DEVELOPMENT OF A NONINVASIVE *IN VIVO*
GLUCOSE SENSOR BASED ON MID-INFRARED
QUANTUM CASCADE LASER SPECTROSCOPY

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Abstract

Diabetes mellitus impacts over 340 million people across the world. Diabetics must monitor their glucose levels multiple times a day and currently, the most accurate method of doing so involves pricking one’s finger, an often-painful procedure. This dissertation documents research aimed at developing a noninvasive in vivo glucose sensor, with the motivation of implementing a pain-free means of measuring glucose for diabetics. We utilize a novel approach based on mid-infrared (mid-IR) Quantum Cascade (QC) laser spectroscopy. While previous optical solutions using near-IR light between 1300-1900 nm have failed due to limits on glucose absorption feature specificity, the mid-IR region between 8 – 10 μm contains strong absorption features with cross-sections up to four orders of magnitude greater than their near-IR counterparts. Although mid-IR spectroscopy has traditionally been neglected for in vivo applications due to the lack of light sources capable of sufficient skin penetration, the advent of powerful QC lasers allows us to overcome this limitation. Here, theoretical background of light-matter interaction is presented, followed by discussion of experimental research progressing towards noninvasive glucose sensing. First, the feasibility of mid-IR noninvasive glucose sensing is shown through measurements of angular scattering patterns in skin, which show that QC laser light can penetrate deep enough into skin. Next, we show clinically accurate sensing of physiological glucose concentrations in vitro using partial least squares regression (PLSR) analysis on mid-IR transmission spectra of proxy solutions for dermal interstitial fluid. Finally, we record breakthrough results showing clinically accurate glucose prediction capability in vivo with human subjects using a setup featuring hollow-core fiber optics, liquid nitrogen cooled detection, and PLSR analysis. This sensor is then made more
compact by replacing the fibers with an integrating sphere that significantly increases the collection efficiency of scattered light from skin. This allows the sensor to be housed on a mobile cart, and it removes the system’s nitrogen dependency by allowing the use of a thermoelectrically cooled detector, all while maintaining glucose sensing accuracy. We conclude with an outlook for the improved portable sensor to move on from laboratory trials with volunteers to large-scale trials in diabetes clinics.
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Chapter 1

A Novel Approach for Noninvasive in vivo Glucose Sensing

Diabetes mellitus is a condition that impacts over 340 million people across the world [1], including over 29 million Americans [2], according to the World Health Organization and the United States Center for Disease Control. Furthermore, the number of diagnosed diabetics is rapidly increasing and will continue to increase due to combinations of poor diet and genetics, according to both government and independent studies [1-6]. The glucose molecule plays a significant role in the life of individuals afflicted with diabetes; diabetics are unable to regulate their glucose levels to safe levels through their metabolism alone. Diabetics are defined as individuals whose blood glucose concentration does not naturally decrease below 140 mg/dL within two hours after eating [2, 3, 47, 8]. In many cases, due to irregular glucose metabolism, diabetics can also reach a state where their blood glucose content is dangerously low (below 70 mg/dL) – this condition is
referred to as hypoglycemia. The inability to metabolize glucose leads to both short-term inconveniences and the development of long-term complications for diabetics.

Diabetics are faced with daily adversity, as they need to carefully portion every meal to ensure a modest sugar consumption, and in the case of diabetics who develop the condition later in life, abandon foods they enjoy and are accustomed to. Even with a proper diet, their condition can make them prone to episodes of dizziness, nausea, and shock. Over the course of a diabetic’s lifetime, the disease takes a toll on the body through various metabolic disturbances; a diabetic is more prone to high blood pressure, heart disease, blindness, nerve damage, kidney failure, and the need for amputation due to ulcers [8-17]. Despite these seemingly bleak outcomes, diabetes can be regulated if an individual is regularly aware of their blood glucose levels and adjusts their diet and takes proper medication accordingly.

Monitoring blood glucose concentration is essential for a diabetic. This needs to be done three to five times a day in order to ensure that glucose levels do not become dangerously high or low [18]. Diabetics regulate their condition through a combination of insulin supplements, orally administered medication, and diet regulation. The vast majority of diagnosed diabetics need insulin and/or medication to safely deal with their condition, as shown in Fig. 1.1. Currently, the most accurate and accessible method for obtaining blood glucose concentration readings is through the use of an electrochemical sensor. These sensors convert an electric signal, produced after glucose within a sample reacts with an enzyme, into a numerical value for glucose concentration [18-23]. Technology for electrochemical sensors has matured to the point where pocket-sized handheld monitors with
Fig. 1.1: Breakdown of different forms of diabetes regulation by a sample of the United States diabetic population. Diabetes is usually regulated through orally administered medication and/or insulin supplements. Only 16% of sampled diabetic were able to regulate their condition through diet alone. In any case, multiple readings of blood glucose concentration are required daily in order for proper regulation. The statistical information shown in this figure has been obtained courtesy of the US CDC [2].

United Stated Food and Drug Administration (FDA) approved accuracy can be purchased for around $50. FDA approved self-monitoring blood glucose meters are required to achieve accuracies that deviate no more than 10% from a lab-standard reference measurement of a given glucose concentration between 30-400 mg/dL. However, the functionality of such monitors requires measurements of blood samples placed onto test strips, meaning that diabetics need to draw blood from their fingers multiple times a day in order to manage their diabetes.
To this day, invasive techniques are the only accepted forms of glucose monitoring. While automated implantable monitors that can inject insulin into a diabetic's body at the required time have become available [24], these still need to be embedded within the skin while remaining somewhat bulky, causing some discomfort. Furthermore, users of completely automated systems are at the mercy of either computational or mechanical malfunctions, which may result in catastrophe. Because of this, diabetics who use implantable devices usually measure their glucose with finger pricks as well. The ideal way to manage diabetes would be the ability to quickly obtain accurate, pain-free measurements of blood glucose concentrations at any given time, which would leave the individual at liberty to decide upon the proper action to undertake. Current commercial electrochemical sensors allow for quick and accurate measurements, but they do not satisfy the pain-free condition. The realization of an accurate and portable means of noninvasively sensing glucose, which is the motivation for this thesis research, would thus tremendously enhance the quality of life for the hundreds of millions of diabetics throughout the world.

Because of the lack of a convenient, pain-free glucose sensing mechanism in the commercial market, techniques for noninvasive glucose sensing have been studied for years, primarily optical methods. The glucose molecule, C₆H₁₂O₆, is a large molecule with many bonds, which leads to several distinct resonances in various wavelength regions [25-29], including a near-IR band between 1500-2500 nm and distinct mid-IR features between 8 – 10 µm (Fig. 1.2). The goal of optical noninvasive glucose sensing techniques, whether they are absorption, scattering,
Fig. 1.2: a) Normalized absorption spectra of glucose (red) and water (blue) between 1500 – 2500 nm, as shown in [25] (©IEEE). Windows of low water absorption and high glucose absorption, such as 1500-1900 nm and 2100-2300 nm are the primary targets for near-IR based noninvasive glucose sensing research. b) Normalized absorption spectra of glucose measured in the mid-IR between 1000-1250 cm\(^{-1}\) (corresponding to 8 – 10 μm). Mid-IR glucose absorption features have a higher cross-section and larger specificity.

Raman, or photoacoustic based, is to utilize those resonances; higher glucose concentrations will absorb more light at resonant wavelengths. The near-IR and mid-IR wavelengths for glucose absorption each have unique strengths and weaknesses.

Light in the 1500-2500 nm near-IR region has high penetration capability into skin, due to relatively low water absorption; the skin's dermis is 70% water. Also, the existence of commercial fiber-optic technology for the wavelength region allows for precise beam
delivery and collection, and powerful light sources are commercially available at relatively cheap prices in this region, allowing for the rapid advancement of sensor development. However, the absorption features in the near-IR are only moderately strong. On the other hand, mid-infrared vibrational absorption features for many molecules (including glucose) have been quantified to be the strongest known, with up to four orders of magnitude larger absorption cross sections than their near-IR counterparts. This has led to the mid-IR wavelength region to be dubbed as a molecular “fingerprint” region [30]. This advantage is mitigated by the high water absorption in this region; the penetration depth of light with wavelengths greater than 3 μm in skin is extremely shallow. For this reason, some researchers have expressed doubt that the mid-IR region would be suitable for in vivo biomedical study [31, 32]. Therefore, for the past two decades, the near-IR wavelength region between 1300-1900 nm had emerged as the area of choice to focus efforts for a noninvasive glucose sensor.

Due to the immeasurable impact a successful sensor would have, many groups attempted to solve the problem with unique optical approaches such as photoacoustic sensing, Raman spectroscopy, and direct absorption spectroscopy [33-41]. Despite demonstration of accurate in vitro sensing capabilities [27-29], however, progress plateaus because of fundamental limitations of the near-infrared region. Between 1300-1900 nm, the absorption features of other proteins and acids overlap glucose features, which lead to daily calibration requirements and an inability to effectively isolate the contributions of glucose in vivo, despite the use of advanced multivariate analysis [34-37]. Therefore, a noninvasive sensor is not commercially available yet, and progress in optical noninvasive glucose sensing has been stagnant, until now.
In order to break through the barriers limiting progress on noninvasive glucose sensing research, we approach the problem from a new and previously unreported perspective – the mid-IR wavelength region. This approach is made possible through the advent of commercialized Quantum Cascade (QC) lasers, which can output high enough pulsed power to penetrate deeper into the skin than any other previous mid-IR light source while remaining under thresholds for skin damage. These lasers have progressed rapidly, beginning with their first demonstration in 1994 [42], which led to countless optimizations to their design, culminating in broadband, efficient, highly tunable, and powerful lasers in the present day [43-48]. Applications driving QC Laser development and commercialization throughout the past decade include environmental trace gas sensing [49-51], military countermeasures [52], and \textit{in vitro} diagnostics through breath sensing [53-54]. We seek to extend the bounds of QC Laser spectroscopy into a new realm – \textit{in vivo} diagnostics – through the development of our glucose sensor.

\textbf{1.1: Thesis Organization}

The unsolved problem of accurate and accessible noninvasive glucose detection fits nicely with the strengths of the newly available QC lasers and the physical benefits of stronger absorption features in the mid-IR. We reasoned that the unprecedented power from QC lasers would help overcome high water absorption and allow for the detection of light scattered from skin at depths previously deemed unreachable. We set out to develop a noninvasive glucose sensor with this new technology. Due to a lack of literature in the mid-IR region regarding \textit{in vivo} sensing of biological molecules, including glucose, we would first need to show evidence that light from QC lasers could penetrate deep enough into skin
for the sensor to be feasible \textit{in vivo}. Next, we needed to demonstrate a proof of concept by showing that mid-IR absorption spectroscopy is sensitive enough to detect physiologically relevant concentrations of glucose \textit{in vitro}. If those two steps proved to be successful, we would be able to run trials on human volunteers. The following chapters of this thesis document the journey we took in developing a mid-IR based noninvasive glucose sensor. Chapter 2 offers a theoretical overview of the wavelength dependence of material properties, and how these material properties impact scattering and absorption. In Chapter 3, experimental determinations of scattering properties in porcine and human skin are discussed, confirming the ability of QC laser light to penetrate into the dermis layer of skin. Chapter 4 documents an experimental demonstration of clinically accurate glucose sensing on physiologically relevant glucose solutions \textit{in vitro}. Finally, Chapters 5 and 6 report the success of laboratory demonstrations of noninvasive \textit{in vivo} glucose sensing on human volunteers.
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Chapter 2

Theoretical Background:

Wavelength Dependence of Material Properties

and Impact on Scattering and Absorption

In order to tackle the challenge of noninvasively detecting glucose *in vivo* in human subjects using mid-infrared (mid-IR) spectra, we need to begin with a general understanding how light interacts with materials. From this, we can delve into the unique effects caused by the use of mid-IR light, which is a novel approach in this field. For glucose sensing, as well as any type of spectroscopic sensing, the physical mechanisms at play involve light penetration into a medium (skin in our case), interaction molecules (scattering, transmission, reflection), and absorption when coming into contact with the molecule of interest (glucose). Thus, this chapter will cover general light-matter interaction, the interesting cases of scattering, and finally the laws that govern absorption.
Please note that, where applicable, bolded variables in expressions represent vectors, while non-bold terms represent scalars.

### 2.1: Wavelength Dependence of Material Properties

Because light is a form of electromagnetic radiation, its interaction with any type of matter is governed by the effect that the forces of its incident electric and magnetic fields have on the electric charges of the matter. These interactions are dependent on the wavelength of the light, the size and composition of the matter, and the field strength. In electromagnetic theory, these material properties are quantified into a complex function $\varepsilon$, with real and imaginary components, both dependent on frequency. This can be expressed as:

$$\varepsilon(\omega) = \varepsilon_1(\omega) + i\varepsilon_2(\omega)$$ \hspace{1cm} (2.1)

Here, $\varepsilon(\omega)$ defines a material’s permittivity, which expresses the force between two charges in the material. When an electromagnetic field (i.e. light) interacts with a matter, the force of its electromagnetic field displaces electrons inside the matter. The electrons oscillate with the incident field, while the particles in the atomic nuclei usually do not react unless the light is of a very low frequency (wavelength greater than 15 µm, which corresponds to frequencies of 20 THz and smaller) [1]. The displacement of the electrons causes the atoms inside the matter to become an electric dipole, which is defined as a separation of opposite (positive and negative) charges.

The electric field contribution of these dipoles leads to a net polarization of the material, which is usually denoted with $\mathbf{P}$. This term is related to the electric field $\mathbf{E}$ using the following relation:
\[ P = \varepsilon_0 \chi E \] 

(2.2)

In this expression, \( \varepsilon_0 \) is an electromagnetic constant (permittivity in vacuum – approximately 8.85 pF/m), and \( \chi \) is a tensor that represents the susceptibility of the material, or in other words, its response to an applied field. Expression 2.2 can be expanded into a Taylor series with each term representing the summation of an increased order of \( \chi \) and \( E \). For typical field strengths (\( |E| < 100 \text{ V/m} \)), only the first term contributes significantly to the net polarization, and this the material is said to behave linearly. At larger fields, however, condensed matter is impacted by higher order terms, and thus said to display optical nonlinearities [2].

