the frequency dependence of the dielectric function, and illuminate the target sample with a broadband source. Wavevector matching will occur at frequencies that match the intrinsic sample wavevectors, and the frequency dependent attenuation can be measured via a spectrophotometer as in [12].
2.3 Optical properties of dielectric materials

In practice, the dielectric function of a media is not directly measurable, so instead a closely related value, optical reflectance is measured instead. Optical reflectance is a composite of the refractive index, \(n(\omega)\), and extinction coefficient, \(\kappa(\omega)\), of a material. At normal incidence, the refractive index and extinction coefficient are related to optical reflectance by [26]:

\[
r(\omega) = \frac{n(\omega) + i\kappa(\omega) - 1}{n(\omega) + i\kappa(\omega) + 1}.
\]

(2.23)

Optical reflectance is related to the dielectric function as:

\[
N(\omega) \equiv n(\omega) + i\kappa(\omega) \equiv \sqrt{\varepsilon(\omega)}.
\]

(2.24)

Where \(N(\omega)\) is the complex index of refraction. Although (2.23) is useful, classic Kretschmann style surface plasmon resonance sensors operate at a fixed frequency and varying angle. To describe this relationship, we use the Fresnel reflectivity and transmissibility coefficients. The Fresnel coefficients describe the behavior of light across system boundaries, and are described by the following relations for reflectance of perpendicular and parallel polarized waves [8]:

\[
r_{\perp} = \frac{N_1 \cos(\theta_{\text{incident}}) - N_2 \cos(\theta_{\text{refracted}})}{N_1 \cos(\theta_{\text{incident}}) + N_2 \cos(\theta_{\text{refracted}})},
\]

(2.25)

\[
r_{\parallel} = \frac{N_2 \cos(\theta_{\text{incident}}) - N_1 \cos(\theta_{\text{refracted}})}{N_2 \cos(\theta_{\text{incident}}) + N_1 \cos(\theta_{\text{refracted}})}.
\]

(2.26)
Perpendicularly polarized, or transverse electric, light cannot excite surface plasmon resonance because the oscillations of the electric field do not couple with the longitudinal modes of vibration of the sample media [12]. They are screened from the physical device using a polarizer, and the analytical solution will similarly subsequently focus on parallel polarized waves. Using Snell’s law, which relates the angle of incidence and refraction of light in isotropic media, it is possible to substitute $\Theta_{\text{incident}}$ for $\Theta_{\text{refracted}}$, and re-write (2.26) in terms of incident angle. For a two-layer, nonmagnetic, isotropic, semi-infinite dielectric media, the Fresnel coefficient for a parallel polarized incident waves is [17]:

$$
R_i = \frac{\left(\frac{N_2}{N_1}\right)^2 \cos(\theta) - i\sqrt{\sin^2(\theta) - \left(\frac{N_2}{N_1}\right)^2}}{\left(\frac{N_2}{N_1}\right)^2 \cos(\theta) + i\sqrt{\sin^2(\theta) - \left(\frac{N_2}{N_1}\right)^2}}
$$

(2.27)

For reflections, the percent reflected power is the square of the Fresnel coefficient. For physical systems, (2.27) can also be written in the form [8]:

$$
R_i = \frac{N_1 \sqrt{1 - \left(\frac{N_1}{N_2}\right)^2 \sin^2(\theta_{\text{incident}}) - \frac{N_1}{N_2} \cos(\theta_{\text{incident}})}}{N_1 \sqrt{1 - \left(\frac{N_1}{N_2}\right)^2 \sin^2(\theta_{\text{incident}}) + \frac{N_1}{N_2} \cos(\theta_{\text{incident}})}}
$$

(2.28)

When $N_1 < N_2$, external reflection results, and when $N_1 > N_2$, internal reflection occurs. If $N_1 = N_2$, then no reflection occurs because there is no optical boundary to interact with.
Plotting (2.28) for both $N_1 = 5$, $N_2 = 1$, and $N_1 = 1$, $N_2 = 5$:

![Graph of internal and external reflection](image)

**Figure 6: Internal and External Reflection of a Parallel Polarized Wave, after [17].**

Kretschmann style plasmon resonance sensors belong to a class of instrument known as attenuated total internal reflection spectrometers, so it is important to the design of these sensors that the layer system allows for total internal reflection over the angular range of interest. The minima and initial maxima of the internal reflectance curve are the principal ($\Theta_p$) and critical angle ($\Theta_c$) respectively, and are defined as:

$$\theta_p = \tan^{-1}\left(\frac{n_2}{n_1}\right),$$  \hspace{1cm} (2.29)

$$\theta_c = \sin^{-1}\left(\frac{n_2}{n_1}\right).$$  \hspace{1cm} (2.30)
Given a normalized first layer index of refraction, $N_1 = 1$, the system reflectance varying $N_2$ and $\Theta$ can be visualized as:

![Figure 7: Reflectivity over $0 < N_2 < 10$, and $0^\circ < \Theta < 90^\circ$ at constant $N_1 = 1$.](image)

A two-dimensional projection of Figure 7 illustrates the $N_2$ dependence on achieving total internal reflection as Figure 6 showed the angular dependence.

![Figure 8: Reflectivity over $0 < N_2 < 100$ at constant $N_1 = 1$, and $\Theta = 45^\circ$, after [17].](image)
2.4 Fresnel coefficients for a multi-layer system

In absorbing media, such as the gold layer used in surface plasmon resonance sensors, the reflectivity will not drop to zero at $\Theta_p$, and $\Theta_c$ will not occur at a sharp boundary. As such the reflectance at normal incidence, (2.23) can be rewritten as reflectance for two layer systems as [17]:

$$ R = \frac{(n_2 - n_1)^2 + n_2^2 \kappa_2^2}{(n_2 + n_1)^2 + n_2^2 \kappa_2^2}. $$

(2.31)

Using this adjustment, more general reflection coefficients can also be rewritten for multilayer systems such as used in the physical setup [9]. The Fresnel reflection coefficient between layer i and j is:

$$ r_{ij} = \frac{N_j \sqrt{N_j^2 - N_{\text{ambient}}^2 \sin^2(\theta)} - N_i \sqrt{N_i^2 - N_{\text{ambient}}^2 \sin^2(\theta)}}{N_j \sqrt{N_j^2 - N_{\text{ambient}}^2 \sin^2(\theta)} + N_i \sqrt{N_i^2 - N_{\text{ambient}}^2 \sin^2(\theta)}}. $$

(2.32)

The reflectance is still the square of the Fresnel reflectivity coefficient as with (2.27) and (2.28). The absorptive term also contributes to a phase shift at each layer by a phase factor $\phi_i$ of:

$$ \phi_i = \frac{2 \pi d_i}{\lambda} \sqrt{N_i^2 - N_{\text{ambient}}^2 \sin^2(\theta)}. $$

(2.33)

Where $d_i$ is the thickness of layer i. For use in numerical simulations, the Fresnel coefficients of an entire layer system can be simultaneously solved using Abelsès matrix representation. For parallel polarized systems, the characteristic matrix describing each layer is [10, 18]:

21
\[
M_i = \begin{bmatrix}
\cos\left(\frac{2\pi}{\lambda} N_i h_i \cos(\theta_i)\right) & \frac{1}{i} \left[-i \frac{\sin\left(\frac{2\pi}{\lambda} N_i h_i \cos(\theta_i)\right)}{\sqrt{\frac{\mu_i}{N_i^2}} \cos(\theta_i)} \right] \\
-i \sqrt{\frac{\mu_i}{N_i^2}} \cos(\theta_i) \sin\left(\frac{2\pi}{\lambda} N_i h_i \cos(\theta_i)\right) & \cos\left(\frac{2\pi}{\lambda} N_i h_i \cos(\theta_i)\right)
\end{bmatrix}
\]

(2.34)

The field strength in an n-layer system and their Fresnel behavior is:

\[
Q_i = M_i^{-1} \prod_{i=1}^{n-1} M_i Q_{i-1}
\]

(2.35)

Where \(Q_i\) is defined as the matrix of the field strengths at each layer as:

\[
Q_i = \begin{bmatrix}
H_y^0 \\
E_z^0
\end{bmatrix}
\]

(2.35)

And the components of \(Q_i\) are the tangential fields at the \(i^{th}\) boundary. For the classical Kretschmann style prism-metal-air system, Figure 9, with the optical characteristics listed in table 1, plotting the solution to the matrix system shows surface plasmon resonance behavior from evanescent field formation.

Figure 9: Simple prism-metal-air Kretschmann SPR layer system.
Table 1: Optical properties of the Kretschmann system presented in Figure 9.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Index of Refraction</th>
<th>Layer Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. N-BK7 90° Prism [50]</td>
<td>1.15109</td>
<td>Semi-infinite</td>
</tr>
<tr>
<td>2. Gold Substrate [42]</td>
<td>0.19715-i3.0899</td>
<td>50 nm</td>
</tr>
<tr>
<td>3. Air (at 20° C and 101.325 kPa) [38]</td>
<td>1.00027</td>
<td>Semi Infinite</td>
</tr>
</tbody>
</table>

Figure 10: Predicted reflectivity for a simple Kretschmann system excited by a 632.8 nm parallel polarized laser source.

The expected minimum of the model is approximately 44.2°, which near the reported minima of similar layer systems [27, 44]. If a thiol monolayer with the
properties listed in table 2 is created on the gold surface, the additional layer will also interact with the evanescent wave of surface plasmons. This causes a shift in the minimum of the surface plasmon resonance curve. Notably, the point at which total internal reflection occurs, the peak near 40°, will remain constant as shown in Figure 11.