If there are no nonlinear effects to be considered, the force of the light’s time-harmonic electromagnetic field causes the displaced charges to function as a damped harmonic oscillator. In such a model (Fig 2.1), the total electrical force ends up being a summation of a force that includes removal from inertia, damping caused by friction, and a spring-like repulsive force that causes the oscillations. Here, a charge with mass \( m \) oscillates between two points with displacement \( r \), brought out of inertia by the electric field, but propelled towards its starting location due to a spring-like force with a spring constant \( D \). Its oscillations are dampened by
Fig 2.1: Forces acting on an oscillating dipole with an incident electric field. Here, the blue circle represents a charged particle being attracted to an opposite charge to its right in a damped harmonic oscillator model. The force of the particle leaving inertia, which is quantified by a product of its mass and acceleration, is countered by an elastic force with spring constant D. An additional damping force accounting for perturbations caused by other interactions and lattice defects in the system is represented by $m \gamma v$, which is a product of mass, velocity, and frictional constant $\gamma$. The total contribution of these interactions results in the electrical force $eE$. These interactions are also expressed in expression 2.3.

Friction, which depends on its velocity $v$ and a frictional constant $\gamma$. The summation of these interactions can be expressed as follows:

$$\sum_j m_j \frac{d^2 r_j}{dt^2} + m_j \gamma_j \frac{d^2 r_j}{dt} + D_j r_j = \sum_j e_j E(t)$$  \hspace{1cm} (2.3)
The solutions of these differential equations are covered in standard electromagnetics and optics textbooks. A solution for $r_j$ can be substituted into the macroscopic polarization relationship (Eq. 2), which yields an expression for the susceptibility $\chi$ with respect to $\omega$. Based on the relationship between net polarization, permittivity, and susceptibility, a frequency dependent expression is obtained for the material dielectric constant $\varepsilon(\omega)$, as follows:

$$\varepsilon(\omega) = 1 + \sum_j \frac{\omega_p^2}{\omega_j^2 - \omega^2 - i\gamma_j}$$

(2.4)

Here, $\omega$ represents the frequency of the light, while $\omega_p$ represents the plasma frequency, which quantifies the rate of electron oscillations in a material. This parameter depends on properties such as mass, electron lifetime, and conductivity; discussions regarding plasma frequency can be found in typical condensed matter textbooks [3,4].

The dielectric constant of a material is the basis for defining material properties in electromagnetics. Often when dealing with light absorption and scattering, it is convenient to express this constant in the form of the refractive index (typically denoted by $n=n+i\kappa$), which is a dimensionless measurement of the propagation of light within a material. Maxwell’s relation [1] states that refractive index relates to dielectric constant in the following manner:

$$n(\omega) + i\kappa(\omega) = \sqrt{\varepsilon_1(\omega) + i\varepsilon_2(\omega)}$$

(2.5)

As can be seen in expression 2.5, the refractive index is a complex term as well. The real part, $n$, represents light propagation capability, while the imaginary part, $\kappa$, quantifies light absorption within the medium. Both parts are frequency dependent.
As can be seen from expressions 2.1 through 2.5, a material’s properties depend on the frequency of the electromagnetic wave it interacts with. This frequency dependence means a wavelength dependence also exists, due to the relationship between the frequency of an electromagnetic wave and its wavelength:

$$\omega = \frac{c}{\lambda}$$

(2.6)

Here, $\omega$ represents frequency, $\lambda$ represents wavelength, and $c$ represents the speed of light (approximately $3 \times 10^8$ m/s).

### 2.2: Light Scattering Theory (Mie’s Theory)

The previous section discussed the basic mechanisms that lead to a wavelength dependence of material properties. Now, we will examine the changes that occur to the light itself after interaction with matter. We know from introductory physics that light incident onto an interface with bulk material can reflect, transmit, and be absorbed. For the majority of cases where experiments are done on bulk material, the basic principles (Snell’s Law) can be applied to describe the parameters of the light’s electromagnetic field at different points of the interaction. However, when interacting with particles with sizes about the order of the light’s wavelength, more interesting phenomena occur, and they exceed the complexity of a direct trigonometric relation.

The most fundamental analysis of light’s behavior at an interface, known as light scattering, can be done by determining the results of interaction with a single particle. Depending on the field of study, the term “single particle” can vary in meaning from a single
electron to a molecular compound. Fundamental light scattering theory, called Mie theory after its founder, Gustav Mie, begins with the derivation of an appropriate wave equation from Maxwell’s equations, standard in electromagnetic theory, listed below:

\[
\nabla \cdot \mathbf{E} = \frac{\rho}{\varepsilon_0} \tag{2.7.1}
\]

\[
\nabla \cdot \mathbf{B} = 0 \tag{2.7.2}
\]

\[
\nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t} \tag{2.7.3}
\]

\[
\nabla \times \mathbf{B} = \left(\frac{1}{c^2}\right) \left[ \frac{\partial \mathbf{E}}{\partial t} + \frac{1}{\varepsilon_0} \frac{\partial \mathbf{P}}{\partial t} \right] \tag{2.7.4}
\]

A few notes about the equations: Expression 2.7.1 holds true in the cases of isotropic and homogeneous matter; the more general form uses the electric field displacement vector \( \mathbf{D} \). Here, \( \rho \) represents a material's charge density. In the case of anisotropic materials or graded index materials (often used to make optical fibers), the general term must be used. Also, expression 2.7.4 is applicable for a dielectric (non-conducting) material. In this case, we can use the net polarization vector \( \mathbf{P} \) instead of introducing a new vector for current displacement, because dielectrics contain no free charge and thus the current density ends up becoming a time derivative of polarization.

Expressions 2.7.1 through 2.7.4 can be algebraically manipulated to result in a second order differential equation that allows one to solve for the electric (or magnetic, if one chooses) field. This is a standard procedure that many electromagnetics and optics
texts show step-by-step [6, 7]. It begins with taking the curl (\(\Delta \times\)) operator of both sides of expression 2.7.3. The most general final outcome is known as the Helmholtz equation, specified below:

\[
\nabla^2 E + k^2 E = 0 \tag{2.8}
\]

This second order differential equation is often known as a wave equation, since the solution for \(E\) expresses how the electromagnetic wave propagates. For a typical case (and the case considered for this project), a harmonic wave expression for \(E\), in the form of \(E = E_0 e^{i(kz-\omega t)}\) is used, where \(k\) represents the wave propagation vector, which is wavelength dependent, as seen in expression 2.9.
In the case of light scattering discussion, the electric field in expression 2.7 is solved for in spherical coordinates, which is advantageous because it allows for an elegant solution. The conversion from Cartesian to spherical coordinates is an exercise covered by typical Calculus texts [8]. The usual variables used in the conversion are shown in Fig 2.2. Conversion of the Helmholtz equation (Expression 2.8) to spherical coordinates yields:

\[
\left[ \frac{1}{r^2 \partial r} \left( r^2 \partial \right) + \frac{1}{r^2 \sin \theta \partial \theta} \left( \sin \theta \partial \right) + \frac{1}{r^2 \sin^2 \theta \partial \phi^2} \right] E + k^2 E = 0
\]

In light scattering theory, the field \( E \) is represented by a general function in spherical coordinates, namely \( E = R(r)\Theta(\theta)\Phi(\phi) \). Maxwell's boundary conditions are maintained, where the combination of incoming and scattered light are equal to the amount of light at the integration surface. Solving the resulting differential equations in spherical coordinates yields solutions for both the magnitude of light scattering (called scattering efficiency), as well as the angle (called scattering anisotropy). The final closed-form expressions can be very complex, and are typically “condensed” in the following manner [1]:

\[
Q = \frac{2}{x^2} \sum_{l=1}^{\infty} (2l + 1)(|a_l|^2 + |b_l|^2) \tag{2.11.1}
\]

\[
\cos \theta = \frac{4}{Qx^2} \sum_{l=1}^{\infty} \left[ \frac{l(l+2)}{l+1} Re(a_l a^*_{l+1} + b_l b^*_{l+1}) + \frac{2l+1}{l(l+1)} Re(a_l b^*_{l}) \right] \tag{2.11.2}
\]
Here, $Q$ represents the fraction of scattered light versus incoming light as a function of particle size. The variable $x$ is known as a size parameter, which includes both the wave's propagation constant and the particle's radius as follows:

$$x = \left(\frac{2\pi n}{\lambda}\right)a$$

Here, $n$ is the refractive index, $\lambda$ is the wavelength, and $a$ is the radius of the particle. The term inside the parentheses in expression 2.12 is the wave vector $k$ seen in expression 2.9. The multipole order, meaning the number of summations that are taken (the upper limits of expressions 2.11.1 and 2.11.2), is inversely related to the ratio between the particle radius and the wavelength. These values have been empirically found and condensed into a piecewise function [9]. The variables $a$ and $b$, represent Bessel functions (a) and their associated Legendre polynomials (b), and $l$ represents their order. Note that the order of the Bessel function order corresponds to the upper limit of the expression; the larger the particle is with respect to the wavelength, the more orders of Bessel functions that need to be summed.

Bessel functions are solutions to second order differential equations in the form of expression 2.11. As such, these functions appear extensively in optical theory, since any wave propagation equation will be derived from Maxwell’s Equations, and thus have a similar form to the Helmholtz Equation [8]. In the case of light scattering theory, the particular combinations of the Bessel functions in expressions 2.11.1 and 2.11.2 occur because of the boundary conditions in place for energy conservation at the interface [1]. To provide an understanding of Bessel function behavior with respect to an independent
variable (in this case we use $x$, the size parameter seen in expression 2.12), Fig. 2.3 shows a plot of five orders of Bessel functions across $x = 0$ through 10.

Here we see a general oscillatory pattern, but with higher frequency oscillations (versus $x$) as the order increases. As the order of $l$ in expressions 2.11.1 and 2.11.2 increases (due to a smaller ratio between particle radius and wavelength), higher orders of these functions enter into the summation, yielding interesting scattering behavior in terms of both scattering efficiency and angular scattering. This behavior is shown and discussed in Figs. 2.4 and 2.5, which plot applicable cases for light scattering from skin.
Fig. 2.4: Scattering efficiency (Q) versus size parameter (x) with a refractive index n = 1 (a typical value for skin). A size parameter of $2\pi n$ (approximately $x = 9.5$ in this case) represents the case of the wavelength equaling the radius of the scattering particle. If the wavelength $\lambda$ is kept constant, the progression of x from 0 to 200 represents a decreasing particle size. Note that the oscillatory behavior stabilizes as x (and correspondingly, $\lambda/a$ ratio) increases.

Expressions 2.11.1 and 2.11.2 are the standard final-form expressions of Mie theory for spherical particles. Since this theory is derived from Maxwell’s equations, it is considered to be the most general form of scattering theory, applicable to all size parameters, and thus is the most well-known and applied scattering theory. Note that the expressions presented here arise from solving the Helmholtz equation in spherical coordinates; solutions for other shapes (especially rectangular) are much more complex and often require numerical solutions from computer scripts to fully solve [9 - 12]. In fact, while Mie’s most notable
Fig. 2.5: Evaluation of angular scattering intensity ($I=|E|^2$), based on solutions to expression 2.11.2, with the geometry shown on top. Angular scattering plots show scattering intensities with respect to scattering angle $\theta$ with the labeled particle radius ($a$) to wavelength ($\lambda$) ratios. Here, we see that slight changes in size parameter when wavelength and particle radius are on the same order can cause dramatic differences in angular scattering properties. When the wavelength is much larger than the particle radius, an isotropic forward scattering pattern emerges. The angular profile for this case can also be obtained through Rayleigh scattering theory, which will be discussed shortly in section 2.3.
contribution came in the form of the closed solution for spherical particles, real-life cases do not usually adhere exactly to the perfectly spherical case, and thus modeling of light scattering for various scenarios has evolved into its own robust research field. However, the spherical scenario is a very useful tool in biomedical optics because of the orientation of cellular structures; Mie's theory on spherical particles is used as the basis for models that are very prominent in methods for optical imaging in skin [10].

2.3 Light Scattering Theory (Rayleigh’s Theory)

When being introduced to light scattering theory, many students are taught Rayleigh's theory [13]. Rayleigh theory applies to cases where the wavelength is much larger (over an order of magnitude) than the radius of the scattering particle. It is useful in many applications, and is much easier to evaluate than Mie theory, mainly because its final form does not involve combinations of Bessel function products. The answer to the often-asked question “why is the sky blue?” can be answered by Rayleigh theory, particularly its well-known tenet that scattering intensity scales inversely to the fourth power of the incident wavelength.

Although the Rayleigh and Mie theories are often discussed as separate entities to be used for different situations of scattering particle sizes, the more general Mie theory has the same results as Rayleigh theory for wavelength sizes much larger than the scattering particle (as is expected, since it is derived from Maxwell's equations). Rayleigh theory arises from the electromagnetic ansatz for far-field dipole radiation, found in standard electromagnetics texts [6, 14], which is expressed as follows:
\[ E = \frac{k^2 \mathbf{p} \sin \gamma}{r} e^{-ikr} \]  \hspace{1cm} (2.12)

Here, \( k \) is the wave’s propagation constant, \( \mathbf{p} \) represents the dipole moment, \( \gamma \) is the radiation angle (and represents both parallel and perpendicular planes), and \( r \) is the dipole displacement vector (same as in Fig. 2.1). In Rayleigh’s theory, this expression is rewritten in the form of intensity \( (I = |E|^2) \) and has the right side of the equation relate to the initial intensity in the following manner:

\[ I = \frac{k^4 |A|^2 (\sin^2 \gamma_\parallel + \sin^2 \gamma_\perp)}{r^2} I_0 \]  \hspace{1cm} (2.13)

In expression 2.13, the dipole moment term \( \mathbf{p} \) is replaced by the term for polarizability, \( A \). Polarizability relates dipole moment and incident electric field as follows:

\[ A = \frac{\mathbf{p}}{E_0} \]  \hspace{1cm} (2.14)

The parallel and perpendicular components of \( \gamma \) split the radiation angle into each plane of the electromagnetic field. This separation is convenient for conversion to spherical coordinates [8]. After conversion to spherical coordinates and substituting expression 2.9 for \( k \), the following expression is obtained:

\[ I = \left( \frac{2\pi n}{\lambda} \right)^4 \left( 1 + \cos^2 \theta \right) \frac{1}{2r^2} I_0 \]  \hspace{1cm} (2.15)

Expression 2.15 is the usual final form of Rayleigh theory, and from it the wavelength and angle dependencies can be extracted easily. The relationship of this expression to Mie theory can be verified readily. A fit on a Mie Q vs. x curve (Figure 2.4) from \( x=0 \) to a value of \( x \) representing \( \lambda = 50*a \) (this value will be refractive index dependent) will show a 4th power fit, which is expected since \( x \) is inversely related to
wavelength. On the angular side, a Mie calculation where the wavelength is larger than 20 times the particle radius (Fig. 2.5d) shows the $1+\cos^2 \theta$ behavior seen in expression 2.15. Mathematically, expressions 2.11.1 and 2.11.2 can be evaluated in the Rayleigh regime - when the appropriate upper summation limit is used for a particle radius to wavelength ratio greater than about 15, one can realize that only the 1st orders of the appropriate Bessel functions contribute to the scattering efficiency and angle. As such, the expression for Q results in a $\lambda^4$ relationship, and the associate scattering anisotropy can be mapped to a $1+\cos^2\theta$ expression.