**Table 2: Optical properties of a thiol monolayer.**

<table>
<thead>
<tr>
<th>Layer</th>
<th>Index of Refraction</th>
<th>Layer Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiolate from [24]</td>
<td>1.45</td>
<td>2.5 nm</td>
</tr>
</tbody>
</table>

**Figure 11: Prism-gold model from figure 9 exhibiting a shift in plasmon resonance angle when including a 2.5 nm and 5 nm thiolate layer.**

The effective principal angle, when the reflectivity is zero in the prism-air system, and critical angle, the point at which total internal reflection occurs, have
constant reflectivity values regardless of layer thickness in this model. This can be seen by superimposing the reflectivity of the prism-air system over Figure 11.

**Figure 12: Prism-gold-organic model with prism-air reflectivity curve overlay.**

The matrix formulation of the Fresnel coefficients includes information on both the thickness of the layer system, and the dielectric functions of the media in the layer system, so the angular position of the minima of the surface plasmon resonance curve is also affected by changes in the index of refraction of the adlayer as seen in Figure 13.
Figure 13: Prism-gold model exhibiting a shift in plasmon resonance angle when including a 12 nm \( n = 1.4 \) and 12 nm \( n = 1.8 \) layer.

Although the point of total internal reflection remains the same, the shape of the overall curve is different for materials of different indices of refraction because the optical properties of individual layers in the system affect the effective principal angle.

In systems including a dielectric layer, there is an imaginary component in addition to the real index of refraction, as mentioned in (2.24). Varying the extinction coefficient of the dielectric in a layer system affects the width and depth of the reflectance curve for values typical of classic gold and silver substrates as seen in Figures 14, and 15.
Figure 14: Prism-gold model with increasing dielectric extinction coefficients in $\Delta \kappa$ 0.5 increments.

Figure 15: Prism-gold model with decreasing dielectric extinction coefficients in $\Delta \kappa$ 0.5 increments.
Increasing the extinction coefficient of the dielectric in the layer system, Figure 14, shows a narrower but still distinct surface plasmon resonance effect that occurs at a lower angle. Decreasing the extinction coefficient, Figure 15, shows a broader response curve that shifts towards higher angles. As the extinction coefficient approaches zero, the observable surface plasmon resonance behavior fades, and the curve begins to model a reflective surface similar to a mirror. For variations in dielectric function near gold, the effect of extinction coefficient on the curve is most prominently its width and location.

The real component primarily contributes to the depth of the curve minima [44], so a metal with a small real index of refraction, and a large extinction coefficient would result in a narrow and deep curve minimum. This is useful because it allows for finer experimental measurements of the location of the minima. For surface plasmon resonance studies with a 632.8 nm source, typically a Helium-Neon laser, using a silver substrate results in the deepest and narrowest resonance curve. Gold is often used instead because it is easily functionalized for use in biochemical applications and has only a slightly wider baseline curve. Table 3 lists some metals that have been used experimentally [14, 30], and their reflectivities at 632.8 nm are plotted in Figure 16. Typically, aluminum, platinum, and chromium are used at other frequencies where they produce a more typical surface plasmon response. These predicted responses have been experimentally verified elsewhere [14, 30, 45].
Table 3: Indices of refraction at 632.8 nm for common surface plasmon resonance sensor substrates.

<table>
<thead>
<tr>
<th>Substrate Material</th>
<th>Index of Refraction</th>
<th>Figure 16 Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silver Substrate [42]</td>
<td>0.13455-i3.9865</td>
<td>1 (leftmost)</td>
</tr>
<tr>
<td>Gold Substrate [42]</td>
<td>0.19715-i3.0899</td>
<td>1 (rightmost)</td>
</tr>
<tr>
<td>Copper Substrate [42]</td>
<td>0.24905-3.4102</td>
<td>1 (center)</td>
</tr>
<tr>
<td>Platinum Substrate [30]</td>
<td>2.33166-i4.1452</td>
<td>2 (above)</td>
</tr>
<tr>
<td>Chromium Substrate [30]</td>
<td>3.13515-i3.3101</td>
<td>2 (below)</td>
</tr>
</tbody>
</table>

Figure 16: Reflectivities of 50nm thick substrate media from table 3 replacing the metal layer of table 1 with a laser source of a wavelength of 632.8 nm.
In addition to shifts induced by the optical properties of the media and adlayer thickness, the thickness of the substrate, as a member of the layer system, can also affect the shape of the curve. It has been experimentally determined that for a 632.8 nm light source, a gold layer of approximately 50 nm thickness is usually optimal [14, 30]. If the substrate layer is too thin, then the resonance curve minimum shifts to a higher angle, exhibits a wider curve width, and has a higher reflectivity at its minimal value. If the layer is too thick, the curve shifts to a slightly lower angle, but also has a higher reflectivity at the curve minima, as seen in Figure 17.

Figure 17: Effect of layer thickness of a gold substrate on resultant surface plasmon resonance curve.

In situations when the substrate or adlayer grows very thick, the system may exhibit waveguide behavior in lieu of surface plasmon resonance. Waveguide modes
can be excited by both parallel and perpendicularly polarized light, and do propagate. While it is possible to manipulate the layer system to exhibit both waveguide behavior and surface plasmon resonance as a method to further characterize the system, it is not a feature of the classic Kretschmann style surface plasmon resonance sensor.

![Waveguide behavior](image)

**Figure 18:** Prism-gold model exhibiting waveguide behavior from a 900 nm thick layer of index of refraction $n = 1.4$.

The waveguide behavior exhibited in Figure 18 can be distinguished from surface plasmon resonance because there is no peak at the critical angle prior to the dip in reflectivity despite the first minima being near the expected location for a thinner film as seen in figure 11. Waveguide behavior may also include higher modes of oscillations, characterized by multiple local minima in reflectivity over an angular sweep.
2.5 Determining field depth and adlayer thickness

With this understanding of the relationship between angle, index of refraction, and dielectric function, it is useful to characterize the depth of penetration of a surface plasmon from the substrate surface into the analyte media, and the effective thickness of a layer in contact with the dielectric substrate. From equations (2.2), and (2.16), there is an oscillating electric field that is continuous and differentiable across the dielectric boundary, and decays exponentially from the boundary surface. From [17], the distance required for the exponential field amplitude to decay to \( e^{-1} \), referred to as the penetration depth or decay length, is:

\[
\begin{align*}
    d_p &= \frac{\lambda}{2\pi \left[ \sin^2(\theta) - \left( \frac{n_2}{n_1} \right)^2 \right]^\frac{1}{2}} \\

    &\text{(2.36)}
\end{align*}
\]

From the Drude model, the skin penetration depth is inversely proportional to the wavevector of the media, so better conductors will have a faster decay rate and correspondingly smaller penetration depth. If the film thickness is less than the penetration depth of the surface plasmon, then the thickness of the analyte can be determined by linearly interpolating between two or more known thickness to angle relationships for similar materials because the relationship is roughly linear [24, 28]. However, it is also possible to iteratively solve for an additional layer in the system, or analytically solve for layer thickness as [24]:

32
\[ d = \left( \frac{l_d}{2} \right) \ln \left( 1 - \frac{R}{R_{\text{max}}} \right) \]  \hspace{1cm} (2.37)

Where \( R_{\text{max}} \) is the maximum response from an infinitely thick adlayer, and \( l_d \) is the decay length. Rather than use (2.36), an estimation of decay length from Maxwell's equations is used [24]:

\[ l_d = \left( \frac{\lambda}{2\pi} \right) \text{Re} \left( \sqrt{\frac{\eta_{\text{eff}}^2 \varepsilon_{\text{metal}}}{\eta_{\text{eff}}^2 + \varepsilon_{\text{metal}}} - \eta_{\text{eff}}^2} \right) \]  \hspace{1cm} (2.38)

Where \( \eta_{\text{eff}} \) is the effective index of refraction of the analyte material.

### 2.6 Kinetic Measurements

In addition to measuring the reflectivity over an angular sweep, it is also possible to observe the binding and unbinding of an analyte from a surface by measuring the change in the minima of the surface plasmon resonance curve over a time period. The characteristics of the resulting curve depend on both how the analyte(s) and surface(s) interact, and the rate at which they do so. For example, the most simple surface-analyte interaction is when the surface does not change, only a single analyte exists, and it is absorbed or removed based on its equilibrium rates. The equilibrium conditions of the system can be described using the Langmuir model [23]:

\[ [A] + [B] \xrightleftharpoons[k_d]{k_a} [AB] \]  \hspace{1cm} (2.39)

The time dependent representation of (2.39) is:
$$\frac{d[AB]}{dt} = k_a [A][B] - k_d [AB].$$

(2.40)

Visually, the behavior is similar to figure 16:

Figure 19: Typical kinetic surface plasmon resonance curve. (1) Initial baseline, (2) binding to the surface until equilibrium is reached, (3) disassociation from the surface as a buffer wash is introduced, and (4) reestablishment of the baseline value Signal vs. Time.

In more complex cases, it is possible to observe the decay of the substrate, variations in the binding curve if two possibly competing analytes are introduced, or multiple binding curves if multiple subsequent layers are formed. For these more general cases, (2.40) can be rewritten as:

$$d\left[\text{substrate(s) and analyte(s)}\right] = \sum (\text{reactants})(\text{association constant for those events}) - \sum (\text{products})(\text{disassociation constant for those events})$$

(2.41)
3

Experimental Design

3.1 Physical Design

This surface plasmon resonance sensor uses a modified Kretschmann prism arrangement. The sensor, shown in Figure 20, uses equipment rails for all of its optical components to allow for any future additions to the design. Components and materials used in fabrication of the experimental setup are listed in the bill of materials in Appendix D.

Figure 20: Layout of experimental system. (1) Laser mount, (2) Helium-Neon Laser, (3) Steering mirrors, (4) Polarizing cube, (5) Iris, (6) Photo-detector, (7) Prism and mounting hardware, (8) Goniometers

The left side of the device, labeled items 1 through 5 in Figure 20, includes the laser, mirrors, polarizer, and iris, and is used to locate and condition the beam prior to
interacting with the prism and detector. The laser and first steering mirror are located at the rear of the device. Although both are adjustable, they should not have to be adjusted during the day to day operation of the sensor. The second steering mirror is mounted on the front track along with a polarizing cube, and an iris used in the laser alignment process detailed in Appendix A. The iris was left on the track after the alignment process to serve as a beam stop for any off-axis reflections in order to minimize the chance of specular reflections off the second steering mirror.