The understanding of light scattering mechanisms helps determine the probable pathways light will take upon entering the skin after interacting with the molecules making up tissue. As light traverses through different layers of skin, it interacts with molecules of different sizes, shapes, and densities, and thus scatters in unique ways depending on the composition of the skin region. In the visible (390 – 700 nm) and near-IR (700 – 2500 nm) wavelength regions, researchers have developed extensive Monte Carlo models [10] to model scattering from various areas with the human body for the development of near-IR diagnostic systems. In the mid-IR region (3 µm to 20 µm), optical diagnostic techniques have not advanced as far as in the near-IR, primarily due to high water absorption, as discussed in Chapter 1. However, we used the principles of light scattering discussed in this chapter to determine the penetration depth of mid-IR light between 8 – 10 µm in skin to confirm the feasibility on noninvasive glucose sensing in the mid-IR. This is discussed in Chapter 3.
2.4: Light Absorption (Beer-Lambert Law)

While information about scattering properties provides information about the depth of light in skin, molecules can be identified and quantified through absorption. The term absorption refers to any case where a molecule is excited by energy from an outside source. In the optical case, for a basic two order process, light hits the molecule, and in turn the molecule takes energy from that light if the light’s energy matches the difference of two discrete energy levels of the molecule.

Fig. 2.6: Schematic of the absorption process. Incident light with intensity $I_0$ is transmitted through a container containing Molecule “A”. After transmitting a path length $x$ through the container (for the purposes of this schematic, assume no scattering events occur inside the container), the intensity of the light varies based on wavelength. If the wavelength is non-resonant with Molecule “A”, it is not absorbed in the container and the output intensity $I = I_0$. If the incident light is resonant with Molecule A, much of it will be absorbed in the container, leaving the output intensity $I$ much smaller than $I_0$. 
and conforms to selection rules governing quantum mechanics. If the light’s energy does not match the difference between any two levels, the molecule will not absorb the energy. Note that the exact discretization of different molecules depends on their atomic composition, and it is an advanced topic beyond the scope of this thesis. Discussions of the topic can be seen in Refs. 3 and 4. The reason absorption is such a powerful tool for molecular identification is that no two molecules have energy levels that are exactly equal. This independent discretization is a fingerprint in the sense that specific molecules will always absorb certain energies of light [15], and no other molecule will behave exactly the same way towards a given wavelength of light. Furthermore, at non-resonant wavelengths, the same molecules will not absorb light, meaning that molecules can be identified and distinguished through spectral information showing which wavelengths of light are absorbed.

Although no two molecules have the exact same discrete energy levels, all absorption events are not equal in strength. A conventional metric to determine absorption strength is the absorption cross-section, which quantifies the probability that light of a particular wavelength will be absorbed. This parameter depends on a molecule’s size and its refractive index. Vibrational resonances, which occur in the mid-IR, are measured to have the strongest known absorption cross-sections for various gases [16]. This is one of the primary reasons we choose to detect glucose using mid-IR light; while traditional optical approaches use near-IR absorption features between 1.5 to 2.5 µm to detect glucose, we utilize the vibrational resonances between 8 to 10 µm.
The quantification of the absorption process allows for identification of several parameters based on the measurement being performed. The entities involved in identifying absorption are incoming light intensity, outgoing light intensity, interaction path length, concentration of absorber, and absorption cross-section. We start by labeling the absorption probability through the use of an absorption coefficient with units of inverse length, denoted as $\mu_a$. This coefficient is a function of the absorbers concentration per unit volume and the absorption cross-section, which quantifies strength of absorption at the respective wavelength. This can be seen in expression 2.15, with $c$ representing absorber concentration (per unit volume) and $\sigma$ representing absorption cross-section area.

$$\mu_a = c \ast \sigma(\lambda) \tag{2.16}$$

Then, we identify the rate of change of light intensity as it traverses through a small portion of an absorbing medium with a given absorption coefficient as follows:

$$\frac{dI}{I} = -\mu_a dx \tag{2.17}$$

Integrating this standard first order differential equation gives:

$$I(x) = I_0 e^{-\mu_ax} \tag{2.18}$$

Expression 2.18 is known as the Beer-Lambert law [10], where $I$ represents light intensity and $x$ represents interaction path length. A combination of expressions 2.16 and 2.18 show that the absorption process is dependent on light intensity, wavelength and molecular concentration, which will prove instrumental to the identification of glucose concentration through spectral measurements. These measurements are discussed in Chapter 4.
2.5: Summary

This chapter has covered the basic fundamentals of how light interacts with matter. We must appreciate the role that the wavelength of incident light plays in these interactions; material properties that govern light-matter interaction change with wavelength, oftentimes in a complex fashion. Understanding this wavelength-dependence allows for the determination of how light will scatter through molecules and molecular compounds of different sizes (relative to the wavelength), allowing one to predict the general light propagation path through a turbid medium. As the light scatters through the medium, it will interact with many different molecules, all of which absorb different amounts of light at different frequencies. Eventually, one collects the light after it has undergone these interactions, and from its spectral and spatial information determines many interesting parameters such as propagation path length and molecular concentration. The following chapters discuss how these physical tools are used to determine glucose concentration in human skin using mid-IR light. Chapter 3 focuses on spatial and spectral scattering properties of human and porcine skin, and Chapter 4 discusses the sensitivity of \textit{in vitro} glucose sensing with mid-IR light through absorption measurements.
References


Chapter 3

Mid-IR Scattering from Porcine and Human Skin

In this chapter, the application of the light scattering theory discussed in Chapter 2 is shown in experimental scenarios with porcine and human skin. An understanding of mid-IR light interaction with skin is one of the necessary steps for in vivo sensing of tissue molecules that absorb in this wavelength region, such as glucose, which shows four distinct absorption peaks between 8 and 10 μm [1,2], particularly to determine the penetration capability of mid-infrared light into skin. The optical absorption spectroscopy used to determine glucose concentrations from human spectra relies on incident mid-IR light travelling through the dermis layer of skin before scattering out to a detector.

Glucose monitoring is essential for treating diabetics, as discussed in Chapter 1, and non-invasive methods using near infrared light have been studied for over a decade [3-5] together with Monte Carlo simulations modeling photon transport in tissue [6,7]. In the
mid-IR region, however, research on the scattering of light within turbid biological media such as skin is much more limited [4,8]. In fact, the results of our project for determining penetration capability of mid-IR light in skin, discussed in this chapter, led to the first published work detailing angular scattering patterns from skin using mid-IR light [9]. The primary reason for the lack of published work regarding mid-IR light interaction with skin – and, in general, any in vivo application of mid-IR light on humans – is the high (but flat) magnitude of water absorption in this wavelength region [10], which results in very weak penetration in skin from conventional mid-IR light sources. However, this thesis project takes advantage of QC lasers, coherent light sources that were introduced in 1994 and are rapidly developing (as discussed in Chapter 1). QC lasers emit light with unprecedented power for the mid-IR wavelength range, which makes them ideal for transcending the water absorption barrier.

3.1: Mid-IR Light Interaction with Porcine Skin

Here, we study mid-IR light interaction with skin, particularly angular and spectral profiles of back-scattered light, to determine if mid-IR light penetrates sufficiently deep (into the dermis layer of skin) for the realization of a noninvasive glucose monitor. Skin has a layered structure with significant differences in composition between each layer (Fig. 3.1). The topmost layer is the stratum corneum, which is typically typically 15 μm thick and contains pockets of densely packed cells [11]. Underneath the stratum corneum is the epidermis, between 100 –
Fig. 3.1: a) Diagram of upper skin layers, with relative shapes and sizes of scatterers present in each. The stratum corneum and epidermis consist of relatively homogenous arrays of spherical scatterers, while the dynamic dermis layer contains scatterers of various sizes, shapes, and densities.

175 μm thick, in which spacing between cells decreases rapidly with increased depth [11]; inter-nucleus spacing decreases from 40 μm at the top of the epidermis to 5 μm at the boundary between epidermis and dermis. The dermis layer is under the epidermis, and it is the most active layer of skin. The dermis has small, tightly packed cells, as well as larger structures such as collagen fibers and nerves. Most importantly, the dermis contains capillaries and is highly permeable, leading to an interstitial fluid (ISF) glucose concentration that correlates to blood glucose levels more accurately than epidermal ISF [10,12]. Porcine skin can be used as a proxy for human tissue for purposes of analyzing light matter interaction because of the similarities between the two in layer thicknesses and refractive indices [13].
Analysis conducted from porcine skin is thus advantageous because it allows for examination of the light scattering mechanisms without incurring noise from involuntary motions that humans exhibit. This noise can be very uncorrelated and random [14], which hinders the calculations needed to quantify penetration depth (important for glucose measurements, since penetration depth impacts the light’s path length through skin) from scattering patterns. Upon determining the mid-IR light penetration from experiments with porcine skin, we ventured to conduct the same experiments with human volunteers and observed similar patterns, along with a visible impact of noise from involuntary motions.

The primary light interaction mechanism inside a turbid medium like skin is scattering [15,16], and there is also a strong signal attenuation resulting from flat water absorption occurring in the 8 – 10 μm region of interest. Spatial properties of back-scattered light will depend on the composition of the region the light scattered from since scattering properties are highly dependent on particle size and shape (see Chapter 2). Because skin layers are unique in composition, with respect to particle size, density, and shape (Fig. 3.1), analysis of back scattering from skin can provide information about the region of the skin the light was collected from. Furthermore, an integration of collected scattered light across all angles divided by the incident light power can be used along with the appropriate absorption coefficient in Beer-Lambert’s law (see Chapter 2) to determine the penetration depth and validate the determination of scattering region through analysis of scattering patterns.
Fig. 3.2: Schematic of the experimental setup. The mirror was placed on a flip mount. When the QC laser was used, the mirror was positioned upright, and when the FTIR was used, the mirror was flipped down.

3.1.1: Experimental Setup and Method

Experiments were conducted using a bi-axial rotational device, which combines two motors and a motion controller to allow for changes in both the detector angle and the sample angle, with a possible precision of up to 0.001°. A schematic of the experimental setup is shown in Fig. 3.2. Due to the 0.0625 numerical aperture of the optics in front of the detector, a 1° detector angle resolution was selected as an appropriate step size to obtain sufficient spatial sampling while avoiding oversampling. The numerical aperture quantifies the half-angle of light acceptance into the optical component (lens in this case) and can be calculated through a trigonometric relationship using the lens' focal length and radius. The following expression is used to calculate numerical aperture:
\[ NA = n \sin \theta \] 

(3.1)

Here, NA represents numerical aperture, \( n \) denotes the refractive index of the medium the lens is operating in, and \( \sin \theta \) is the ratio of lens radius to focal length.

The light sources used in this experiment were a pulsed external cavity QC laser from Daylight Solutions Inc., tunable from 8.4 to 9.9 \( \mu \text{m} \) (with resolutions of up to 0.01 \( \text{cm}^{-1} \)) and a broadband IR source from a Fourier Transform Infrared Spectrometer (FTIR). A calibrated liquid nitrogen cooled mercury cadmium telluride (MCT) detector was used to collect the light reflected and scattered from the sample. The QC laser, operated in pulsed mode with 1% duty cycle and a 100 kHz repetition rate, output on the order of 100 mW peak power at each wavenumber step, while maintaining an average incidence of 1 mW onto the skin with intensities on the order of solar radiation (and thus well below ANSI regulations for laser exposure to skin). Fig 3.3 shows the power spectrum of the QC laser as well as the emission spectrum at four tuning points.

The incoming beam was focused onto the sample surface, and the detector was first aligned to collect specular reflections of light from a rough, sanded aluminum surface, in order to calibrate the setup and ensure proper angular alignment. We ensured that the position of specular reflection was accurate to within ±0.02 degrees. Fig 3.4 shows the detected reflections versus both angle and wavenumber for the rough metal surface. This metal piece was then replaced by porcine skin. The detector angle \( \theta \) was swept from 20° to 100° with respect to the incoming beam. In order to collect as much scattered light as possible and have an equal angle range on each side of the specular reflection angle, we chose to have the
Fig 3.3: Power spectrum of the Daylight Solutions External Cavity QC laser used in this study. Emission spectrum at four tuning points are shown at specific wavenumbers where glucose absorbs mid-IR light through vibrational resonances, in order to show their relatively narrow line widths and highlight the wavelength selection capability of the laser. These wavenumbers are 1036 cm\(^{-1}\) (red), 1080 cm\(^{-1}\) (blue), 1116 cm\(^{-1}\) (green), and 1152 cm\(^{-1}\) (violet); further discussion about glucose absorption features at these particular wavenumbers is given in Chapters 4 and 5.

Sample angle \(\phi\) rotated to 30°, meaning the specular angle 2\(\phi\) was located at \(\theta = 60°\), allowing for collection of scattered light in a ±40° range. Other sample angles were also examined, but behaved similar to \(\phi = 30°\).

Light intensity was collected versus angle and wavenumber for over thirty different skin samples using either the QC laser or FTIR, and certain trends are evident from that data set. Fig. 3.5 shows examples of typical scattering patterns from four porcine skin samples. First, the diffuse scattering from skin leads to a
Fig. 3.4: Scattered light collected versus angle and wavenumber for a rough metallic aluminum block used to calibrate the bi-axial rotation device. Here, the sample was held at 30°, which leads to a specular reflection when the detector is at 60° on the rotating arm of the device (refer to Fig. 3.2). Across the entire wavelength tuning range, the maximum light was collected at the specular reflection, with scattered light being detected up to 10° away from this point. The half-width half-maximum of the scattered light was 6°.

much wider scattering angle range than for the control aluminum block, which is easily visible from a comparison of Figs. 3.4 (aluminum) and 3.5 (skin). Furthermore, important differences between the FTIR and QC laser scattering can be noticed. The scattering patterns obtained with the FTIR have a very broad maximum around the specular reflection angle, with one representative data set shown in Fig. 3.6a. At 1120 cm⁻¹, this profile had a half width half maximum of 32° (Fig. 3.6c). This particular wavenumber was chosen because it was a region that contributed a relatively high intensity of light throughout our experiments.
Fig. 3.5: Examples of typical scattering patterns versus angle and wavenumber for four different porcine skin samples, where the sample angle $\phi$ was kept at 30° (meaning that specular reflection would occur at $\theta = 60°$. While modulation patterns across $\theta$ were the prominent scattering features, each example shown differs in the number of modulation patterns, peak scattering angle, and distance between scattering maxima. This indicates complex scattering from directional scatterers.