The second portion of the experimental setup, located at the lower right of Figure 20, contains the prism and sample on a central goniometer, and a detector on a swingarm on the secondary goniometer. Rather than change the angle of the incoming beam by moving the laser, this design rotates the prism relative to a stationary incoming beam. This allows for a large fixed optical path, so items such as the beam polarizer can be added more easily. To maintain focus on the beam, the detector then moves both to compensate for its position relative to the prism, and the changing angle of incidence of the beam relative to the prism. The prism-detector-goniometer system alignment follows the procedure in Appendix C, and uses the prism hypotenuse at a $45^\circ$ position relative to the beam as a home position.
The goal of the alignment is to square the sample and detector system with the beam path, which itself is aligned to be parallel to the table. As demonstrated in the appendices, significant improvements to the alignment were made, and the remaining misalignment was further improved by adjusting the detector goniometer to use the calibration line to determine its position relative to the prism stage.
With an aligned system, the next step in validating the physical setup is to verify the detector independently so that the photodetector baseline can then be used to characterize and validate the rest of the optical system. The components and wiring diagram for the photodetector is in Appendix H. The voltage response of the detector was measured while the detector was inside the laser enclosure with the room lights off to establish a low-light baseline, presented in Appendix E. Following this, the procedure was repeated under the same conditions with the laser source on to check for power fluctuations from the source. These results, reported in Appendix F, suggest that there are significant fluctuations in the output power of the laser during its warm-up period, which is normal and expected according to the manufacturers’ literature [21]. From the results, contained in appendix F, to minimize the variation in the measured data, there is approximately a 50 minute warm-up period for this laser source prior to using the sensor although the system will produce satisfactory results after the recommended 20 minute warm-up period.

To prepare the device for use, follow the sample mounting procedure in Appendix G, ensure that all of the electronics are on, and then run the system control software, detailed below.
3.2 Software Design

In addition to the physical system, a software data collection and control program was written to handle typical experiments. The goniometer motion controller, and photodetector data acquisition board are both controlled from an accompanying computer running LabVIEW 2010. As with the physical system, the code was written to be as modular so that future extensions to the system do not require a rewrite of the code. The program, reproduced in full in Appendix I, is capable of measuring photodetector response over an angular sweep, measuring variations in reflected intensity at an angle over time, and tracking the angle of the minimum measured response over time.

The user interface of the program is organized into four tabs, an introductory tab with operating instructions, Figure 22, a tab for preforming an angular sweep of reflectivity values, Figure 23, a tab for measuring the reflectivity at a fixed angle over time, Figure 24, and a tab for tracking the reflectance minima, Figure 25.
Figure 22: Software user interface front panel.

Figure 23: Reflectance versus angle tab.
Figure 24: Reflectance versus time tab.

Figure 25: Curve minima versus angle tab.
The first tab is a case selection that determines which of the following three logic paths the program uses. Each subsequent tab corresponds to an operating mode of the physical system. Each tab will ask for the system parameters required for that measurement.

**Table 4: Required software parameters for running the test procedures.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Angular sweep</th>
<th>Time sweep</th>
<th>Minima tracking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial angle</td>
<td>Initial search angle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final angle</td>
<td>Number of samples</td>
<td>Search window width</td>
<td></td>
</tr>
<tr>
<td>Increment size</td>
<td>Sampling rate</td>
<td>Samples per search</td>
<td></td>
</tr>
<tr>
<td>Samples per increment</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Given the input parameters from Table 4, the logic followed by the portion of the program used on the angular sweep, time sweep, and kinetic scan tabs are shown below in Figures 26 through 28.
Figure 26: Flowchart for software behavior during an angular sweep.
Figure 27: Flowchart for software behavior during a time sweep.
Figure 28: Flowchart for software behavior while minima tracking.
In the control and data acquisition program, the motion controller, a Newport ESP301, uses two byte operational codes with a two byte prefix and two byte postfix to communicate with the controller rather than standard LABView commands because this is the native language of the motion controller. The photodetector sampling and communication with the data acquisition board is pre-configured through the associated LabVIEW Measurement and Automation program. This simplifies the program by allowing the output data to be sampled by calling the existing LabVIEW reference. Furthermore, any changes in the photodetector are handled by the measurement and automation utility so the control and data acquisition program can function independent of changes to the specific configuration of the sensor.

Each of the procedures will run and produce a graphical output and a data file containing the angle and measured voltage of the sensor at that angle. This can then be related to the properties of the system being tested by comparing it to the layer system model for that system.
Experimental Results

4.1 Baseline results

To validate the experimental system, the sensor was first tested with a clean gold backed slide to establish the baseline behavior of the system. The slide was cleaned using the procedure in Appendix B, and mounted in the sensor using the procedure outlined in Appendix G.

The sensor prism system can be modeled using the layers detailed below in Figure 29 and Table 5. The resulting reflectivity versus angular position model, using the procedure detailed in Section 2, is shown in Figure 30.

Figure 29: Full experimental Kretschmann SPR layer system.
Table 5: Optical properties of the Kretschmann system presented in figure 26

<table>
<thead>
<tr>
<th>Layer</th>
<th>Index of Refraction</th>
<th>Layer Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. N-BK7 90° Prism [50]</td>
<td>1.5151</td>
<td>Arbitrary</td>
</tr>
<tr>
<td>2. Type FF Index-matching fluid [6]</td>
<td>1.4766</td>
<td>≈100 nm</td>
</tr>
<tr>
<td>3. Quartz glass slide [42]</td>
<td>1.4570</td>
<td>≈1000000 nm</td>
</tr>
<tr>
<td>5. Gold Substrate [42]</td>
<td>0.1972-i3.0899</td>
<td>50 nm</td>
</tr>
<tr>
<td>6. Air (at 20° C and 101.325 kPa) [38]</td>
<td>1.00027</td>
<td>Semi Infinite</td>
</tr>
</tbody>
</table>

Figure 30: Predicted response reflectivity for the modified layer system detailed in table 5, excited by a 632.8 nm parallel polarized laser.
Using this more complete model, there is a lower maximum reflectance than with the simple model presented in Section 2. This is due to energy losses to waveguide modes. The large intermediate microscope slide allows for higher order waveguide modes to develop.

Baseline measurements of a clean gold slide were taken, five of which are shown below in Figure 31. Four trials measured from 30° to 60° in 0.05° steps, sampling 20 times per step. One trial swept from 40° to 50° in 0.02° steps, sampling 20 times per step. These parameters were chosen to be more thorough than the 0.1° increment used in a similar surface plasmon resonance sensor design [48]. Each trial required approximately 20 minutes to complete, which is quick enough that the decay of measured laser power, modeled in Appendix F, is negligible. The measured sensor voltage was transformed to percent reflectivity for comparison with the model system.

![Graph](image)

**Figure 31:** Clean slide baseline measurements.
Fitting Figure 31 to the layer model shown in Table 5 was accomplished by first vary ing the prism index of refraction to match the point of total internal reflection. Next, the chromium layer thickness was varied until a match the shape of the curve between the point of total internal reflection and minimum was achieved. Finally the gold layer thickness, real, and imaginary component parameters were used to match the depth and width, depth, and width of the curve respectively [44,48]. Changes to the layer system properties are shown in Table 6 with changed entries bolded. The larger index of refraction of the prism and gold extinction coefficient are representative of the bulk effect of losses across all layers. The model fit is shown in Figure 32.

**Table 6: Properties of the layer model after fitting to experimental results.**

<table>
<thead>
<tr>
<th>Layer</th>
<th>Index of Refraction</th>
<th>Layer Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. N-BK7 90° Prism [50]</td>
<td>1.5</td>
<td>Arbitrary</td>
</tr>
<tr>
<td>2. Type FF Index-matching fluid [6]</td>
<td>1.4766</td>
<td>≈100 nm</td>
</tr>
<tr>
<td>3. Quartz glass slide [42]</td>
<td>1.4570</td>
<td>≈1000000 nm</td>
</tr>
<tr>
<td>5. Gold Substrate [42]</td>
<td>0.17-i3.55</td>
<td>65 nm</td>
</tr>
<tr>
<td>6. Air (at 20° C and 101.325 kPa) [38]</td>
<td>1.000272</td>
<td>Semi Infinite</td>
</tr>
</tbody>
</table>
Figure 32: Clean slide measurements fit to the full layer model.

The experimental curves have a minimum at $43.75^\circ \pm 0.01^\circ$ with a standard deviation of 0.061°, and match the model system with a relative error of 0.23%. In addition to the fit, using this model is reasonable because the deviations from the original model are all favorable such as a narrower experimental curve width than predicted by the model. The more distinct, narrower curve will lead to higher precision measurements when measuring and tracking the curve minima in future experiments, and will allow for a more repeatable baseline. The non-zero reflectivity at the minimum is a result of imperfections and roughness at each layer interface, and would be lessened with a fresh gold slide.
4.2 Angular shift from a self-assembled thiol monolayer

With a consistent baseline, the system can be tested with a known sample to evaluate its performance. Using a clean slide, a self-assembled thiol monolayer was created on the gold surface. The shift in the minima of the surface plasmon resonance curve was measured using the same sampling method used for the baseline, and the results were compared to the baseline to validate the sensors performance.

The self-assembled monolayer was created by immersing the gold backed slide in a mixture of 3 mg of 1-Octadecanethiol per 10 ml of EtOH, sonicating for three minutes, and letting sit for a day. The mixture was then sonicated again, the slide was removed, rinsed in EtOH, and dried in N\textsubscript{2}. The slide was used immediately afterwards.

The resulting experimental measurements show a shift of $0.47° \pm 0.1°$ from the baseline measurements, shown in Figures 33 and 34. Grey points represent baseline measurements, and black points represent measurements taken after adding the thiol monolayer to the gold surface.
Figure 33: Baseline and Gold with thiol monolayer experimental results.

Figure 34: Detail of baseline and gold with thiol experimental results near resonance ($\Delta \theta = 0.45^\circ$).

The gold with thiol sample is fit to the theoretical curve by using the previous baseline model with an additional layer between the gold and environment layers. This layer represents the bulk properties of the thiol. The index of refraction of the additional
layer is held constant, and the thickness is increased until a match is found with the experimental data. The chosen thiol has an index of refraction of $n = 1.4589$ at 20°C, and an expected layer thickness of 2.1 nm ± 0.2 nm [37].

![Graph showing reflectivity vs. angle](image)

**Figure 35: Gold with thiol monolayer measurements fit to the full layer model.**

The model layer thickness that matches the model minimum to the experimental minimum indicates the deposited layer has a thickness of 2.5 nm ± 0.2 nm. This is physically reasonable. Although not identical, it may differ from the value in [37] for reasons discussed in the next section.
4.3 Possible sources of uncertainty and error

Most likely, the monolayer in literature was at a 30° angle to the surface [37]. If the monolayer formed on the slide was slightly different, it would result in a different measured length.

Another source of uncertainty in the measured minimum is the discretization of the data. According to the manufacturer, the goniometers have an absolute accuracy of 0.030° ± 0.015°. There is some variability when translating the angular accuracy of the goniometers into the accuracy in measuring the thickness of an analyte layer because there is also a dependence on the optical properties and existing thickness of the layer system, so there is no one value for the uncertainty this introduces. For the specific layer system used in the experiments above, this limits the sensor to a resolution of approximately 0.05 nm for small changes in layer thickness.

A possible source of error is the temperature dependence of the measurements. In addition to the wavelength dependence of a materials index of refraction, there is a dependence on the temperature of the system and changes in layer composition of that system. Temperature is mostly held constant by the building climate control system, but is subject to some variation. The average ambient temperature for the experiments over the entire span of tests was 23.6 °C, with a 6.5 °C range as shown in figure 36.
Figure 36: Sampled ambient temperature near sensor over a 4 month period, plotted by time of day the sample was taken.

This variation in temperature can cause a shift in the minimum of the surface plasmon resonance curve of $3.3 \times 10^{-5}^\circ$. From additional measurements, the layer system can be up to 2 °C warmer after an extended period of use, and the combined effect can cause an error in the measured layer thickness of up to 0.05 nm.

A final possible source of error is a result of the label-free nature of surface plasmon resonance sensors. “Label-free” means that the surface plasmon resonance curve is sensitive to any changes in the layer system or sensing surface regardless of if they are intentional or not. To illustrate the effects of a “dirty” gold surface, a clean slide was mounted in the sensor and left for one month. The laser enclosure was left over the device, but otherwise no steps were taken to prevent the slide surface from becoming dirty.
Figure 37: Detail of the curve minimum of a slide left in the environment relative to a recently cleaned and recently cleaned and thiolated slide.

Although the slide looked visibly clean after the waiting period, there is a consistent and noticeable shift in the curve minima from the freshly cleaned slide results. This contamination is equivalent to a 1 nm thiol layer. Some thiols form monolayers that are approximately 1 nm in height, so an unintentionally dirty slide can introduce significant error [15]. However, for the validation tests, the slides were used immediately after cleaning, so this is likely not a source of error in the reported data.
5.1 Thoughts on the manufacture and testing of a surface plasmon resonance sensor

The goal of the project was to manufacture a surface plasmon resonance sensor, and then validate its performance so that it can be used as a tool in other experiments. The device was built from existing lab materials, and tested against a known sample. The resulting experimental baseline shows measurements consistent with a correctly functioning device, and the measured shift in the minimum of the curve for the test case demonstrated a system precision of 0.2 nm.

Sources of error were explored and addressed. Remaining sources of error were characterized by the uncertainty they introduced so that if necessary, future work on the system could be focused and efficient. This sensor will increase the capability of the laboratory to perform surface plasmon resonance based experiments. The project was successful in achieving its goal, and will contribute to the work done by the research community at Duke.
6

Future Work

6.1 Improvements to the sensor

The system, while producing repeatable, consistent, and physically reasonable data, does have room for further improvement. These improvements can be divided into two general classes, improvements to further minimize uncertainty in the system, and changes to add new capabilities to the system.

6.2 Improvements addressing error in the sensor

Improvements possible within the current system include recalibration of the system with higher precision. While the current alignment is sufficient, any increase in the accuracy of the alignment of the components will further decrease errors. It may be possible to achieve this by laying out stationary components with a micrometer, and welding them in place so that there is no chance for the components to shift over time.

Another area for improvement to the current sensor is the design of the photodetector. Currently, the wiring ties the 9 Volt power source and one of the photodiode leads to the non-inverting input and ground pin of the op-amp. An improvement would be to isolate the battery from the op-amp input. The battery leads would instead go to the supply pin for the op-amp through a 5 Volt regulator, and the ground provided by the BNC cable. The BNC ground is tied to the actual ground
through the data acquisition board. The photodiode could either go to the input pins to provide a differential measurement, or to the input pins and ground to provide a constant reference relative to the ground. Currently, when providing power to the op-amp, the resulting data is vertically mirrored so that the location of the minima is represented as a peak in the data. The measured curve also lacks the distinct peak at the point of total internal reflection although there is a minimum at that angle.

![Figure 38: Measured response when powering the photodetector op-amp.](image)

A cleaner amplified signal may allow for better comparison with the theoretical model layer system.

### 6.3 Improvements adding further functionality to the sensor

One source of error examined but not fully addressed is the power fluctuation of the laser source. To compensate for this beyond observing an adequate warm-up period, a beam splitter and second fixed photodiode could be used to measure the
power fluctuations of the beam. The data could then be scaled to this dynamic baseline to remove the influence changing output intensity may have on the system [39].

It is also possible to compensate for thermal and bulk composition effects on the system using a reference beam that does not interact with the analyte. This can be accomplished by using a beam splitter to send the beam through a different section of the prism system that is outside of the test area. The beam, split from the same source, would reflect power fluctuations of the source. The measured response would also reflect any change in layer composition and temperature because both beams interact with the same physical layer system [39]. Similar approaches include using a split beam to interact with a duplicate set-up that serves as a reference cell although this does assume that the differences between the sample and reference cells are negligible [33].

In addition to controlling for the variation in temperature when measuring reaction kinetics, it can also be important to work in a certain temperature range, such as when trying to mimic biological conditions. To accomplish this, a thermocouple and thermoelectric cell such as a peltier device could be attached to the mounting hardware for the prism, or a smaller device could be mounted directly onto a portion of the sample slide that does not interact with the laser beam.

Although not exhaustive, these possible changes to the system should serve as a useful set of possibilities when considering potential changes to the device.
Appendix A: Aligning a laser-mirror system

Although the procedure described in “Walking the beam” [3] is useable, it suffers from two shortcomings that can be addressed with a more specific procedure. First, because the procedure relies solely on mirror angle, the guaranteed convergence relies on the assumption of infinitely large plane mirrors. Unless initial mirror positions are chosen well, the laser beam can track off of the mirror system entirely while adjusting the system. Second, it lacks the degrees of freedom to adjust the beam path prior to the final mirror.

By acknowledging the additional degrees of freedom afforded by translating the mirror parallel to the work surface, the beam path can be aligned for both final, and intermediary positions. Using this, it is possible to keep the beam parallel to the hardware rails and underlying bolt pattern on the table. This is advantageous because future additions to the system such as a beam chopper, additional lenses, or a beam splitter to another detector, can now be mounted to the rail system without realignment. The below procedure also constrains the system to the height of the final detector, again so future changes are simpler to install.

Describing the system such that $d_1$ through $d_4$ are the intra-element spacing distance, $\Theta_1$ and $\Theta_2$ are mirror angle relative to the incoming beam, and $O_1$ and $O_2$ are the beam offsets relative to the center of each iris, then the system can be drawn as such:
Figure 39: Laser-mirror layout for the SPR system, also showing two irises to be used in the beam alignment procedure, and a misaligned second mirror.

If we treat the mirrors as black-box devices that change the beam angle by the same behavior as a real mirror, the system can be re-drawn on a line. By examining a simplified version of the system, it will be clearer why:

Figure 40: Simplified one-mirror system.

It is trivial to write the offset at each iris:

Offset 1: \[ d_2 \tan(\theta_1) \]  
Offset 2: \[ (d_2 + d_3)\tan(\theta_1) \]

As is shown, the ray only has one in-plane degree of freedom. If the irises were offset from the centerline of the laser, as they are in the actual setup, one mirror can not position the beam correctly. To position the beam, a two-mirror system should be used:
Figure 41: Simplified two-mirror system.

Using the same procedure as the one-mirror system, the offsets containing both influences from the first, and second mirror, can be written.

Offset 1: 
\[ (d_2) \tan(\theta_1) + (d_1) \tan(\theta_1 + \theta_2), \]  \hspace{1cm} (A3)

Offset 2: 
\[ (d_2) \tan(\theta_1) + (d_3 + d_4) \tan(\theta_1 + \theta_2). \]  \hspace{1cm} (A4)

By using the first mirror to influence beam position, and the second mirror to influence beam angle, it is possible to correctly position the beam. Hypothetically, by measuring the angle of the beam leaving the laser source, and the angle of the first and second mirror, it is possible to align the beam for the target at 180°. However, remembering that in the physical setup, \(d_2\) acts as a horizontal offset relative to the bottom rail, it is possible, and desirable to change both the position and angle of the mirrors to reach the final alignment.