Experiments performed with rough sanded aluminum samples yielded a reflection profile featuring a sharp peak at the specular reflection angle $2\phi$ and a half width half maximum $\leq 6°$. The fact that scattered light from skin is not concentrated at the specular reflection angle indicates that the light is being scattered from within skin rather than simply being reflected off the surface.
Fig 3.6: Scattering profiles for porcine skin samples with respect to wavenumber and detector collection angle $\theta$, with the sample angle $\phi$ kept constant at 30°. a) Results of a typical experiment using the FTIR. b) Results of a typical experiment using the QC laser. Note that the QC laser input power was approximately 100 mW at 1120 cm$^{-1}$, while the FTIR's was 7 mW integrated, so the normalized values in b) represent larger absolute values compared to those in a). c) Slice of FTIR (blue) and QC laser (red) scattering pattern versus $\theta$ for 1120 cm$^{-1}$ and $\phi = 30^\circ$. 
For the QC laser experiments, the scattering pattern was very different; the maximum reflection was recorded up to 30° away from 2ϕ. A trend for these experiments were modulation patterns across the θ axis. One representative experiment is shown in Fig. 3.6, with 10 scattering maxima occurring across the swept angle range at 1120 cm⁻¹ (Fig. 3.6c) for this particular experiment. For a given dataset, there could be anywhere between 5 and 15 maxima across θ for a specific λ and the absolute maximum recorded scattering intensity would occur between θ = 35° and θ = 90° (Fig. 3.5). Fourier analysis with respect to cos(θ) showed a lack of periodicity in the data, which eliminated the possibility of thin film effects originating from layer boundaries being the cause of the modulation patterns. Furthermore, it also eliminates the possibility of the modulations simply resulting from diffraction caused by the undulated epidermal-dermal junction.

### 3.1.2: Analysis of Modulation Patterns in Angular Scattering

The modulation patterns apparent in the QC laser experiments were further evaluated by analyzing the effects of changes in incident beam power on the scattering pattern. For the case of purely surface and boundary reflections, the detected (P_{det}) and input (P_{in}) powers should share a linear relationship, which was verified by shining the QC laser source tuned to 1120 cm⁻¹ on the reflective aluminum surface with the detector positioned at the specular reflection angle 2ϕ.

When the same experiment was conducted on a porcine skin sample, there was a nonlinear increase for P_{det} versus P_{in} throughout the regions where strong scattering was detected. This can be seen in Fig. 3.7a), which shows P_{det} with respect to both θ and P_{in} for an experiment with the QC laser tuned to 1120 cm⁻¹ shining
onto the porcine skin sample. Scattering maxima, such as θ = 80° and 87°, were considered angles of high scattering, and the minima, such as θ = 48° and θ = 73° were considered regions of low scattering. Fig. 3.7b) shows the power of the detected light normalized to the corresponding input power for the swept θ range. On average, the detected signal was attenuated on the order of 10^4 for each angle measured, consistent with the expected absorption coefficient. Analysis of P_{det} versus P_{in} for two peaks (θ = 80° and 87°) and two troughs (θ = 48° and 73°) shows an approximately exponential rise in detected power as input power is increased; the recorded power at the peak angles rises faster than at the troughs (Fig. 3.7c).

A larger incident beam power results in a stronger signal at a given depth of an absorbing medium, as governed by the Beer-Lambert relationship [16]. Since the detector used in the study is kept the same, increasing the beam power results in light backscattered from deeper within tissue being more intense than the detector response threshold. This is the primary reason we detect stronger backscattering around 1100 cm⁻¹, with a decline for wavenumbers less than 1050 cm⁻¹ and greater than 1160 cm⁻¹; the QC laser power spectrum peaks at 1120 cm⁻¹ and declines towards the limits of its tuning range. As input power (and detectable depth) increases, more scattering is detected at certain angles, indicating that the light reaches a region containing strong yet isolated scatterers. The essential randomness of the modulation pattern between different skin samples follows the random orientation of directional (non-spherical) scatterers.
Fig 3.7: Effect of input beam power at 1120 cm$^{-1}$ on detected scattered light from a porcine skin sample (different from the sample used to obtain the results shown in Fig. 3.6) kept constant at $\phi = 30^\circ$.  

a) Detected power versus input power and $\theta$.  

b) Detected power normalized to the respective input power, for purposes of quantifying absorption. Note that the angles of strong scattering differ from those in Fig. 3.6b.  

c) Detected power versus input power at 1120 cm$^{-1}$ and $\phi = 30^\circ$ for $\theta = 80^\circ$, $87^\circ$, $73^\circ$, and $48^\circ$. These specific angles were chosen to highlight the increased influence of directional scatterers at larger skin depths.
At angles of peak scattering, such as at $\theta = 80^\circ$ or $87^\circ$, the subset of randomly oriented directional scatterers scattering back at the instantaneous detection angle was greater than at a scattering minimum, such at $\theta = 73^\circ$ or $48^\circ$. The steeper rise of $P_{\text{det}}$ versus $P_{\text{in}}$ at higher input powers can be attributed to the detection of backscattering from a greater depth; the deeper the backscattering occurs, the more likely that certain scattering angles will be more conducive to light reaching the detector before attenuating below the sensitivity threshold. This explains the presence of modulation patterns across the $\theta$ axis when using the QC laser. The lack of polarization dependence as confirmed from additional measurements is consistent with scattering at random angles.

It should be noted that these patterns do not originate from Mie scattering of large, spherical particles because the location and number of maxima varied as different skin samples were used (refer to Fig. 3.5). Mie theory states that the volume scattering from such an array will always peak at the same angles [17]. Even considering that the density and orientation of scatterers in skin vary from sample to sample, the presence of Mie dominated scattering would yield similar patterns as many more trials were conducted. The trend of our data indicated that so far, no angle over most of the swept $\theta$ range was favored over others. Furthermore, there was no consistency with respect to the number of scattering maxima that occurred. Being that the number of scattering peaks in Mie theory is a function of scatterer size, one would expect that at least this parameter would remain fairly consistent in Mie-dominated scattering from different samples of the same region of skin.
Fig. 3.8: Comparison of scattering patterns versus wavenumber and $\theta$ for QC laser peak input powers of 100 mW (top) and 30 mW (bottom). The shift from modulation patterns with well define peak scattering angles towards a broader, more homogeneous scattering profile versus angle as QC laser power is lowered indicates scattering from a shallower region of skin, similar to what is seen in FTIR scattering patterns.

The lack of modulation patterns when using the FTIR, which is evident when comparing Figs. 3.6a (FTIR) and 3.6b (QC laser), is also explained by comparing the power spectral densities of the two sources. The FTIR beam power is 7 mW integrated over its broad wavelength range, so the absolute power at 1120 cm$^{-1}$ is far less than a milliwatt. Thus, the FTIR light scattered back from deeper in skin is much weaker than that from the 100 mW QC laser light, meaning that the majority of scattered light detected in the FTIR experiment originates from scattering centers close to the surface of the skin, consistent
with the more homogeneous structure of the top layers of skin [11]. Experiments conducted versus wavenumber and angle for 30 mW peak power QC laser light show that the modulation patterns, while still present, become less angularly resolved, and start to blend into each other to form a broader region of peak scattering (Fig. 3.8). This indicates that the featureless scattering profile obtained with the broadband FTIR source is indeed a function of power and not the incoherent broad spectrum range of the light source.

3.1.3: Penetration of Mid-IR Light into Skin

In order to determine the penetration depth of the mid-IR light, microscopy studies of human and porcine skin were consulted for an understanding of the types of scatterers in each layer, which is summarized in Fig. 3.1. The stratum corneum, 15 μm thick on average, contains pockets of densely packed cells [11], which accounts for the isotropic scattering obtained close to the surface. In the epidermis, the spacing between cells decreases exponentially with increased depth [11], with inter-nucleus spacing going from 40 μm at the top of the epidermis to 5 μm near the boundary with the dermis layer. The dermis contains smaller, more tightly packed cells, as well as directional scatterers such as collagen fibrils and capillaries.

As a beam with a given diameter penetrates deeper into skin, it will come into contact with an exponentially growing number of possible scatterers [18]. Potential scatterers in the stratum corneum and epidermis are primarily cell organelles, which have diameters over 10 times smaller than the wavelengths being analyzed and have spherical shapes, meaning they would backscatter light as described by Rayleigh theory. The large relative spacing of scatterers in the epidermis [18] makes it more likely for light to travel
forward, rather than scatter. Within the dermis, scatterers do not come solely as small spherical particles, but also come as rod-like structures composed of many sub micron aggregates, such as collagen or capillaries. Work done using Rayleigh scattering theory on rod-like particles provides an estimate of their volume scattering [19] and confirms that such particles scatter selectively with respect to angle. Reflections from long and thin capillaries would also be angle dependent, based on their orientation. Skin is known to be a very heterogeneous medium [20], so, random or near random orientations of collagen and capillaries in different samples is expected, which explains why angles of scattering maxima varied between skin samples.

Based on this information, we conclude that the observed patterns of light scattered off of porcine skin with respect to wavenumber and scattering angle using both a QC laser and FTIR indicate that scattering originating from within the skin is the primary contributor of detected light. Thin film effects and surface reflections were eliminated as potential major contributors. Furthermore, a comparison of scattered light power versus angle for low and high input powers shows an isotropic scattering at shallow skin depths along with directional scattering at greater depths. Combining the scattering patterns with knowledge about contents of the different skin layers, we conclude that mid-IR light is scattered and detected outside the skin with easily achievable mid-IR laser power, such as 100 mW peak power, low milliwatt average power, and a commercial detector. This is based on the fact that the stratum corneum is conducive to isotropic scattering, the dermis favors forward transmission, and the dermis layer is conducive to directional scattering.

Additionally, a quantitative estimate for mid-IR penetration depth can be obtained through integration of the light scattered across all angles at a particular wavelength (we
Fig. 3.9: Summary of results with respect to penetration of mid-IR light from QC laser and FTIR sources. From angular scattering patterns, it can be determined that the QC laser light backscatters from a region of complex and orientation-dependent scattering bodies, which can be found in the dermis layer.

need to select a wavelength that is only subject to water absorption and not that of any molecules prevalent in skin). This quantity gives an output light power that can be used in Beer-Lambert’s relationship. Since we know the input power, we can obtain the absorption coefficient of water from literature, and we know the water composition and relative thicknesses of relevant skin layers, a value for path length can be extracted. This path length divided by two (accounting for forward travel, as well as backscatter) provides an estimate for the depth within skin that the light scattered from. When this calculation is done on a representative dataset from a porcine sample, we obtain skin depths around 150 μm, which includes a 50 μm trip through the dermis and thus agrees with the assertion that
the mid-IR light traverses through the shallow regions of the dermis, which is a requirement for effective glucose sensing with ISF.

Fig. 3.9 shows the diagram of upper skin layers with respective scattering bodies (as in Fig. 3.1), and it is updated with a summary of our findings with respect to penetration depths of mid-IR light from QC laser and FTIR sources.

3.2: Mid-IR Light Scattering from Human Skin

Due to the positive results of the scattering study on porcine skin, which showed the feasibility of mid-IR in vivo sensing in skin, we extended this project to include analysis of scattering from human skin [21]. In in vivo human studies, additional variables are present (with respect to the in vitro study on a porcine skin sample). These variables include the fluctuation of back-scattered signal with time and the effect of positional displacement caused by involuntary human micro movements. This additional noise is uncorrelated and can appear at random, adding another layer of challenge to in vivo measurements (Fig. 3.10). Solutions for resolving this issue are discussed in Chapter 5, where we tackle glucose sensing in humans head on!

The experimental setup for the mid-IR light scattering study on humans was kept the same as for the porcine one (refer to Fig. 3.2), only the porcine skin sample was replaced by a human volunteer’s arm. Experimental procedures maintained standards set by Princeton’s Industrial Review Board (IRB). Volunteers kept their arm as steady as possible while still remaining comfortable. Fig 3.11 shows the results from a human subject; although the scattering patterns from the FTIR and QC laser contain more noise
Fig. 3.10 a) QC laser spectra of back-scattered light from porcine skin mounted on a stable aluminum block. b) QC laser spectra of back-scattered light from the same skin sample, this time held by a human arm. In both cases, a free-space optical system was used for light collection. The instability seen – the loss of repeatability in the spectra as well as the loss of resolvable spectral features – indicates the lack of effectiveness of a free space optical system for collecting back-scattered light from skin after the introduction of additional positional variation.

due to the added variable of human motion, the scattering patterns show similar trends to those seen from porcine skin. Backscattering of FTIR light yields a smooth scattering profile versus angle that fits to a Rayleigh profile, while QC laser backscattering shows more complexity with modulation patterns, which results from scattering at a deeper depth. Given that porcine and human skin are very similar in layer thicknesses and refractive indices, the same conclusion can be drawn from the human studies – mid-IR light from a pulsed QC laser can penetrate deep enough for feasible glucose sensing.
Fig 3.11: a) Measurement of infrared light interaction with human skin *in vivo* using FTIR spectroscopy. b) Measurement of mid-IR light interaction with human skin using QC laser spectroscopy. For both datasets, the sample angle, $\phi$, was kept at 30°. Angular scattering patterns from both light source types were similar to those seen in porcine skin, yielding the conclusion that pulsed QC laser light was backscattered from the upper regions of the human dermis.
Fig. 3.12: Scattered light intensity collected from porcine skin with three different melanin contents. The overlapping of spectra between the three tones indicates a weak melanin absorption in the mid-IR. This is a beneficial property for prospective in vivo glucose sensing. Note that for the human scattering data shown in Fig. 3.11, a Caucasian skin tone is represented. Experimental data was also taken from a volunteer with dark brown skin tone, and the results showed the typical FTIR/ QC Laser scattering characteristics discussed in Sections 3.1 and 3.2.

3.2.1: Lack of Melanin Impact on Mid-IR Skin Spectra

An additional study we conducted with porcine skin was determining the impact of melanin on mid-IR spectra. Melanin, a protein that affects pigmentation in humans as well as pigs, is known to absorb strongly in the near-IR, and thus an individual’s skin color can cause significant spectral differences [16]. In the mid-IR, however, its impact is far less pronounced [16, 18]. We demonstrated that this is indeed the case, as we analyzed the
backscattered skin spectra of different colored pigs (pink, light brown, dark brown) at a constant position.

Spectra recorded for these porcine skin samples showed a consistent shape from 1000 cm\(^{-1}\) to 1220 cm\(^{-1}\), which indicated that the absorption present at those wavelengths resulted from factors independent from melanin concentration (Fig. 3.12). This is an important result that adds to mid-IR glucose sensing feasibility, as it removes the need to include skin tone as a variable in spectral analysis when calculating glucose concentrations. Because skin is a dynamic and heterogeneous medium, increased complexity in spectral analysis of interstitial fluid can hinder its accuracy [12].

### 3.3: Summary

In summary, we utilize spatial and spectral information of back-scattered light from porcine and human skin to determine the depth within the skin the light has scattered from in order to assess the feasibility of noninvasive glucose monitoring with mid-IR light. Because dermal ISF is reported to have strong correlations with blood glucose levels (whereas epidermal ISF does not), light must penetrate into the dermis layer for accurate sensing glucose sensing. We find that back-scattered FTIR (broadband with low power density) light returns a broad Rayleigh angular scattering profile, while pulsed QC laser light with >100 mW peak power returns back-scattering profiles showing angularly selective modulation patterns. The Rayleigh scattering patterns obtained when using FTIR light indicate scattering from the upper layers of skin, which consists of homogeneous layers of spherical scatterers. The modulation patterns seen using QC laser light indicate
back-scattering from large, angularly selective scatters which only exist in the heterogeneous and dynamic dermis layer. Path length analysis based on recorded signal attenuation and Beer-Lambert’s Law validate the conclusion that QC laser light I back-scattering from the dermis layer of skin.
References


Chapter 4

Clinically Accurate Measurement of Glucose in vitro

We continue the assessment of the feasibility of noninvasive in vivo glucose sensing using QC laser absorption (between 8 – 10 μm) spectroscopy by demonstrating the capabilities of the technique in vitro. The aim of this research is to develop a novel sensor that can enhance the quality of life for the 340+ million diabetics in the world [1]. In Chapter 3, we discussed the analysis of scattering patterns of light on porcine and human skin to conclude that QC laser light was powerful enough to penetrate roughly 50 μm into the dermis layer, deep enough to interact with interstitial fluid (ISF) containing relevant glucose content. The next requirement for a proof of concept of mid-IR glucose sensing viability is the ability to sense physiologically relevant glucose concentrations within ISF proxies in vitro with an adequate sensitivity.

Spectroscopic techniques for sensing glucose rely on quantifying the amount of light
absorbed at particular resonant frequencies. Traditionally, optical noninvasive *in vivo* glucose detection studies have been focused on the near-infrared (near-IR) [2-4] due to the presence of resonant glucose overtone and combination bands combined with low water absorption in that region, which allows for greater penetration of light into skin. However, absorption features of other biological absorbers such as hemoglobin and amides are also relatively broad and strong in the near-IR, leading to the necessity of complex multivariate analysis to extract the impact of only glucose on the spectrum obtained from backscattered light. The unpredictability of concentrations of these other absorbers leads to chance temporal correlations and the need to calibrate data using complex sets recorded over multiple days [5]. Work using Raman spectroscopy with near-IR light has also been reported, but the method has its own obstacles to overcome, such as the relatively weak signal associated with Raman scattering [6]. Furthermore, recent work using optical coherence tomography in the near-IR to sense glucose *in vitro* has been reported [7], but so far there have been no reports of *in vivo* work with this method.

The mid-infrared (mid-IR) is promising for the field of noninvasive *in vivo* glucose detection, as the glucose molecule contains fundamental vibrational resonances between 8 - 10 μm [8], which are not overlapped by other biological absorbers except water. Water is a broad featureless absorber throughout the near and mid-IR, but its absorption coefficient is roughly four orders of magnitude greater at 10 μm than at 1 μm, which has been the biggest challenge for researchers.
Fig. 4.1: Timeline of significant work done in the field of *in vitro* glucose sensing in the mid-IR region. The work presented in this Chapter (highlighted in the figure) is the most detailed work published on *in vitro* mid-IR glucose sensing.

Focusing on noninvasive *in vivo* glucose detection in the mid-IR regime [9]. However, recent developments in mid-IR light source technology, including pulsed QC lasers able to provide high peak powers on the order of hundreds of milliwatts while maintaining average powers on the order of a few milliwatts [10] have provided the capacity to obtain more robust signals from skin regions where mid-IR light had previously been considered to be undetectable [11].

Prior studies on detection of glucose *in vitro* via transmission measurements show the promising capabilities of mid-IR glucose sensing but leave room for improvement. The studies are either confined to narrower boundaries of concentrations (40 to 140 mg/dL versus our range from 1 to 400 mg/dL) [13], do not use techniques conducive to *in vivo*
measurements (photoacoustic spectroscopy requires more incident power than our method, which may lead to problems with tissue heating and/or damage during in vivo measurements) [9,14], or had too few measured samples to establish the technique's sensitivity limit with respect to detectable concentration (only 8 concentrations in the physiological range were used in the regression fit) [15]. A summary of significant previous work and a comparison to the work presented here is shown in Fig 4.1.

4.1: In vitro Glucose Sensing Using Mid-IR Transmission Spectra

In this chapter, we show that glucose concentrations can be predicted with clinical accuracy throughout and below the entire physiological concentration range (50-400 mg/dL) with respect to a Clarke Error grid [16] in aqueous, serum, and Intralipid solution using mid-IR transmission spectra. The Clarke grid (Fig. 4.2) is a means of categorizing the glucose concentration output from a sensor based on its deviation from the actual value of glucose concentration (known from control experiments). Clinical accuracy is defined as predictions having less than 20% deviation from the actual concentration when outside the hypoglycemic range (< 70 mg/dL blood glucose level) or a prediction value in the hypoglycemic range when the actual concentration is also in the hypoglycemic range. Serum is used here to simulate ISF, as it has been reported to be a valid ISF proxy [17]. Intralipid (combination of linoleic and alpha-linoleic fatty acids) is used as another biological background solvent. Through the use of these background solvents, we
Fig 4.2: Standard Clarke Error grid; this is the most prevalent metric for the qualification of glucose concentration outputs from experimental glucose sensors with respect to the actual concentration value obtained from control experiments. The regions from A to E denote what harm, if any, would be caused to a human subject who was given a false glucose reading from the experimental monitor. The individual regions are described as follows: A - clinically accurate reading, B - result that would lead to benign action or inaction, C - results that would lead to unnecessary corrections, D - results that would lead to inaction when action is necessary, and E - results that would lead to treatment opposite to what should be given. Sensor readings that fall within regions A and B are regarded as safe.
experimentally establish that glucose can be isolated in various biological fluids using mid-IR light.

4.1.1: Experimental Setup and Method

Solutions with different glucose concentrations were created from 10% (equivalent to a 10,000 mg/dL concentration) glucose solution obtained from TekNova, which was serially diluted with the background material (water, serum, or Intralipid) to create concentrations ranging from 1 mg/dL to 10,000 mg/dL. Serum was obtained from Animal Technologies, Inc., while Intralipid was obtained from Sigma Aldrich.

In vitro transmission spectra of glucose solution samples were acquired in the following manner; light from a mid-IR source was transmitted through a 100 μm path length pressure-sealed liquid cell containing the solution. A liquid nitrogen cooled mercury cadmium telluride (MCT) detector was used to collect the transmitted light. The two mid-IR light sources used were an IR source contained in a Nicolet Fourier Transform Infrared Spectrometer (FTIR), which emits incoherent broadband (650 - 4000 cm⁻¹) continuous wave light with an integrated power of 7 mW and a Daylight Solutions External Cavity QC Laser, tunable from 1000-1200 cm⁻¹ which emits pulsed (100 kHz with 1-5% duty cycle) coherent light single mode (full width-half maximum < 1 cm⁻¹) with a beam diameter on the order of a millimeter and average power of approximately 5 mW and peak powers on the order of 100 mW (dependent on wavenumber). Spectra were acquired using software that was interfaced to each source - OMNIC for the FTIR and LabView for
Fig 4.3: Schematic of experimental setup used to sense glucose \textit{in vitro} using mid-IR spectra. Glucose solutions were created in the noted background solvents and placed into a pressure-sealed liquid cell. Light from the mid-IR source was shined through the cell containing the solution, and the transmitted light was collected with an MCT detector. The detector’s signal was converted to spectra and appended into either a calibration or prediction dataset for partial least squares regression analysis.
the QC Laser. The spectrum collected for each trial run resulted from the averaging of 100 voltage readings from the MCT’s preamplifier collected for each wavenumber. The experimental setup is shown in Fig 4.3.

Ideal blood glucose concentrations in the human body range from 70-140 mg/dL before meals and peak at approximately 200 mg/dL for two hours after meals. Diabetics can reach the hyperglycemic range (considered by the American Diabetes Association to be a blood glucose concentration greater than 240 mg/dL), in which case they would have to take medication and/or exercise to bring glucose levels down [18]. Calibration and prediction using transmitted mid-IR spectra of concentrations too high to be physiologically relevant (500, 1000, 5000, and 10000 mg/dL) and concentrations of 1, 5, 10, 50, and 100 mg/dL were initially done to gauge the spectral changes over across a large concentration scale. Later, experiments were conducted using only physiological concentrations, with a denser set of nominal concentration values - at least 18 unique concentrations calibrated between 1 to 400 mg/dL.

The analytic method used for quantifying the predictability of the different glucose solutions was partial least squares regression (PLSR) - more specifically the SIMPLS algorithm for PLSR [19] built into MATLAB. We used a subset of two or more spectra of a given known concentration for calibration, and the rest of the spectra taken were used for prediction. The accuracy of each individual prediction was determined based on their placement on a Clarke grid, with the aforementioned definition for clinical accuracy. The mean predicted value for a given subset of prediction spectra (with the same expected concentration) was the data point plotted on the grid, while error bars represented the standard deviations in predicted values. Along with analyzing how similar prediction
values of a given subset of spectra were to each other, we also analyzed how similar the mean predicted value was to its expected value. To accomplish this, we used the standard error of prediction (SEP) metric, defined as the following:

\[
SEP = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (C_a - C_p)^2}
\]

Here, the N represents the number of predictions for a given concentration, the \(C_a\) denotes the actual concentration, and \(C_p\) denotes the predicted concentration.

4.1.2: PLSR Prediction of Glucose Concentrations

Both FTIR and QC laser light were used for in vitro glucose sensing because of their inherent advantages. FTIR light is broadband, which allowed for the identification of multiple glucose resonances across a wide frequency range. Furthermore, the FTIR chamber allows for plug-and-play use of transmission accessories, assuring optimal alignment of a liquid cell, making it a desirable apparatus for control studies. On the other hand, the power density of pulsed QC laser light is required for sufficient penetration into skin for in vivo glucose studies, so accurate glucose sensing with the laser light is necessary for validation of the feasibility of the technique on humans. Although the QC laser tuning range was much smaller than the FTIR spectral range, we were able to tune the QC laser through two of the glucose resonance features identified through the FTIR study, as discussed below.

Initial FTIR transmission spectra for aqueous, serum, and intralipid solutions with concentrations in a range from 10 to 10,000 mg/dL (specifically 10, 50, 100, 500, 1000, and
10000 mg/dL) show glucose absorption features that become more apparent with higher concentrations (Fig. 4.4a). Since the spectra shown are normalized to a water background, dips in the signal seen around 1040, 1080, 1125, and 1160 cm⁻¹ are attributed to glucose absorption. When prediction spectra were analyzed with the calibration vector, we obtained a nearly 1:1 linear relationship between predicted concentrations and expected concentrations; $R^2$ values of .999 (water – Fig. 4.4b), 1.00 (serum – Fig. 4.4c), and 1.00 (Intralipid – Fig. 4.4d) were obtained. However, when using only concentrations up to 100 mg/dL for prediction, $R^2$ values deviated by up to 10%, showing that the near-perfect linear relationship stemmed from the overwhelming signal to noise ratio of the absorption features of the higher concentrations.

For FTIR experiments focusing in on the physiologically relevant range of glucose concentrations (50-400 mg/dL) in aqueous and serum solutions, a denser range of nominal concentration values was used. This was done because a more robust calibration matrix consisting of more spectra improves prediction accuracy. The concentrations used for calibration and prediction of glucose in aqueous solution can be seen in Table 4.1. Some intermediate concentrations (112, 135, 225, and 337 mg/dL) were created using mixtures of two other concentrations in order to make a denser prediction concentration set. The expected concentrations that are listed in bold are the ones used for calibration.
Fig. 4.4: a) Representative transmission spectra of aqueous glucose at concentrations from 10 – 10,000 mg/dL measured by FTIR spectroscopy. b-d) Prediction of glucose concentrations using calibrations ranging from 1 through 10,000 mg/dL in water (b), serum (c), and Intralipid (d). The red lines and corresponding fitting equations show changes to the linear fit when only concentrations between 1 and 100 mg/dL are used for calibration and prediction.

Each concentration listed had three prediction spectra tested. From the table, one can see that for every respective concentration > 50 mg/dL, the average values of the three predicted concentrations were between 80 - 120% of the expected value, and the SEP were within 20%. Since the SEP of both concentrations that are included in calibration and those that are just used for prediction are under 20% of the known concentration value, we show
that our calibration set is robust enough for this concentration range. Furthermore, we show that there is no prediction bias favoring concentrations that were used for calibration versus those only used for prediction, which is important for eventual *in vivo* predictions in humans because one cannot expect de facto concentrations to be the same as the ones used in calibration.

**4.1.3: Determination of Mixing Error**

An additional measurement was conducted to determine the significance of errors introduced from imprecise serial dilution. Since serial dilution results in each sample containing portions of a larger concentration sample, an error in mixing introduced at some point would propagate throughout the rest of the entire range of concentrations. While this would not change the deviation between predicted values of a given concentration, it would mean that the nominal values of the concentrations themselves would not be what we expected them to be.

In order to obtain a gauge of the mixing error present in our experiment and to ascertain that our results were not the cause of an unexpected bias, we made a new batch of solutions for 500, 400, 300, and 200 that were independent of both the solutions used for the dataset in Table 1 and each other (they were not created through serial dilution). Two separate, completely independent mixtures for each concentration were made. We then made a third mixture for each concentration by making 1:1 mixtures of the two. Spectra were taken for the three mixtures of each concentration and then predicted using the calibration of the dataset in Table 4.1 (from 1-400 mg/dL). As seen in Table 4.2, the predicted concentration for the third mix was always between the predicted
concentrations of the two independent mixes. Furthermore, the SEPs for the independent data set were comparable to those obtained in Table 1 for the respective concentrations. This shows that the noise in the spectra generated from imprecise mixing is much smaller than the noise generated from the electronic and mechanical interferences present in the experimental setup. Based on the fact that these predictions were obtained using an independent calibration set, we can eliminate mixing error and unwanted bias resulting from serial dilution as significant sources of error in the data.