To align the beam, first move the laser source onto the track facing the target with the two irises between the laser and the target. Mount a mirror to the target surface, and adjust the laser by itself such that the beam reflected off the mirror hits the laser aperture. This behavior means the laser is at the same height, and perpendicular
to, the mirror surface. Now adjust the irises so that they allow the beam to pass through their center when closed to the beam diameter.

Now re-mount the laser in its operating position, and move the irises onto the rear track with the laser. The laser should be in reasonable alignment, but verifying will demonstrate the alignment procedure. Begin with the laser in its un-calibrated state:

**Figure 42: Laser-iris system with a misaligned laser.**

Now raise or lower the laser so that the beam passes through the first iris when that iris is closed to the laser beam diameter.

**Figure 43: Laser-iris system aligned with the first iris.**
Next, open that iris as necessary, and adjust the pitch of the laser so that the beam passes through the second iris.

![Figure 44: Laser-iris system aligned with the second iris.](image)

Now iterate between vertical alignment with the first iris, and rotational alignment with the second iris until the desired level of alignment is reached.

![Figure 45: Laser-iris system aligned with both the first and second irises.](image)

This procedure of iterating between translation and rotation is the same as will be used in the subsequent mirror alignment.

Mount the first mirror onto the track holding the laser, and mount the two irises on the second track so that they are parallel to each other, and perpendicular to the beam leaving the laser.
In this configuration, by iterating the translation of the irises and the rotation of the mirror, then the system will converge on a beam path that bends 90° at the first mirror by allowing the degree of freedom introduced by the iris translation to result in an arbitrary distance, $d_1$. Note that $d_1$ does not influence the alignment of the system as per (A3), and (A4).

Once an acceptable level of error in beam distance from the centerline has been reached, mount the irises on the second rail so that the final beam path passes through the apertures, and the second mirror is where the irises were during the alignment of the first mirror. Noticing that translation of the irises will not be effective, use translation of a slightly off-center second mirror coupled with the rotation of that mirror to translate and rotate the beam. The prism and detector are attached directly to the equipment table rather than either of the equipment rails. To ensure that the second beam approaches the prism and detector at the correct angle, the final alignment should consider alignment against irises located both on the equipment tack, and directly on the
table surface. If the two are not in agreement, adjust the track position rather than mirror position so that the system is in alignment with both the track, and underlying equipment table.

![Diagram of laser-mirror-iris system for aligning the second mirror.](image)

**Figure 47: Laser-mirror-iris system for aligning the second mirror.**

Once the iterations to align the second mirror are finished, the alignment procedure is complete. Although aligning the mirrors one at a time by using a rotational and translation degrees of freedom may require more iterations than aligning the system only using the two degrees of freedom afforded by mirror rotation, the final alignment, as stated, will be convenient when adding other components such as the polarizing cube in use on the device.
Appendix B: Cleaning procedure for gold coated slides

The cleaning procedure for SPR chips was provided in personal communications with a peer at Duke University, Greg Hardy, on January 11th, 2012. The cleaning procedure makes laboratory-specific references, and is reproduced here with additional comments to illustrate the cleaning process.

To clean an existing gold-coated SPR slide:

1. In cases where the slide is particularly dirty, pre-clean visible dirt with Milli-Q water and a squeeze bottle of 2% by weight Sodium dodecyl sulfate, \( \text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na} \), abbreviated SDS.

2. Incubate the gold coated coverslip in 2% by weight SDS for 30 minutes.

3. Sonicate the incubation vial containing the slide and SDS solution for 3 minutes at a power level of 3. SDS is a surfactant, and is used to clean the surface of oils. It may however leave streaks on the glass unless further cleaning is performed.

4. Remove the slide from the vial using tweezers to grasp an area that will not be interacted with by the laser, and wash in Milli-Q H\(_2\)O, taking care to hold the slide such that possible contaminants from the tweezers do not wash across the surface of the slide.

5. Dry the slide with Nitrogen, N\(_2\).
6. Place the slide on a support that does not touch the center area of the slide (which will eventually interact with the SPR laser). A small glass petri dish is appropriate. Do not use a plastic dish in the UV/Ozone cleaner.

7. Place the dish and slide into the UV/Ozone cleaner, open the lab vacuum line slightly, and turn on the cleaner for 10 minutes. It will be useful to lift the top of the cleaner out of the way prior to step 3, so the chamber is already accessible. The UV/Ozone cleaner and subsequent wash are to remove hydrocarbons that may have accumulated on the surface. After 10 minutes, turn off the lamps, and open the vacuum line further to clear the ozone from the chamber before opening it.

   a. While the slide is in the UV/Ozone cleaner, fill a vial with acetone in preparation for sonicating the slide in acetone after the UV/Ozone cleaner.

   b. Additionally, while the slide is in the UV/Ozone cleaner, prepare RCA solution for later use. To note, only step one of RCA SC-1 will be performed—do not prepare any HF or HCl for the subsequent RCA Cleaning steps. RCA Cleaning was chosen in lieu of using Piranha (varying combinations of H₂SO₄ and H₂O₂) because the two preform similar functions in this procedure and RCA is ‘milder’. RCA Solution is:

      i.  5 Parts Milli-Q water (H₂O)

      ii. 1 Part Hydrogen peroxide (H₂O₂)

      iii. 1 Part Ammonium Hydroxide (NH₄OH)
iv. Heat the RCA solution to 80° C, a very light boil.

8. Sonicate the slide in acetone, (CH₃)₂CO, for 3 minutes at power 3.

9. Rinse excessively with Milli-Q water, following the previous procedure to avoid contaminating the sample from the tweezers. Empty the acetone from the vial, and rinse similarly well.

10. Place the slide in the vial, and fill with RCA solution. Let sit for 5 minutes.

11. Dispose of the RCA solution in the sink, and rinse the slide and vial excessively with Milli-Q water.

12. Dry with N₂.

13. Place back into the UV/Ozone cleaner for 5 minutes, using the previous procedure.

14. Rinse, don’t sonicate, with acetone.

15. Rinse in Milli-Q water.

16. Dry with N₂.

17. Use immediately, or store in a clean location.
Appendix C: Aligning the prism-detector goniometer system

The goniometer system consists of an inner goniometer that actuates the prism, and an outer goniometer that controls the photodetector. The inner goniometer also contains a horizontal translation stage, and a pitch and roll stage for fine adjustments, and the outer goniometer has a three-axis translational mounting platform. Beginning with a properly aligned laser-mirror system, from following the procedure outlined in appendix A, the goniometer system can be aligned relative to the incoming beam by following a similar procedure.

![Figure 48: Simplified goniometer system sketch, showing prim column, detector swing-arm, and potential beam path.](image)

Recall first that from an initial alignment achieved with a bubble level, the prism pitch and roll stage was leveled relative to the beam by reflecting the beam off a mirror.
mounted in the sample clamp, and adjusting the pitch and roll until the reflected beam followed the same path as the incoming beam. It is important to preserve this prior alignment, so do not adjust the pitch and roll of the system when aligning the goniometers.

In an ideal system with a mirror in the sample location, the angle of the incoming and outgoing beam relative to normal to the mirror surface would be identical. That is to say that:

\[ \text{Ideal: } \theta_{\text{detector}} = 2\theta_{\text{prism}} \]  \hspace{1cm} (C1)

To this end, the goal of the goniometer system alignment is to shift the system prism angle versus detector angle curve closer to the ideal curve. The initial hypothesis is that deviation in the y-intercept from its ideal value of 0 is caused by misalignment of the goniometers relative to each other, and deviation from the ideal slope of 2 are a result of off-axis misalignment of the prism relative to the beam, beam walk caused by changing path lengths as the prism rotates, and additional effects including temperature. Deviations in y-intercept should result from relative misalignment of the goniometers because translations on the y-axis represent rotation of the stages relative to each other. Deviations in slope are more ambiguous because it is coupled with information about the prism, allowing factors not represented by just the angular position of the prism to influence it.
To test this hypothesis, an initial angular sweep of the prism from -15.0° to 15.0° was conducted. The detector angle was stepped in 0.1° increments from -30.0° to 30.0°, noting the angle at which the beam was visually centered on the detector. The following scatterplot shows the 61 initial samples, a least-squares trend line, and a dashed line representing the ideal curve.

![Scatterplot showing initial prism-detector angular relationship.](image)

**Figure 49: Initial prism-detector angular relationship.**

The resulting trend line is:

\[
\text{Initial: } \theta_{\text{detector}} = 1.877\theta_{\text{prism}} - 0.946 \quad (C2)
\]

The trend line has a coefficient of determination of \( R^2 = 0.99997 \), which means that 99.997% of the variation of the required detector angle is explained by variation in the prism angle. This is useful because it implies that by changing the relative alignment of the goniometers, a change fully described by the linear model, that the system behavior can be improved. Checking the residual error of the linear model of the system reveals a
non-random residual indicative that while the linear model may fit the system well, there are additional nonlinear effects.

Figure 50: Residual error of the initial prism-detector system.

Despite the evidence of non-linear effects, the high coefficient of determination suggests that the non-linear effects are much less influential than the linear effects. Furthermore, addressing the linear effects of misalignment will make way for a more clear path to address the nonlinear effects later.

To begin the alignment procedure, rotate the detector out of the beam path, and place the irises on the table in the position of the ideal beam path. Loosen the bolts affixing the goniometer to the table, and shift and rotate the goniometer as far as possible to one corner.
Alternate between shifting the goniometer left and right, and rotating as per the same procedure followed for aligning the mirrors, align the goniometer as much as the play in the system allows. The prism column includes a translational stage, so it is more important to achieve a beam path perpendicular to both iris openings rather than one that passes through one at the cost of and angular misalignment. Once this point is reached, carefully but firmly tighten the bolts on the prism goniometer.