The accuracy in the data was extracted via plotting the predicted concentration versus the expected concentration on a Clarke error grid. Fig. 4.5 (top left) shows the Clarke plot of the FTIR data for aqueous glucose solutions. The following are the descriptions for the respective regions:

- **A**: clinically accurate readings
- **B**: results that would lead to benign action or inaction by the user
- **C**: results that would lead to unnecessary corrections
- **D**: results that would lead to action when inaction is necessary
- **E**: results that would lead to treatment that is opposite to what should be called for

Average values of predictions were clinically accurate throughout the entire 1-400 mg/dL region, and we obtained accurate and practical (non-negative) concentration values for solutions containing as low as 20 mg/dL concentrations of glucose.

FTIR experiments on glucose solution in serum were conducted in the same manner as those for the aqueous solutions. Due to the increased viscosity of the serum, which sometimes contained small solid particles, it was more difficult to mix with equal precision
Fig. 4.5 (top right) shows the predicted versus expected value of serum solutions plotted on the Clarke grid. The results resemble those obtained for aqueous solutions, with slightly smaller standard deviations for 150 and 200 mg/dL, and slightly larger deviations for concentrations under 50 mg/dL (hypoglycemic range). Once again, we observe a cutoff at 30 mg/dL, below which we do not always obtain non-negative prediction concentrations.

Table 4.1: Average prediction values and standard errors of prediction (SEP) for FTIR transmission spectra on aqueous glucose solutions of respective concentrations. Bolded concentrations indicate that two samples of that concentration were used for calibration. Note that the SEP was calculated with each individual predicted value, not the average.

<table>
<thead>
<tr>
<th>Expected Concentration (mg/dL)</th>
<th>Average Predicted Value (mg/dL)</th>
<th>Standard Error of Prediction (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>400.00</td>
<td>369.80</td>
<td>35.53</td>
</tr>
<tr>
<td>350.00</td>
<td>342.44</td>
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<td>300.00</td>
<td>289.86</td>
<td>22.12</td>
</tr>
<tr>
<td>250.00</td>
<td>237.76</td>
<td>20.79</td>
</tr>
<tr>
<td>225.00</td>
<td>232.91</td>
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</tr>
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<tr>
<td>Value</td>
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<td>Column 2</td>
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<td>----------</td>
<td>----------</td>
</tr>
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<tr>
<td>150.00</td>
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<td>15.32</td>
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<td>135.00</td>
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</tr>
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<td>120.00</td>
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<td>60.00</td>
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<td>50.00</td>
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</tr>
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<td>10.00</td>
<td>0.06</td>
<td>19.97</td>
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<td>-2.65</td>
<td>20.21</td>
</tr>
<tr>
<td>1.00</td>
<td>4.64</td>
<td>9.91</td>
</tr>
</tbody>
</table>
Table 4.2: Predicted concentration versus expected concentration for an independent batch of solutions calibrated using the dataset shown in Table 1. Mix 1 and 2 denote separate, unrelated mixtures for each concentration, while Mix 3 is a 1:1 mixture of mixes 1 and 2.

<table>
<thead>
<tr>
<th>Expected Concentration (mg/dL)</th>
<th>Average Predicted Value (mg/dL)</th>
<th>Standard Error of Prediction (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>Mix 1: 523</td>
<td>39.06</td>
</tr>
<tr>
<td></td>
<td>Mix 2: 445</td>
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<tr>
<td></td>
<td>Mix 3: 468</td>
<td></td>
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<tr>
<td>400</td>
<td>Mix 1: 337</td>
<td>38.50</td>
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<tr>
<td></td>
<td>Mix 2: 421</td>
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<td></td>
<td>Mix 3: 406</td>
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<td></td>
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<td></td>
<td>Mix 3: 282</td>
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<tr>
<td>200</td>
<td>Mix 1: 205</td>
<td>15.81</td>
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<tr>
<td></td>
<td>Mix 2: 174</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mix 3: 193</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 4.5: Predicted glucose concentrations versus expected (actual) concentrations plotted on Clarke error grids. **Top row:** aqueous solution (left) and serum solution (right) measured with FTIR. **Bottom row:** aqueous solution (left) and serum solution (right) measured with QC laser spectroscopy.
Fig. 4.6: First loading vectors for calibration sets in serum (left) and water (right), for FTIR (top row) and QC laser (bottom row) spectra, with arrows denoting wavenumber regions of the most prominent absorption features.

### 4.1.4: PLSR Loading Vector Analysis

PLSR analysis of the first loading vectors of calibrations for both aqueous and serum solutions can be seen in Fig. 4.6. The first loading vectors highlight the wavenumbers where glucose absorption was most prominent, and one can see that these locations remained constant regardless of background solvent.

Lastly, we predicted aqueous glucose concentrations from QC laser spectra. This was necessary because of our determination that our cooled detectors could not detect FTIR light scattered from within the dermis layer of skin, but that pulsed QC laser light with just over 100 mW of peak power (yet average power on the order of the FTIR source’s integrated power, which complies with ANSI standards for acceptable skin exposure) incident onto the skin could be detected from such depths [9]. As such, it was important
that we established an ability to accurately predict glucose concentrations using the QC laser as well.

Experiments were conducted in the same manner as for the FTIR predictions, yet over a narrower range of wavenumbers than for the FTIR, due to restrictions in the laser’s tuning range. According to the loading vectors obtained in FTIR data, the best location to focus in on was the glucose absorption feature around 1080 cm\(^{-1}\), which was the most prominent feature seen in the loading vector analysis for FTIR data. Thus, the concentrations predicted from QC Laser transmission were based on data between 1075 and 1085 cm\(^{-1}\).

Fig. 4.5 (bottom row) shows the predicted versus expected glucose concentration using the QC laser for both aqueous (left) and serum (right) solutions on a Clarke error grid. We observe slightly larger error bars on the QC laser data (compared to the FTIR data for both aqueous and serum solutions). We attribute this to the weaker signal to noise ratio seen in our QC laser data, which is a product of some mode-hopping artifacts caused by the laser tuning mechanism. Even though the standard deviation between individual prediction values for a respective concentration was slightly greater than for FTIR data, we see clinically accurate predictions to as low as 40 mg/dL. Furthermore, comparisons of the first loading vectors in Fig. 4.6 for FTIR (top) and QC laser (bottom) show similar regions of prominent glucose absorption features with respect to wavenumber.
4.2: Summary

In summary, we show that glucose concentrations spanning the entire range of human hypo- to hyperglycemia can be predicted to clinical accuracy (less than 20% deviation from the actual concentration when outside the hypoglycemic range or a prediction value in the hypoglycemic range when the actual concentration is also in the hypoglycemic range) \textit{in vitro} when contained in biological fluids by using mid-infrared transmission spectra. Mid-infrared glucose absorption features are strong enough to where concentrations as low as 30 mg/dL can be predicted consistently using either a broadband mid-infrared source or a QC laser, which is the highest sensitivity documented for glucose sensing in this wavelength region [20]. The optical powers we use in this study (7 mW integrated for the FTIR broadband source and around 5 mW average for the QC laser), as well as the path length of the liquid cell (100 μm), are kept in accordance with acceptable power levels and expected propagation path lengths in human skin. These results thus indicate that noninvasive glucose sensing with mid-IR light is feasible.
References

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Chapter 5

Demonstration of *in vivo* Glucose Sensing Capability in Human Subjects

The assessments of glucose sensing capability with mid-IR QC laser spectroscopy discussed in Chapters 3 and 4 culminate into a working device capable of clinically accurate sensing glucose *in vivo* in human volunteers! In this chapter, the steps we took to build a working sensor based on fiber-optic light delivery and collection are discussed, with a focus on the primary challenges encountered when dealing with live human spectra, as opposed to mounted porcine skin or liquid solutions contained in a transmission cell. Design improvements to increase the signal to noise ratio (SNR) as well as the system’s stability are discussed. These optical enhancements lead to breakthrough results showing that PLSR analysis of back-scattered mid-IR spectra from human subjects can predict glucose concentrations to clinical accuracy.
5.1: Added Challenges with Human Spectra

Working with human spectra leads to additional challenges that are not present when conducting *in vitro* studies with stationary objects such as mounted porcine skin or liquid cells. Signal quality and stability is significantly lower for *in vivo* measurements, primarily due to the impact of an additional noise source – involuntary motions. In an *in vitro* experiment, the main sources of noise occur due to the electrical, thermal, and mechanical properties of the setup components. Such noise is well understood and follows Gaussian statistical trends, which allows the impact of the noise to be diminished through additional sampling and filtering [1]. In an *in vivo* study, however, the sample itself can cause additional fluctuations to the signal through involuntary motions caused by the neural feedback loop. Kinesiology studies have shown that these fluctuations, which can cause significant changes in signal magnitude, are position-based [2]. Additionally, they do not conform to the same statistics that govern electronic and thermal noise, which makes it necessary to collect a large number of samples so that a means for quantitative outlier identification based on an existing database for a typical *in vivo* measurement can be employed to counteract this additional source of noise.

Evidence of noise from involuntary human tremors can be highlighted through an experiment that compares the steadiness of a human arm to that of a metallic mount. Fig. 5.1 shows the differences in spectral quality when a free-space optical setup is used to collect light back-scattered from porcine skin using both a metallic mount (left) and with the porcine skin attached to a human arm (right). When the porcine skin is mounted, consecutively recorded spectra across the
Fig. 5.1 a) QC laser spectra of back-scattered light from porcine skin mounted on a stable aluminum block. b) QC laser spectra of back-scattered light from the same skin sample, this time held by a human arm. In both cases, a free-space optical system was used for light collection. The instability seen – the loss of repeatability in the spectra as well as the loss of resolvable spectral features – indicates the lack of effectiveness of a free space optical system for collecting back-scattered light from skin after the introduction of additional positional variation.

QC laser tuning range show repeatable spectral shapes with distinguishable features that can be resolved. On the other hand, when the porcine skin is attached to a human arm, the spectra lose all repeatability and distinguishability. Because light was back-scattered from the same sample for both studies (with the only difference being the mounts), the additional noise encountered when using a human arm to hold the skin sample indicates that the free space optical system was not a sufficient means of capturing backscattered light from the porcine skin after the introduction of positional perturbations caused by the
involuntary tremors. In Chapter 3, the importance of steady beam positioning on the skin sample was discussed - angular scattering properties can vary significantly with even centimeter order changes to incident beam position [3]. When the human tremors cause a relative position shift, the free-space optical system encounters interferences between such patterns, causing the noise seen.

Given that the differences in absorption feature width and magnitude between glucose concentrations in the physiological range are very small and require several iterations of regression analysis to resolve [4] (discussed in Chapter 4), we face the situation of a low signal in a noisy background in the best scenario. Complicating an already weak SNR system with the additional noise in Fig. 5.1b would make it impossible to resolve glucose spectral features for accurate concentration predictions. Therefore, a free-space optical system was not practical for an in vivo glucose sensor; we needed a larger SNR, as well as increased stability. Here, stability is quantified through repeatability of spectra – consecutively recorded spectra under the same controlled conditions should have as low a variance as possible. We implemented hollow-core fiber based light delivery and collection in multiple design iterations into our setup in order to improve SNR and stability. The quality of the sensor designs was quantified through percent of clinically accurate predictions on a Clarke Grid [5] as well as the SEP metric (discussed in Chapter 4).

5.2: Fiber-Based in vivo Glucose Sensor

Fiber-optic based sensors are used in many in vivo biomedical applications, including glucose sensing, due to their flexibility and small size. Fibers allow for precise
beam delivery and collection, and their tips can be designed to accommodate for contact with various surfaces [6-13]. Fibers can be bundled to enhance light collection while occupying a relatively small amount of space. Small fiber contact probes that can either be embedded into skin or attached to the surface of skin allow for the maintenance of beam position with respect to the skin even while the subject moves. The flexibility fibers provide with regards to space consumption and portability, as well as the ability for precise control of beam delivery and collection, make them a valuable and heavily used tool in biomedical optical studies. The optical principles governing light collection in a fiber-based system are similar to those in a free space system; the amount of light that an individual fiber can collect is governed by numerical aperture. The numerical aperture of a fiber is dependent on the refractive of the fiber’s core material, which in turn is designed to tailor to a specific wavelength range [14].

Using fibers does have drawbacks that merit consideration - in addition to losses from surface reflections that also occur with free-space optical components, fibers are prone to bandwidth limitations additional loss while light propagates through the core. Bandwidth limitations can occur from intermodal dispersion, caused by variations of velocities of different modes in a fiber, or from intra-modal dispersion, which originates from the line width of a mode. Loss can occur from absorption and scattering mechanisms, primarily through optical properties of the fiber material and impurities, as well as the interaction of light with acoustic signals generated by the light propagation. These mechanisms are described and discussed in detail in standard optics texts [14 – 16]. In addition, curving the fiber will lead to additional loss. Although there are many loss
mechanisms to consider, fiber lengths in our sensor were relatively short, on the order of 10 cm, and as such, we were not heavily impacted by fiber loss.

We utilized fibers in our glucose sensor to ensure delivery of the QC laser light to the skin in a fixed position and to facilitate the collection of back-scattered light even as the subject moved (both voluntarily and involuntarily). Miniaturization efforts came about later (see Chapters 6 and 7) and were not a primary motivation for fiber usage at this time. In the mid-IR between 8 – 10 μm, two types of cores are predominant – chalcogenide and hollow (air) core [17]. We chose to work with the hollow-core fibers primarily the ease of handling; their larger core diameter (approx. 500 μm) and air-friendly outside coating allowed for straightforward handling and manipulation of bare fibers. This allowed for rapid changes and additions to our fiber probes and comparison of spectral stability without the need for special jacketing apparatus. Chalcogenide fibers were much smaller and required specialized equipment to modify while not providing any benefits in terms of signal loss. They were also much less durable and prone to fracturing than hollow core fibers. The major disadvantage of the hollow core fiber was numerical aperture. However, the disadvantage of having smaller (5° acceptance for hollow core vs. 30°+ acceptance for chalcogenide) numerical aperture could be mitigated by bundling fibers into a probe, as discussed below.
Fig. 5.2: Evolution of our mid-infrared noninvasive glucose sensor from an open path system using free space optics to a system implementing a contact probe with a seven fiber bundle (one fiber for beam delivery and six for collection). Throughout each step, signal stability and SNR were improved; a summary can be seen in Table 5.1. The increased SNR and stability in the final design is a result of the contact probe mitigating effects of involuntary physiological tremors.

5.2.1: Designing an Optimal Fiber-Based Glucose Sensor

The ideology for fiber-based *in vivo* noninvasive glucose sensing is similar to the *in vitro* study discussed in Chapter 4, but instead of collecting transmitted light through a liquid cell, we collect back-scattered light from skin. Prior to conducting *in vivo* studies, the fiber setup was optimized for stability and signal quality. To this end, the fiber-based sensor had its own stages of evolution, starting with only a delivery fiber and growing into a bundled multi-fiber probe with one fiber for laser light delivery and six for collection
encased into a probe that was held in contact with the skin (Fig 5.2). Stability for each iteration of the setup was quantified through the average standard deviation (denoted as $\sigma$) of 50 consecutive measurements on the same sample, and SNR was measured by dividing the average detected signal magnitude with a scattering sample present to the average detected signal magnitude with no sample. Table 5.1 shows a summary of these metrics for each stage of the probe; $\sigma$ was decreased 40% and SNR was increased by a factor of 9 when going from a free-space open path setup to a multi-fiber bundle with contact probe.

Table 5.1: A summary of the evaluation metrics used to determine the quality of each iteration in our hollow core fiber-based noninvasive glucose sensor. Throughout the evaluation, all aspects of the setup aside from fiber-related delivery and collection mechanisms were kept constant. Here, $\sigma$ denotes the average standard deviation of 50 consecutive spectra. Desired qualities for a system are low $\sigma$ and high SNR.

<table>
<thead>
<tr>
<th>Setup Type</th>
<th>$\sigma$ (mV)</th>
<th>SNR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open Path</td>
<td>.168</td>
<td>184.5</td>
</tr>
<tr>
<td>Open Collection</td>
<td>.176</td>
<td>39</td>
</tr>
<tr>
<td>Single-Fiber Collection</td>
<td>.199</td>
<td>10.2</td>
</tr>
<tr>
<td><strong>Fiber Bundle Collection</strong></td>
<td><strong>.101</strong></td>
<td><strong>1667.5</strong></td>
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</tbody>
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Fig. 5.3: Geometry of fiber numerical apertures and angular scattering profiles from skin. The combination of fiber acceptance angle with angularly selective scattering from skin leads to an optimal distance to keep the collection fiber away from the skin surface. This diagram assumes the delivery and collection fibers are adjacent, which was the case in our system.
Fig. 5.4: The benefit of using multiple collection fibers arranged around the delivery fiber. If the peak angular scattering feature at a given position of skin occurs around 270° with respect to the incident beam, a single fiber in a suboptimal orientation will not collect the majority of the back-scattered light. A multiple fiber bundle does not need to be repositioned for collection of peak angular scattering features.

Furthermore, it is worth noting that a comparison of stability and SNR metrics (Table 5.1) for a single collection fiber with the multi-fiber bundle shows that a single fiber is not adequate for achieving high SNR; this is due to the angular scattering profile of QC laser light back-scattered from skin being broad with unpredictable peaks at different positions – one would need to reposition a single fiber with a 6° acceptance angle to match the scattering geometry every time a measurement was taken, which is impractical for in vivo studies due to the time and additional equipment required. Additionally, we experimentally determined that an optimal distance of 5.8 mm from a subject’s skin surface existed for collection of back-scattered light from skin with hollow core fibers, and this corresponded with the expected value based on known geometry of our fibers as well as
angular scattering patterns of skin (Fig. 5.3). Multiple fibers arranged in a circle around the delivery fiber help to alleviate these issues since covering a 360° region around the incident allows for the collection of a broader angular range of scattered light (Fig. 5.4).

5.3 In vivo Glucose Sensing with the Fiber-Based Sensor

5.3.1: Experimental Setup and Method

Based on the results obtained for stability and SNR of fiber-based setup designs, we proceeded with in vivo glucose sensing measurements using the fiber bundle contact probe with three healthy human subjects. These individuals started on an empty stomach (glucose levels were typically around 80mg/dL at this time), and we obtained spectra for their low glucose levels. The subjects then consumed twenty jellybeans, which caused their glucose levels to first rise for about a half an hour and then decline again. Consumption of jellybeans is a standard method for efficiently increasing glucose concentrations in humans [19]. Spectra were taken at various points during this variation (calibrated with a commercial electrochemical meter) to obtain measurements for stronger glucose concentrations. This process of controlled glucose level manipulation was used over the span of several weeks to build a dataset for each individual, containing spectra representing a range of concentrations between low and high points (which varied for different subjects – high points ranged from 120 – 160 mg/dL). A diabetic is identified as an individual whose blood glucose concentration does not decrease to less than 140 mg/dL.
Fig 5.5: Schematic of fiber-based setup used to collect mid-IR spectra from human skin. Delivery of QC laser light as well as collection of light backscattered from within the skin are both done through utilization of hollow core fibers coated for optimal transmission of wavelengths from 8 – 10 μm.

within two hours of a meal. For each subject, spectra representing at least nine distinguishable concentrations, as measured using a commercial electrochemical (finger prick) glucose meter (One Touch Ultra 2), were included in the dataset.

Mid-IR spectra were taken using the setup depicted in Fig. 5.5: Light from a pulsed Daylight Solutions Inc. external cavity QC laser with a tuning range between 8 – 10 μm was focused into a hollow-core fiber (500 μm diameter, by Opto-Knowledge Systems, Inc.) responsible for delivering light onto a region of the human palm between the thumb and index finger. Laser light incident onto skin was maintained at 55kHz and 1% duty cycle with peak powers of 50 - 125 mW (depending on wavenumber), resulting in average powers that were on the order of the solar radiation and within limits set by ANSI for
acceptable radiation intensity on skin. Furthermore, the peak intensity incident onto skin was kept well below reported values for tissue damage thresholds [18]. Backscattered light from the skin was collected using a bundle of six fibers, identical in composition and size to the delivery fiber, arranged in a circular fashion around the delivery fiber and coupled directly to a commercial liquid nitrogen cooled mercury cadmium telluride (MCT) detector. Numerical values for a subject’s blood glucose concentration level were obtained using a commercial electrochemical meter prior to the optical experiment. Up to ten spectra were taken for each concentration, with a single spectral scan taking roughly 20 seconds; upon conclusion of such a set, the subject once again obtained a reading from the commercial meter to ascertain the stability of blood glucose levels throughout the elapsed time period.

Chemo-metric prediction of principle component concentrations in a given dataset usually employs partial least squares regression (PLSR), which we use to obtain predictions for glucose concentrations in this study. PLSR is a technique that combines linear regression with principle component analysis [20], and it is optimal for situations where linearly correlated quantities are measured, such as absorbance versus concentration for this specific scenario. Additionally for this study, prediction analysis was also conducted using linear regression of spectral derivatives, since derivative spectroscopy has been reported to enhance the sensitivity of spectra containing small signal changes in a large noise background [21-25]. By analyzing the derivatives (primarily second derivative) of the absorption spectrum within that region, the breadth and depth of the glucose absorption in a dense medium like ISF, could be better resolved.

Calibration sets contained spectra for four unique concentrations and were chosen as follows. All concentrations measured for a subject were put into a matrix and different
calibration sets were created repeatedly through random assignment. PLSR and derivative spectroscopy were performed independently for each random set. While the concentrations for the spectra not used for calibration would be unknown in a real life setting, having each spectra correspond to a concentration measured with a commercial meter allowed for analysis of the accuracy of the values output from regression analysis. The degree of deviation from an ideal one-to-one relationship between predicted values versus their expected values has been used as a standard metric for determining the usefulness of noninvasive glucose sensors.

5.3.2: PLSR Prediction Results

The standard metric for the determination of acceptable accuracy in a noninvasive glucose sensor has been the Clarke error grid [5]. The grid involves a plot of predicted glucose concentration versus expected (known) concentration, with designated zones representing the degree of harm an erroneous reading would cause for a diabetic. For example, a monitor reading of 100 mg/dL when the actual concentration is 300 mg/dL would be very harmful to a person, as no action would be taken based on the monitor reading, while in reality, the subject would need to take supplemental insulin. Clinical accuracy on the Clarke grid is defined as a
Fig. 5.6: (Top) General depiction of a Clarke error grid, denoting varying regions of prediction accuracy. (Bottom) Clarke error grid plots of predicted versus expected glucose concentrations versus for three different human subjects. Predictions in the green region indicate clinical accuracy, and predictions in the yellow region indicate benign inaccuracies. For an individual subject, clinical accuracy was achieved over 70% of the time, and with results aggregated, clinical accuracy was achieved 84% of the time.

Prediction within 20% of the expected concentration for concentrations greater than 70 mg/dL. For concentrations under this level, considered the hypoglycemic range, a clinically accurate prediction would be a number that is also in the hypoglycemic range.

The achievement of clinical accuracy in glucose detection using human in vivo mid-IR back-scattered light can be seen in Fig. 5.6. We display representative Clarke plots from
three different (non-diabetic) subjects that participated in this study, using second derivative spectroscopy to predict glucose concentrations from mid-IR spectra. One can determine that the vast majority of predictions obtained, over 70% for any given individual and 84% aggregate, fall within the clinically accurate (green) regime of the Clarke grid, for concentrations as low as 75 mg/dL and as high as 160 mg/dL. Comparison of error metrics such as average percent prediction error, median error, and maximum error throughout the many iterations of randomly chosen calibration sets showed consistency – for both PLSR regression and derivative spectroscopy, average errors between best and worst iterations deviated less than 6%. Average and median errors typically differed from each other by 1-2%, with average error generally being generally higher than the median error. This indicates that a small number of inaccurate predictions were decreasing the average accuracy of a set containing a majority of very accurate predictions (this can be ascertained from Fig. 5.6 as well). For both PLSR and derivative spectroscopy, worst case average errors hovered around 20%, the hallmark for clinical accuracy, with best case scenarios having average errors as little as 7%.

In this study, second derivative spectroscopy performed slightly more favorably than PLSR with original spectra (which tended to slightly overshoot predictions), yielding a 4% smaller aggregate average error. Additional error measurements were taken for the expected values themselves; we found the commercial meter to have a 6% variation, on average, for consecutive trials taken with minimal time lapse. Deviations in meter readings were determined to be relatively consistent for both low and high concentrations, which indicates that the reported error did not occur due to measurements during a period of change in the
Fig. 5.7: (Top): Ten mid-IR human spectra, each representing 86 mg/dL glucose concentration (blue) and 112 mg/dL glucose concentration (red). (Bottom): Average values for spectra of both concentrations at three specific wavenumbers: 1080, 1130, and 1165 cm\(^{-1}\). At 1080 cm\(^{-1}\), where glucose is expected to absorb, a correlation between attenuation in scattered signal versus increased glucose concentration is observed to beyond a standard deviation. At the other two non-glucose-specific wavenumbers, such a correlation was not observed.
body’s glucose levels. Accounting for a possible 6% error in expected value would work to further increase the average prediction accuracy obtained by both prediction methods.

5.4 Spectral Variations with Respect to Glucose Concentration

Additionally, we observed that changes in blood glucose levels corresponded to visible changes within \textit{in vivo} spectral features at prominent glucose absorption wavenumbers verified by our \textit{in vitro} work\textsuperscript{4}, primarily around 1080 cm\textsuperscript{-1}. Fig. 5.7 (top) shows batches of ten consecutively and rapidly recorded spectra representing 86 mg/dL (blue) and 112 mg/dL (red), while Fig. 5.7 (bottom) shows analysis of the average values of each batch at three wavenumbers – 1080, 1130, and 1165 cm\textsuperscript{-1}. The spectra deviated up to 13% on average across 1040 through 1180 cm\textsuperscript{-1} with 3% of the noise originating from electronic components, and the rest occurring due to physiological tremors. Changes in detected backscattering due to glucose concentration differences were greater than the error around 1080 cm\textsuperscript{-1}, the location of a central glucose absorption feature. Here, the average signal scattered at the higher glucose concentration was 27.6% lower than the signal scattered at the lower glucose concentration, a significant difference in absorption depth. For the other two regions, where glucose does not contain prominent absorption features, the average scattered signals for both low and high glucose concentrations were
Fig. 5.8: (a): Spectra of backscattered light from a human subject’s palm recorded at equidistant time intervals during a fluctuation of glucose concentration, caused by the consumption of jellybeans. Numerical concentration values were obtained using a commercial monitor. (b): Glucose concentrations predicted from the spectra seen on top plotted versus time, and compared to the curve of measured concentration versus time.

within 5% magnitude and within each other’s standard deviation, indicating that spectral features there were not the result of varying glucose concentrations. Fig. 5.8a shows spectra for a human subject recorded in equal time intervals over the period of an hour, with benchmark blood glucose readings from a commercial monitor taken at the beginning, middle, and end of the experiment.

Fluctuating glucose levels during the experiment were the result of jellybean consumption. The glucose concentration peaks around the middle of the experimental time frame and
decreases towards the end of the hour period, and the absorption feature widths and depths mirror the measured concentration changes. In each case, visual comparison of absorption depth around 1080 cm\(^{-1}\) shows increased depth in the backscattered signal for higher concentrations of glucose. Integrals were taken from 1075 to 1085 cm\(^{-1}\) to quantify the combined effect of increasing absorption depth and width versus concentration. Here, we observed a 16% increase in combined depth and width from 86 to 106 mg/dL, and a 41% increase from 86 to 120 mg/dL, indicating that both absorption depth and width increase with increasing concentration, a trend also seen in measurements of aqueous glucose\(^4\). The correlation of these spectral changes with concentration changes is verified through use of first derivative spectroscopy to predict the concentrations represented by the individual spectra. This analysis is shown in Fig. 5.8b, where one can see the prediction trend following the general trend of measured glucose concentration versus time.

5.5: Summary

In summary, we show that mid-IR spectra obtained \textit{in vivo} from human skin using a fiber-based sensor are significantly more stable than human spectra taken with a free-space optical system. The ability to incorporate multiple fibers into a contact probe that can be held on skin leads to decreased noise from involuntary tremors in human spectra. Spectra taken with our fiber-based sensor yield clinically accurate predictions for blood glucose levels for concentrations between 75-160 mg/dL using PLSR with derivative spectroscopy techniques. Best-case scenarios with given calibration sets yielded average errors only 2% more than those from a commercial electrochemical meter. Furthermore, glucose absorption features in mid-IR skin visibly change with respect to increasing
concentration, as absorption minima increase in depth and width. With these breakthrough results, we establish a foundation for noninvasive \textit{in vivo} glucose sensing based on mid-IR QC laser spectroscopy. Although the primary motivation for using fibers in this version of our sensor was obtaining the best spectral quality possible, the physical advantages that fibers provide opens up future prospects for miniaturizing the sensor to the size of a wearable device. Further details of future miniaturization efforts are discussed in Chapters 6 and 7.
References


Chapter 6

Enhanced Sensor Portability Through Use of an Integrating Sphere and TE-Cooled Detector

While the fiber-based noninvasive *in vivo* glucose sensor presented in Chapter 5 provided breakthrough results showing accurate glucose concentration prediction capabilities using mid-IR light, further enhancements were made in order to achieve higher prediction accuracy by replacing the fiber-optic collection with an integrating sphere that allowed for optimal collection of the broad angular back-scattering profile of mid-IR light from skin (discussed in Chapter 3). Maximum collection of these angular scattering features led to a substantial increase in signal stability, which allowed us to replace the liquid nitrogen cooled detector with a thermoelectrically (TE) cooled detector, eliminating the sensor’s dependency on liquid nitrogen. A combination of the integrating sphere and
TE-cooled detector allowed us to fit the sensor onto a desk-height mobile cart that could be easily transported.