Now, adjust for any offset using the horizontal traverse on the prism column. This should also place the reflective surface on the axis of rotation due to the previously enforced beam alignment constraints.
Once the prism goniometer is aligned, rotate the detector back to its default position on its goniometer. Now, using the same procedure as for the prism, align the system such that the laser is centered on the photodetector, first through rough manipulation of play in the bolts for mounting the goniometer to the table, and finally with the three-axis traverse on the clamping system holding the photodetector. Maintaining the detector alignment, slide its clamping structure closer to the prism stage to minimize the effects that any residual misalignment may have on the system. To verify that the beam is aligned, repeat the sampling procedure initially preformed, generating another regression line to compare to the ideal curve.
Figure 53: Adjusted prism-detector angular relationship.

The post-adjustment relationship, shown with triangles is more similar to the ideal curve than the initial plot, shown with diamonds, a conclusion supported by the linear model of the adjusted dataset.

\[
\text{Adjusted: } \theta_{\text{detector}} = 1.957\theta_{\text{prism}} + 0.146
\]  \hspace{1cm} (C3)

Not only is the post adjustment line closer to the ideal curve, it is also consistent, with a coefficient of determination of $R^2 = 0.9999$, that is 99.99% of the variation in the angle of the prism can be explained by the linear model (C3). The residual plot still reflects a nonlinear behavior, albeit without the same level of confounding effects from misalignment with the linear model. The initial data is again shown in diamonds, and the post-alignment data is shown with triangles.
Figure 54: Residual error of the adjusted prism-detector system.

The residual of the adjusted model is more clear after adjusting to minimize the linear error. Before proceeding with the data analysis, check the residual error against a model of the beam walk as a result of the different thickness of prism experienced by the laser at different angles. Beginning from an expression to draw the triangle hypotenuse:

\[ \text{Hypotenuse: } y = h - x \]  \hspace{1cm} (C4)

Next, we draw a ray from the origin to the surface of the prism, and then express the Cartesian location as a function of theta.

Figure 55: Ray tracing the hypotenuse of a right-angle prism.
Tracing ray: \( y_{\text{trace}} = mx \), \( \quad \text{(C5)} \)

Parameterize slope in terms of \( \theta \): \( y_{\text{trace}} = \tan(\theta)x \), \( \quad \text{(C6)} \)

Combine and Solve: \( h - x = \tan(\theta)x \), \( \quad \text{(C7)} \)

\[
x = \frac{h}{\tan(\theta) + 1},
\]

\( \quad \text{(C8)} \)

\[
y = h\frac{\tan(\theta)}{\tan(\theta) + 1},
\]

\( \quad \text{(C9)} \)

Ray length: \( |\vec{r}| = \sqrt{x^2 + y^2} = \sqrt{\frac{h^2}{(\sin(\theta) + \cos(\theta))^2}} \).

\( \quad \text{(C10)} \)

The prism, a THOR Labs model PS911 N-BK7 Borosilicate glass right angle prism, has a leg length of 25 mm [50]. As such, the apparent thickness of the prism relative to the angle of incidence of the laser beam is:

![Figure 56: Apparent thickness of a PS911 prism relative to incident beam angle.](image)
The curving behavior of the residual data seems to follow a similar pattern as the apparent thickness of the prism, so to check, we can find and compare the curvature of the prism model against the curvature of a model of the adjusted residuals. Rather than attempt to fit a similar model as the actual curvature or the prism, (C11), we approximate both the prism, and residual data with a second order polynomial fit.

Prism Curvature: \[ \kappa = \frac{75}{(\sin(\theta) + \cos(\theta))^3} - \frac{50\sin(\theta)\cos(\theta)}{(\sin(\theta) + \cos(\theta))^4} \left[ 1 + \left( \frac{25(\sin(\theta) - \cos(\theta))}{\sin(2\theta) + 1} \right)^2 \right] \] (C11)

Prism Curvature (2nd order approximation): \[ \kappa = \frac{0.0056}{\left[ 1 + (0.0056\theta - 0.2486)^2 \right]^{\frac{3}{2}}} \] (C12)

Residual Curvature (2nd order approximation): \[ \kappa = \frac{-0.0006}{\left[ 1 + (0.0006\theta - 0.00004)^2 \right]^{\frac{3}{2}}} \] (C13)

Figure 57: Comparison of the curvature of the prism and residual data.
It is clear that while the change in apparent thickness of the prism may influence the beam, that the curvature exhibited by the residual data is not well explained by the curvature of the prism apparent thickness profile. However, an alternative explanation can explain the variation within the curvature of the data.

By plotting a connected scatterplot, the residuals take on a somewhat saw tooth appearance.

![Connected scatterplot of the adjusted residuals.](image)

**Figure 58: Connected scatterplot of the adjusted residuals.**

Comparing the peaks in the plot against the raw data, the peaks often correspond to the last data point before the least significant digit of the data changes, which suggests that some of the remaining error is due to the precision of the data, specifically referring to the 0.1° step size of the detector when determining the angle at which the beam is centered on the photodetector. Plotting the relative difference
between each point and the point immediately next from -15° to 15°, results in a scatterplot with three clear bands.

![Figure 59: Relative difference between subsequent residual points.](image)

Not only does every point on the low band correspond with a change of the least significant digit, but both points on the high band correspond to the only two occasions where the least significant digit changes on both the entry before and after. As such, it is reasonable to state that the oscillations within the remaining error of the residuals can be explained as an artifact of the 0.1° sampling precision.

Although this does not also explain the overall curvature of the residual error, because the linear model, equation (C3), has such a high coefficient of determination, using the current model without further modification is acceptable.

Accepting both the initial and adjusted linear models, it is important to establish that in addition to the models explaining variation in the system, that the adjustments
also made a significant improvement in the system. This can be accomplished by plotting the 95% confidence envelopes for both linear models, and observing in figure 60 that neither line falls fully within the confidence envelope of the other.

![Figure 60: Prism Angle versus Detector Angle Required to Center Beam.](image)

It is also possible to check the plot by using the test for the analysis of covariance between the regression lines, and the regression lines and the ideal curve. Testing first to verify the homogeneity of the two regressions, we find that there is only a 0.001% change that the calculated regression lines correspond to data that does not differ significantly. Although this means that we cannot use ANCOVA, we have proven that the alignment procedure caused a statistically significant change in the adjustment.
We similarly find that while the adjustments resulted in a 3.9 percentage point decrease in the error relative to the theoretical curve, from 6.04% error to 2.15% error, the adjusted line still has a distinct slope from the ideal curve, in part from the pattern in the residual of the model. Notwithstanding, the alignment procedure is successful because it produced a measureable, significant improvement in the data. The calibration curve does not suggest that our device does not follow the ideal relationship from (C1), only that there was a residual physical eccentricity and offset that can be corrected for by using the experimental curve.
Appendix D: Bill of Materials

In the following bill of materials, parts were chosen with a variety of considerations, including prior availability in the laboratory, and compatibility with existing components.

Table 7: Commercial of the shelf items used in physical system.

<table>
<thead>
<tr>
<th>COTS Item</th>
<th>Qty.</th>
<th>Supplier and Specifications</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser and power supply</td>
<td>1</td>
<td>JDS Uniphase 35 mW HeNe 632.8 nm, 0.7 mm diameter laser</td>
<td>1145P</td>
</tr>
<tr>
<td>Equipment Rail</td>
<td>1</td>
<td>Melles-Griot 1m 100 mm wide</td>
<td>07 ORP 007</td>
</tr>
<tr>
<td>Rail Clamps</td>
<td>7</td>
<td>Melles-Griot Rail 40 mm and 65 mm wide rail carriers</td>
<td>07ORP441</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>07ORP443</td>
</tr>
<tr>
<td>Equipment Posts</td>
<td>6</td>
<td>Melles-Griot equipment posts</td>
<td>50-4</td>
</tr>
<tr>
<td>Equipment Post Holders</td>
<td>6</td>
<td>Melles-Griot post holders</td>
<td>07 PHM 007</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Newport post holders</td>
<td>VPH-3</td>
</tr>
<tr>
<td>Equipment Table</td>
<td>1</td>
<td>Newport 4’x3’x2” breadboard</td>
<td>RG-34-2</td>
</tr>
<tr>
<td>1’ x 1’ Upper Table</td>
<td>1</td>
<td>Newport optical breadboard</td>
<td>SA2-11</td>
</tr>
<tr>
<td>Laser Clamp</td>
<td>1</td>
<td>Newport adjustable clamp</td>
<td>ULM-TILT</td>
</tr>
<tr>
<td>Iris</td>
<td>2</td>
<td>Newport 1.5-25 mm aperture</td>
<td>M-ID-1.0</td>
</tr>
<tr>
<td>Goniometer</td>
<td>2</td>
<td>Newport precision goniometer</td>
<td>URS150BPP</td>
</tr>
</tbody>
</table>

86
<table>
<thead>
<tr>
<th>Category</th>
<th>Quantity</th>
<th>Description</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motion Controller</td>
<td>1</td>
<td>Newport USB ICS</td>
<td>ESP301-2G</td>
</tr>
<tr>
<td>1-axis translational stage</td>
<td>2</td>
<td>Newport 1” travel alignment</td>
<td>TSX-1D</td>
</tr>
<tr>
<td>2-axis translational stage</td>
<td>1</td>
<td>Newport 1” travel XY stage</td>
<td>460A-XY</td>
</tr>
<tr>
<td>2-axis tilt stage</td>
<td>1</td>
<td>Newport -3.5° to 14°, -5° to 16°</td>
<td>39</td>
</tr>
<tr>
<td>Mirrors</td>
<td>2</td>
<td>THOR Labs 400-750 nm mirror</td>
<td>BB1-E02</td>
</tr>
<tr>
<td>Mirror Clamps</td>
<td>2</td>
<td>THOR Labs Kinematic mount</td>
<td>KM-100</td>
</tr>
<tr>
<td>Polarizing Cube</td>
<td>1</td>
<td>THOR Labs 420-680 nm polarizing beam splitting cube</td>
<td>PBS251</td>
</tr>
<tr>
<td>Polarizing Cube Clamp</td>
<td>1</td>
<td>THOR Labs cube mount</td>
<td>CM1</td>
</tr>
<tr>
<td>Prism</td>
<td>1</td>
<td>THOR Labs N-BK7 90° prism</td>
<td>PS911</td>
</tr>
<tr>
<td>Data Acquisition Board</td>
<td>1</td>
<td>National Instruments</td>
<td>NI-USB 6259</td>
</tr>
<tr>
<td>PEEK Tubing</td>
<td>5 ft</td>
<td>Upchurch Scientific 1/16 in OD</td>
<td>1531</td>
</tr>
<tr>
<td>Luer-lock fittings</td>
<td>2</td>
<td>Thermo Scientific 10-32 female luer-lock fitting</td>
<td>P-629</td>
</tr>
<tr>
<td>Tubing mounting bolts</td>
<td>2</td>
<td>IDEX flangeless tubing fittings</td>
<td>XLT-111X</td>
</tr>
</tbody>
</table>
Table 8: Raw materials used in manufacture of parts for the physical system.

<table>
<thead>
<tr>
<th>Raw Item</th>
<th>Qty.</th>
<th>Supplier and Specifications</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolts (¼-20)</td>
<td>1 box</td>
<td>McMaster-Carr 1” full thread</td>
<td>91251A542</td>
</tr>
<tr>
<td>Swingarm plate and</td>
<td>2</td>
<td>McMaster-Carr 6”x12”x0.25”</td>
<td>8975K439</td>
</tr>
<tr>
<td>Laser clamp stock</td>
<td></td>
<td>6061 aluminum plate</td>
<td></td>
</tr>
<tr>
<td>Drip Plate</td>
<td>1</td>
<td>McMaster-Carr 6”x12”x0.5”</td>
<td>8975K442</td>
</tr>
<tr>
<td>Prism Column</td>
<td>1</td>
<td>McMaster-Carr 2.5”x12”</td>
<td>8974K771</td>
</tr>
<tr>
<td>Column Base</td>
<td>1</td>
<td>McMaster-Carr 5”x0.25”</td>
<td>9035K39</td>
</tr>
<tr>
<td>1” wide extruded aluminum</td>
<td>12”</td>
<td>McMaster-Carr</td>
<td>4065T101</td>
</tr>
<tr>
<td>1.5” wide extruded aluminum</td>
<td>15”</td>
<td>McMaster-Carr</td>
<td>4065T103</td>
</tr>
</tbody>
</table>

Table 9: Auxiliary and specialty items used in physical system.

<table>
<thead>
<tr>
<th>Auxiliary Item</th>
<th>Qty.</th>
<th>Supplier and Specifications</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass slide</td>
<td>1</td>
<td>VWR Plain Micro Slides</td>
<td>48300-025</td>
</tr>
<tr>
<td>Immersion Oil</td>
<td>5 ml</td>
<td>Cargille Labs Type FF oil</td>
<td>16212</td>
</tr>
<tr>
<td>Photodetector</td>
<td>1</td>
<td>See Table 10 for internals</td>
<td>Custom</td>
</tr>
<tr>
<td>Laser Saftey Glasses</td>
<td>1</td>
<td>Glendale Dalloz Laser Line</td>
<td>GPT LGF 31-40157</td>
</tr>
</tbody>
</table>
Table 10: Photodetector internal components.

<table>
<thead>
<tr>
<th>Photodetector Item</th>
<th>Qty.</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photodiode</td>
<td>1</td>
<td>Centronic OSD 5-0</td>
</tr>
<tr>
<td>BNC Connector</td>
<td>1</td>
<td>Amphenol 31-10 75Ω BNC Plug</td>
</tr>
<tr>
<td>Op-amp</td>
<td>1</td>
<td>Texas Instruments TLC272BCP</td>
</tr>
<tr>
<td>Power switch</td>
<td>1</td>
<td>Unbranded, rated: 6A at 125VAC, 3A 250VAC</td>
</tr>
<tr>
<td>Sensitivity selector</td>
<td>1</td>
<td>TYCO Electronics MTA206N Switch</td>
</tr>
<tr>
<td>Resistors</td>
<td>2</td>
<td>1 MΩ ± 5%</td>
</tr>
<tr>
<td>Resistor</td>
<td>1</td>
<td>0.1 MΩ ± 5%</td>
</tr>
<tr>
<td>Power LED Resistor</td>
<td>1</td>
<td>2.7 k Ω ± 5%</td>
</tr>
<tr>
<td>Potentiometer</td>
<td>1</td>
<td>Acting as rheostat, 0.1 M Ω ± 10% Linear scaling</td>
</tr>
</tbody>
</table>

As noted in Section 3, the initial parts manufacture was completed prior to the project beginning. Table 8, the raw materials used in the manufacture of the device, list a selection of materials that could be used to manufacture the components to illustrate a possible choice. Model and catalog numbers in all other tables are exact to the component used in the project.
Appendix E: Photodetector precision and accuracy

To check the photodetector system precision and accuracy, first a baseline of the detector without the laser was taken. To minimize the influence of other light sources, the test was performed with the enclosure over the system, and the room lights off. The data acquisition board was initially set to measure a floating source. When a LabVIEW data acquisition board is set to measure a floating source, the negative terminal is tied to the system ground through a 4.99 kΩ resistor, and the signal differential is measured.

![Figure 61: Photodetector in darkness, sampling at 5 Hz for 122 minutes using a floating reference.](image)

The banding in the data shows the accuracy of the photodetector-data acquisition system to be, $3.2 \times 10^{-5}$ volts. The photodetector itself outputs an analog signal, so the discrete measurement levels are likely a result of the binning occurring to the data as it is interpreted by the data acquisition board. There is no significant drift in the data over the sampling period. Plotting the samples by occurrence per voltage level results in:
Figure 62: Histogram of occurrence of photodetector voltages in baseline using a floating reference.

There seems to be a slight non-normality in the data, an assumption supported by the off-ideal tails in the quantile-quantile plot which do not fall on the model line.

Figure 63: Normalized quantile-quantile plot of baseline voltage data for a floating reference.
Using the Anderson-Darling test for normality, there is some evidence to suggest that the data does not follow a normal distribution, which agrees with the quantile-quantile plot. However, the non-normality is slight, with a skew of 0.113 relative to an ideal of 0, and a kurtosis of 3.075 relative to an ideal fourth moment of 3.

The photodetector was also tested with the data acquisition board set to measure a grounded reference source, with the results plotted in Figure 64:

![Figure 64: Photodetector in darkness, sampling at 5 Hz for 120 minutes using a grounded reference.](image)

The same digital binning every $3.2 \times 10^{-5}$ volts appears in this data, and again the data has no significant drift in baseline measurement over the sampling period. The distribution of samples by samples per bin is:
Figure 65: Histogram of occurrence of photodetector voltages in baseline using a grounded reference.

The data visually seems normally distributed, and the quantile-quantile plot of the data, Figure 66, seems similarly closer to normal although with slightly larger tails:

Figure 66: Normalized quantile-quantile plot of baseline voltage data for a grounded reference source.
The data collected with the data acquisition board set to measure a grounded reference has a baseline mean of $1.24 \times 10^{-4}$ volts, with a standard deviation of $1.44 \times 10^{-4}$ volts. It is slightly skewed to the right, with a skew of -0.227, and has slightly larger tails than a normal distribution with a kurtosis of 3.165. However, using the Anderson-darling, we find that there is not enough evidence to reject the default hypothesis that the data is distributed normally.

Despite the potential of a more normally distributed curve, using the floating reference measurement setting is preferable for all subsequent tests because the noise on the floating reference baseline curve is also very nearly normally distributed, and the measured values are much more consistent, as seen in Figure 67:

![Figure 67](image)

**Figure 67: Histogram of occurrence of photodetector voltages in baseline for both floating and grounded references.**

For data collection, the data acquisition board should be set to measure a floating reference. For modeling purposes, because the system is near-normal, and for
this purpose slightly better than normal, a population of simulated measurements with the photodetector can be expressed as the sum of the value and a normally distributed noise component where the noise has a baseline mean of $1.18 \times 10^{-4}$ volts, and a standard deviation of $4.18 \times 10^{-5}$ volts:

$$f(v) = v + N(\mu_{baseline}, \sigma)$$

(E1)

Qualitatively, the interpretation of these results is that the photodetector electronics are not tied to the analog ground, so using the floating reference is proper, and that using this reference, the precision and accuracy of the photodetector and data acquisition board system is sufficient to be useful in this experimental system.

This disagrees with the schematic generated by disassembling the detector, shown in Appendix H because the outer contact of the BNC connector should serve as a connection to the analog ground through the cable shield and data acquisition board. This disagreement could possibly result from either a poor ground that is not serving as a true ground, or filtering occurring on the data acquisition board. The LABView documentation specifies that in the grounded reference position, the analog ground is not actually grounded, but it tied to the ground through a 4.99 kΩ resistor [36]. It is possible then that the photodetector ground is not a true ground, and the floating reference setting produced more consistent data, so subsequent experimental trials will use this setting.
Appendix F: Characterization of laser power fluctuations

Given the stable photodetector baseline presented in Appendix E, laser power fluctuations can be characterized by measuring the photodetector voltage at a constant angle over a long time span, and observing changes relative to the baseline. The prism-detector system was left in its home position, and the following data was recorded:

![Graph showing photodetector signal over time]

**Figure 68: Photodetector with the laser on, sampling for 12 hours.**

Most notably, there is a large drop in power in the first hour of recording. For HeNe has lasers this is typical behavior, and the laser manufacturer recommends a 20 minute warm-up period to reach 95% power [21]. The dotted line in figure 69 marks this 20 minute warm-up window.
Figure 69: First hour of the laser-prism-photodetector baseline.