6.1: Enhanced Light Collection with Integrating Spheres

Integrating spheres are optical components designed to facilitate collection of diffusely scattered light [1]. The inner walls of an integrating sphere are roughly coated with material designed to scatter light of a target wavelength equally in all directions (similar to a Lambertian diffuse reflectance model [2]). The sphere contains multiple ports, two at minimum, depending on the application. Integrating spheres are used for a wide variety of optical measurements, ranging from intensity calibrations to characterization of optical coefficients [3-6]. For our noninvasive glucose sensor, a three port sphere was used, one port admitting QC laser light, one port for an individual to rest their arm, and a third port to transfer light to a detector. Upon entering an integrating sphere, light will traverse through the sphere core, scattering every time it hits a wall. Eventually, the light will exit the sphere through the output port, where a detector can capture it. We felt that an integrating sphere would improve the optical collection efficiency of our setup because it is the ideal apparatus for collecting the broad angular range of scattered light that is typical from the interaction of mid-IR light with skin (as discussed in Chapter 3).

In the case of our three-port sphere, light enters through the input port and travels unimpeded until reaching a skin sample. The light that backscatters from skin is then passed through the sphere until reaching the output port, upon which
the detector collects the light. Since all of the scattered light, regardless of scattering angle, is contained within the sphere, the optical collection efficiency is significantly boosted. A tradeoff for the increased light collection is the travel time to the detector; the more times the light has to bounce through the sphere before reaching the detector, the longer the delay is between the incident beam hitting the skin and the scattered light being collected by the detector. In our study, we expected those delays to be on the order of hundreds of nanoseconds; because our sampling rate was 100 kHz, we did not encounter any aliasing from this time delay. This was confirmed by the significantly increased stability of human spectra (discussed below) using the integrating sphere setup.

6.2: *In vivo* Glucose Sensing with the Integrating Sphere Sensor

6.2.1: Experimental Setup and Method

Experiments for testing and evaluation of the integrating sphere setup were conducted exactly in the same manner as the steps used for the fiber-based setup, with the only change being the collection mechanism for the light back-scattered from skin. The sphere used in this study was a commercial, off the shelf product from PIKE technologies, designed for optimal function between 7-12 μm. The inside of the sphere was coated with gold, which is the best reflector of light in this wavelength region. The construction of the sphere allowed for convenient contact with the sample port, meaning that a test subject could rest his/ her arm on the sphere while the data was being acquired. The experimental setup schematic for the
Fig 6.1: Schematic of integrating sphere setup used to collect mid-IR spectra from human skin. QC laser light is delivered through the input port of the sphere, where a mirror reflects it to the sample port, upon which an individual holds their arm (in contact with the sphere). The light back scattered from the arm propagates by scattering repeatedly from the walls of the sphere until it reaches the output port, where an MCT detector is used to collect the light.

Integrating sphere sensor is shown in Fig. 6.1, and a summary of the experimental procedure – the steps taken to achieve prediction results out of mid-IR spectra – are shown in Fig. 6.2.
Fig. 6.2: Summary of steps taken to achieve Clarke plots of glucose concentration prediction values from mid-IR spectra. First, reference spectra are cross-correlated with a slow scan of the laser spectrum to ensure accurate wavenumber resolution, and any necessary shifts in the x-axis are applied to the recorded skin spectra. Next, the skin spectra are smoothed with Savitzky-Golay filtering, allowing us to take less noisy derivatives of the spectra. The spectral derivatives are then analyzed with PLSR, allowing us to obtain prediction values.

6.2.2: Enhanced Spectral Stability

Spectra recorded from the integrating sphere set up showed significantly greater stability when compared to spectra taken with the fiber-based setup. We attribute this to the ability to collect the full angular scattering range of the light back-scattered from an individual, which is the major benefit provided by the integrating sphere setup. Fig. 6.3 shows examples of typical skin spectra acquired
Fig 6.3 a) Typical spectra for the labeled range of glucose concentrations recorded from our hollow-core fiber based setup. b) Typical spectra for the labeled range of glucose concentrations from our integrating sphere collection setup. Significant enhancements to the stability of the system can be visually seen from the integrating sphere spectra, confirmed by σ calculations (.078 for the fiber collection and .0008 for the integrating sphere collection). This order of magnitude improvement in the stability can be attributed to the collection of the total angular scattering range, which is made possible by the integrating sphere.
Fig 6.4: Clarke plots of predicted versus expected glucose concentrations versus for two human subjects using the integrating sphere sensor design. Although similar trends existed between the results for the sphere and fiber setups with respect to amount of predictions in A and B regions, overall standard error of prediction was decreased by 18%, on average.

from each setup for multiple glucose concentrations prior to any smoothing function (in our case, Savitzky-Golay filtering). The spectra collected from the integrating sphere setup are visually smoother, and the σ values support this; the integrating sphere spectra shown vary with a σ of 0.008, while the fiber probe collection spectra vary with a σ of 0.078. The increased stability led to glucose predictions with improved accuracy as can be seen in Fig. 6.4. Although the percentages of predictions with the A range, as well as combined A and B ranges, stayed similar (within 10%) between the integrating sphere data and the fiber-based sensor trials, the metrics of SEP (on average an 18% improvement for the integrating sphere data) as well as median accuracy after k-2 cross-validation across the entire available datasets (6% increase for the integrating sphere data) showed that the improved
stability from the integrating sphere collection significantly improved the glucose prediction capabilities of the sensor.

6.3: Use of a TE-Cooled Detector

Additionally, the increased stability allowed for the use of a TE-cooled MCT detector for data collection, replacing the need for the higher sensitivity (2 orders of magnitude higher \( D^* \)) liquid nitrogen cooled MCT detector used in the fiber-based sensor (see Figs. 5.5 and 5.10). A comparison of Clarke plots from an individual using the integrating sphere system with a liquid nitrogen cooled detector and a TE-cooled detector can be seen in Fig. 6.5. In both cases, predictions were obtained exclusively in the A and B ranges, and prediction errors for one case were within 5% of the other. This was an important step in mobilizing the sensor to make it viable for use with diabetes patients at clinics; removing the need for liquid nitrogen allows for a less bulky and more easily transportable sensor. Since the results obtained from the described laboratory trials demonstrate the capabilities of the sensor to obtain clinically accurate predictions in controlled settings with healthy human subjects, the next step in the development of the sensor will focus on real-world trials on the desired end users (individuals with diabetes).
Fig 6.5: Comparison of a participant’s Clarke plots using the integrating sphere setup and a liquid nitrogen cooled detector (left) and a TE-Cooled detector (right). Analysis of both results show similar standard errors of prediction (each case within 5% of the other), as well as predictions exclusively in accurate/benign regions. Removing the necessity for liquid nitrogen allows the sensor to be more mobile and accessible, a requirement for using it in a clinical setting.

6.4: Summary

In summary, we show that the collection of the maximum range of angular backscattering from skin through the use of an integrating sphere significantly enhances the stability of spectra taken from an individual’s skin. This increased stability leads to an improvement in glucose prediction capability with respect to SEP and clinical accuracy metrics when compared to the fiber-based sensor. Additionally, the higher stability of the back-scattered light signal allows for the use of TE-Cooled detection, removing the need for...
liquid nitrogen, and thus enhancing the mobility and accessibility of our sensor. We conclude that a QC laser based *in vivo* noninvasive glucose sensor utilizing an integrating sphere for back scattered light collection and thermoelectrically cooled detection achieves clinically accurate glucose concentration predictions with healthy human subjects in a lab setting and is ready for testing in a clinical setting with diabetic subjects.
References


Chapter 7

Conclusion and Outlook:

Clinical Trials and Beyond

This thesis has presented a novel solution for a quality of life enhancement for diabetics. We apply QC lasers capable of penetrating into the dermis layer of skin in the mid-IR wavelength range with the aim of utilizing the fundamental benefits of mid-IR vibrational resonances to overcome limitations of past optical sensing techniques. This research shows that the mid-IR wavelength region is indeed suitable for \textit{in vivo} biomedical applications. We have reported original results of mid-IR scattering patterns from porcine and human tissue showing that sufficient mid-IR light penetration in skin can be achieved with a QC laser (Chapter 3). We have also shown that QC laser spectroscopy is powerful enough to accurately sense physiologically relevant glucose concentrations, using PLSR
analysis of \textit{in vitro} mid-IR transmission spectra, at path lengths as small as 100 μm (Chapter 4). Finally, we have demonstrated that these proofs of concept do indeed translate to an \textit{in vivo} sensor capable of sensing glucose concentrations in humans with clinical accuracy (Chapter 5). Although successful \textit{in vivo} sensing is already an important and unprecedented development to both the spectroscopy and biomedical diagnostics fields, we continue to strive for the ultimate goal of providing the hundreds of millions of diabetics throughout the world with an accessible and pain-free means of measuring their glucose levels. With that goal in mind, we demonstrated a more compact and mobile version of the sensor featuring an integrating sphere collection optics and liquid nitrogen free detection (Chapter 6). We continue to design hardware and software improvements for miniaturizing the sensor and making it accessible for home use, as well as for improving accuracy to compete with current commercial sensors.

\textbf{7.1: Clinical Trials}

While we have achieved, to the best of our knowledge, the highest reported accuracy for a noninvasive \textit{in vivo} glucose sensor in the mid-IR [1,2], we plan to build upon the promising early results and further develop the sensor with the immediate goal of obtaining large scale (on the order of thousands of spectra) datasets through clinical trials on diabetics in diagnostic labs and hospitals, as well as the long term goal of achieving accuracy levels that are competitive with commercial electrochemical sensors. Results from clinical trials will provide
Fig. 7.1: Roadmap for enhancement of the sensor for clinical trials. On the hardware side, we have transitioned the system from an optical table to a mobile cart suitable for transport, and we desire to use a QC laser with a flat tuning range in this wavelength region. Furthermore, we aim to miniaturize our integrating sphere to miniaturize the sensor as well as minimize loss in order to increase the SNR and stability of the sensor. On the software side, we will simplify our user interface to allow clinicians with no prior knowledge of the sensor to operate it, implement real-time outlier identification and removal based on our existing dataset, and implement advanced machine-learning techniques to increase prediction accuracy [2-8].

essential feedback necessary for further improvement of the sensor. Transitioning from datasets with tens of spectra from healthy lab volunteers to a databank with thousands of
spectra from a diverse range of diabetics will provide information on possible differences in spectral signatures difference for individuals with exceedingly high glucose content, as well as help determine the ideal amount of calibration required for a desired level of accuracy.

Furthermore, we expect larger datasets to improve analytic capabilities to determine optimal wavelength ranges of detection for a diverse population. This information is necessary for tailoring QC laser scans to an optimal range, leading to shorter scans that make the system easier to use, as well is reduce the impact of noise from involuntary physiological tremors (see Chapter 5).

In order to ready our sensor for the first set of clinical trials, the next phase in development will focus on the customization of hardware in order to further increase SNR while maintaining stability, as well the implementation of more advanced techniques for prediction analysis and outlier removal. A roadmap for these plans can be seen in Fig. 7.1.

7.2: QC Laser and Integrating Sphere Optimization

Plans to further improve the sensor's SNR include utilizing a custom laser with a flat power spectrum in our target wavelength range, as well as miniaturization of the integrating sphere. Currently, both the QC laser and integrating sphere are commercial, off the shelf products not customized for our project. Our laser's power spectrum varies from 100 – 200 mW peak power depending on wavenumber. For in vitro studies, this power difference can be accounted for through a reference spectrum, but for in vivo studies, variations in power often lead to variations in scattering depth (discussed in Chapter 3), which is not an ideal situation for comparing spectra for different concentrations. The ideal
solution to this would be to design a laser with high peak powers and a flat power spectrum between 8 – 10 μm for use in our sensor.

Additionally, the integrating sphere we are using in our sensor is a commercial product designed for FTIR use, which means that the input and output port sizes are larger than is necessary for a QC laser application. The input port has a 3mm diameter, whereas the diameter of our QC laser beam is <1mm. If port sizes were matched to the requirements for our application, the sphere could be made roughly 10 times smaller, improving miniaturization capabilities, and also would lose less light, enhancing the sensor’s SNR. Possible solutions for optimal light delivery to the sphere include connecting the QC laser output window to the sphere input port using a fiber or coupling the QC laser directly onto the input port.

7.3: Machine Learning Algorithms

On the software side, we are looking to enhance prediction accuracy of mid-IR spectra through implementation of recent discoveries in wavelet decomposition and machine learning techniques for biomedical applications [3-6]. Although PLSR has provided us with solid results, it has limitations when the application is extended beyond a linear physical model. It is evident from our in vitro spectra at higher glucose concentrations that the absorption features increase both in depth and width (see Chapter 4), which is not an optimal scenario for PLSR analysis. We are currently working on implementing neural networks [7] and/or support vector regression [8] techniques on wavelet decomposed spectra with the expectation of improving prediction accuracy.
Aside from improving general accuracy statistics, we are seeking to minimize the amount of calibration required for clinically accurate predictions. For the results obtained in our laboratory testing, each participant used their own unique spectra to calibrate future spectra. If the sensor were to be applied to the real world in this state, this would mean that every user would have to calibrate the sensor with at least five finger prick readings with an electrochemical sensor before being able to use it noninvasively. We predict that recording spectra with higher SNR and using advanced regression methods to analyze that spectra will allow our sensor to predict spectra from a diverse population with a pre-set databank, rather than requiring each individual to provide calibration spectra.

7.4: Conclusion

Through the design enhancements discussed in this chapter and a continued dedication to iterative refinement in both sensor miniaturization and accuracy, we seek to transform our noninvasive in vivo mid-IR glucose sensor from a mobile system on a desk-height cart capable of approximately 85% prediction accuracy to a compact, fully portable sensor that one can fit in their hand capable of glucose detection rivaling that of commercial electrochemical sensors. We envision a world where diabetics do not have to prick their finger multiple times a day and instead can rest a hand held device on their wrist for a few seconds and obtain a reading for their glucose concentration.
References


List of Publications


List of Conference Presentations


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