At twenty minutes, there has been a mean 11.5% change in the measured voltage. Between the 20 minutes warm-up and 1 hour, there is another 3.5% change in the mean value. From Figure 68, there is still decay in the signal after 12 hours, so rather than wait for the baseline to possibly become stationary, it is more efficient to find a time after which the error introduced by the drift of measured signal intensity is acceptable.

Plotting the change in the standard deviation of the second trial shows that the variation in the samples decreases until approximately 55 minutes have passed. After this point, the change in the variability of the sampled values is less than 3% of the original sampled mean, and there is little further change.
Figure 70: Change in standard deviation of 1 hour samples with sampling.

By plotting the rate of change of the sampled data, Figure 71, and the drift of the measured mean value over twelve hours, Figure 72, it seems that after the initial warm-up period, there are few visually noticeable sources of error other than the drift of the mean.

Figure 71: Rate of change of the sampled data.
Figure 72: Drift of the measured mean value over twelve hours.

The rate of change in the signal from Figure 71 seems to be distributed about a constant value, which would suggest that the error is a linear process. However, plotting the residuals of a linear best fit line yields:

Figure 73: Residual values of a linear best fit line.
The distinct pattern suggests that the proper model choice is not a linear one. Another option that also correlates well with the data is an exponential decay with a very large time constant. An exponential model has the following residual plot:

![Residual values of an exponential best fit curve](image)

**Figure 74: Residual values of an exponential best fit curve.**

The lack of noise in the pattern suggests that there is an exponential component to the model, and the model explains over 99% of the relationship between measured voltage and time. The remaining linear behavior in the residuals is likely an artifact of the numerical precision used to generate the best-fit curve. This is supported by decreasing the precision and noting that this causes the slope and intercept of the linear residual plot to shift. This remaining numerical error is not important because it falls below the precision required. Rescaling the y-axis to reflect the range of voltages measured after the warm-up period better shows Figure 74 in context.
Figure 75: Rescaled residual values of an exponential best fit curve for the data collected between 50 minutes and 4 hours.

From this, it is likely that this model, shown below, reasonably explains the drift.

$$f(t) = 0.0036e^{-\frac{t}{5x10}} - 0.4907$$ (F2)

In the context of the experiment, the time constant of the drift is such that even for a wide 60° angular sweep, which takes approximately 8 minutes to complete, photodetector drift will introduce less than 0.1% error relative to the measurement signal. Over eight hours, there was only an average of 9.8% drift in baseline measurement, which while higher than the published 2% maximum drift for the laser, is likely caused by a combination of the laser and photodetector [21].
Appendix G: Procedure for mounting slides in the sensor

Before mounting a slide, check that the system is still properly aligned as described in appendices A and C. It is not necessary to re-align the device before using it if it has not been moved after its previous alignment. When mounting a test sample, begin with a clean metal-backed glass slide. For this system, gold-backed slides will be used. Gold is one of the metal surfaces that experiences surface plasmon resonance when excited using a helium-neon laser. It is also easily functionalized for use in biological tests, and is widely used in literature [43].

Gold-backed slides are prepared by using an evaporative deposition process. To bond the gold and glass layers, an intermediary 1 nm thick titanium or chromium layer is often used. Imperfections on any surface in the slide-chromium-gold system will affect the formation of surface plasmons, and the surface interfaces will degrade over time, so it is best to use the slides immediately after manufacture. Despite surface degradation, the slides can be reused for some period provided they are properly cleaned prior to use. A procedure for cleaning gold slides is presented in Appendix B.

Prior to working with the prism, slides, or handling either of the mirrors, observe general laboratory cleanliness procedures, and use clean gloves, taking care not to touch the portion of the surface that will interact with the sample or beam. If working with the laser, check that proper safety procedures are followed, particularly that eye protection
is worn. The laser is a class 3B 30 mW 632.8 nm helium-neon laser, and requires 1.5 OD protection goggles [11].

1. To use the slide in this setup, first remove the prism clamping mechanism, the inner portion of the upper clamp in figure 21 indicated by marker 3.

2. Lay the clamp horizontally on the work surface in preparation for loading the sample.

3. Place the clean gold slide, gold side down, on the fluid cell or across the clamping surface such that the gold that will interact with the laser is not contaminated by touching any surface.
   a. If using the fluid cell, load the fluid cell into the clamping mechanism first, and place the gold slide and prism onto the fluid cell.

4. Using a micropipette, deposit 10 μL of index-matching immersion oil on the glass surface of slide at the location the prism will be placed over.

5. Place the prism in its desired orientation, and place the assembly onto the clamping surface, again taking care not to contaminate the section of the slide that will interact with the laser.

6. Tighten the clamp only until the prism and slide do not move when the clamp is inclined.

7. Lift the entire clamp and slide system, and slide it into the outer clamp structure.

8. Tighten the retaining bolts on the outer clamp structure.
9. If testing the slide system in air, proceed to use the sensor after allowing for a 20 to 50 minute initial warm-up period for the laser source as determined in appendix F.

   a. If using the fluid cell, slide the tubing into the hollow plastic mounting bolts for the fluid system. Place a conical washer onto the end of the bolt, and tighten into the rear of the fluid cell. If they are not already in place, tighten the male Luer-lock to 2.5 mm tubing adapter over the available end of the tubing. Standard Luer-lock syringes will interface with the adapter.

   Depending on the index matching material or oil used, the sample can be left in its vertical mounting bracket for many days without issue. Errors that would require re-cleaning and re-mounting the sample system include using either too much or too little index matching fluid. If too much fluid is used, the fluid can leak onto the gold side of the gold coated slide. Owing to the label-free nature of surface plasmon resonance, the sensor response will shift based on the thickness of the contamination. Using the full simulated system, presented in table 5 and reproduced below in Figure 76 as a model baseline, a 10 nm contaminant film would produce a response similar to Figure 77. With a contaminant layer this thick, the error would typically obscure any measurement of the actual analyte. If too little index matching fluid is used, or air bubbles are trapped in the fluid, the prism and glass side of the slide will not be fully optically mated to each
other. If an air bubble half the height of the index matching fluid is in the beam path, the model system predicts a general increase in variation across all measured values, shown below in Figure 76.

Figure 76: Simulated full layer system response using layers listed in table 5.
Figure 77: Simulated full layer system with contamination on the gold surface.

Figure 78: Simulated full system with an air bubble in the index matching fluid.
Other errors generally also include contamination one of the prism or slide surfaces, and will cause similar effects to those predicted above. Imperfections in the layer system result in an increase in losses through higher order waveguide modes. Imperfection on the surface of the gold will affect the position of the predicted minima, and the width of the curve minima.
Appendix H: Photodetector circuit layout

The photodetector had been manufactured for a prior project, but is included here because of its importance to the experimental setup of the surface plasmon resonance sensor.

Figure 79: Photodetector circuit diagram.

The op-amp in Figure 79 has the following pinout. Note that the three unwired pins correspond to an unused second op-amp.

![Diagram of op-amp pinout]

- Output 1
- Supply voltage $V_{DD} = 5$ Volts
- Negative signal input 1
- Output 2
- Positive signal input 1
- Negative signal input 2
- Ground
- Positive signal input 2

**Figure 80: Op-amp pinout, from [49].**

The photodetector produces a useable signal despite some issues with supply voltage, and has been successfully used in previous experiments including those involving a laser source, so the photodetector will be used unmodified for the experimental trials conducted as a part of this thesis. To note, the system actually produces a qualitatively higher fidelity signal when the photodetector power switch is in its off position, possibly because the photodiode is able to drive the system and produce an output when the power switch is off, and when the power is on, the battery contributes to the signal input.
Appendix I: Sensor control and data acquisition code

The code for the experimental system is written in LabVIEW 2010. LabVIEW is a language which uses drag and drop graphical modules to represent specific code behavior and lines between those blocks to represent data flow rather than lines of code. Figure 81, the image of the code used for the system, is broken into multiple pages of content so that the code is large enough to be readable. Circles with common numbers denote two areas of code that logically follow the other in the program, but are not physically near each other such as switch statements where one case is represented on the page and the other cases are linked with a numbered circle. A pentagon with a number inside corresponds to two sections of code that are physically related, two adjacent sections of code that are split across the page so that the image is large enough to be readable.

The program initialization is the first code logically encountered. Cases of the operating mode switch are represented on the subsequent pages.
Program Overview:
1) Push LabVIEW "Run"
2) Initial minima and width are given to program
3) Motion Begins
4) Data Collection Phase
5) Program takes one set of data samples
6) Program averages and finds the minima of the set
7) Program sets new search center as previous minima
8) Repeat (3) through (6) until stop is pressed
9) Motion Stops
10) Write data to file
11) Program ends

ESP301 Motion Controller Parameters
- Input XON/XOFF
- Input Idle Hardware
- Input HW Hardware
- Output XON/XOFF
- Output Idle Hardware
- Output HW Hardware
- Buffer size: 1024
- \( S = \text{COM} \) port number
- Baud rate: 9600
- Data bits: 8
- Stop bits: 1 bit
- Parity: No parity
- Terminator: CR/LF

The values for the theoretical initial
minima are:
IPA: 1.5327
SPM: 3.0654
ES831 Motion Controller Initialization

Axis 1: Read Character
Loop until No Motion

Main Program Loop

Poll Controller
For Motion Stop

If True, read characters in serial port
Loop until no motion "P" is received

ESP Movement Communication:
Ideally, there is no shift, and
a 1:1 ratio between the stages.
This results from an experimental calibration from
January 24th, 2012.

(See Above)

1.957
0.146
The values for the theoretical minimum are:
IPA: 1.5327
SPA: 3.0651
Figure 81: Control and data acquisition code used by the physical system.

The code has three major sections, labeled with the numbered circles 1 through 3. These three sections correspond to the three operating modes of the system, shown in Section 3, Figures 26 through 28.
Bibliography


135


[36] National Instruments. NI-USB 6259 Data Acquisition Board

[38] NIST Engineering Meterology Toolbox.  


[47] SHOTT. “Optical Glass Data Sheets.”  


