Development of a Wearable Short-Wave Infrared Photoplethysmography Device for Detection and Monitoring of Hemodilution During Postpartum Hemorrhage

Hannah Gruensfelder

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Development of a Wearable Short-Wave Infrared Photoplethysmography Device for Detection and Monitoring of Hemodilution During Postpartum Hemorrhage

by

Hannah Gruensfelder

A thesis presented to
the McKelvey School of Engineering
of Washington University
in partial fulfillment of the
requirements for the degree of
Master of Science

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St. Louis, Missouri
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ABSTRACT OF THE THESIS

Development of a Wearable Short-Wave Infrared Photoplethysmography Device for Detection and Monitoring of Hemodilution During Postpartum Hemorrhage

by

Hannah Gruensfelder

Master of Science in Biomedical Engineering

Washington University in St. Louis, 2024

Professor Christine O’Brien, Chair

Postpartum hemorrhage (PPH), the leading cause of maternal death and morbidity, affects nearly 14 million people worldwide each year, disproportionately impacting racial minorities and people in low resource settings. A timely diagnosis of PPH is key in providing optimal patient care, as an estimated 90% of deaths due to PPH are preventable with early diagnosis and treatment. Early diagnosis is especially critical where there is limited access to blood transfusion and surgical care. There are few tools for diagnosing and monitoring PPH, and it is currently diagnosed by visually estimating blood loss and monitoring vital signs. In many clinical settings, visual estimations significantly underestimate blood loss and often fail to detect internally retained blood. During early hemorrhage, patients experience sudden changes in vascular equilibrium, and the body compensates to keep the heart rate and blood pressure stable despite moderate to large blood loss volumes. In young, healthy patients, such as those of childbearing age, vital signs may only begin to fluctuate with large levels of blood loss and can therefore reliably identify only late stages of PPH, by which time surgical intervention or blood transfusions are required. In addition to detecting and diagnosing PPH early, it is also extremely important to monitor patients after beginning treatment for PPH. Current treatment options
include medications, blood transfusions, and surgical interventions such as laparotomy and hysterectomy, which all can present significant risks to the patient. It is imperative that patients be closely monitored after introduction of treatment, however, patient monitoring is currently limited to in-patient hospital care using serial blood draws and vital sign monitoring devices.

One compensatory mechanism during PPH is hemodilution, in which water from the extravascular space is drawn into circulation to increase blood volume, resulting in a reduced hemoglobin concentration. If hemodilution is severe, it can compromise the patient's ability to oxygenate their tissues and cause coagulopathies, creating a medical emergency. There is an urgent need for a tool that can detect PPH early and monitor its progression, along with the introduction of treatments.

Absorption spectroscopy, already used in many optical device designs, is a promising technique for identifying the best wavelengths for noninvasive monitoring. However, absorption spectroscopy has historically covered only the visible (VIS) to near-infrared (NIR) wavelength ranges. Thus, many optical devices use VIS and NIR components, which can cause inaccurate results in people with skin darkly pigmented by melanin. Melanin strongly absorbs light at shorter wavelengths but is a weaker absorber at short-wave infrared (SWIR) wavelengths, making the SWIR a better wavelength range for illuminating skin with high melanin content. However, because little is known about how biological tissue absorbs in the SWIR, characterizing common biological absorbers spanning the VIS to the SWIR wavelength ranges will provide the spectra necessary to create SWIR optical devices.

Here, we describe the design and development of a prototype wearable SWIR photoplethysmography (PPG) device to detect and monitor hemodilution during and after PPH. This low-cost wearable hemodilution sensor will provide continuous monitoring for early
diagnosis of PPH and will prevent dangerous blood loss. Importantly, the use of SWIR LEDs and detectors will minimize the effects of melanin absorption, minimizing the racial disparities affecting other light-based diagnostics. This device has high potential to be successfully translated as an inexpensive, fully wireless, wearable watch, finger clip, or necklace that will be accessible to patients across the world, particularly where PPH mortality is the highest.
Chapter 1: Introduction

1.1 Postpartum Hemorrhage

Postpartum hemorrhage (PPH), defined as excessive blood loss following delivery, affects nearly 14 million people worldwide each year\(^1\). PPH can occur up to 12 weeks after delivery of the fetus, but most often occurs within 48 hours following delivery. For a single birth followed by PPH, the average amount of blood lost is around 500 mL, and for a Cesarean delivery or delivery of twins, the average loss increases to 1,000 mL\(^1\). The most common causes of PPH including uterine atony, retained placenta, and genital tract trauma\(^2\). In uterine atony, the uterine muscles fail to contract and close blood vessels after birth, allowing excessive bleeding\(^3\). In retained placenta, the placenta is not expelled from the uterus completely after delivery, and the area where it is still attached to the uterus bleeds significantly\(^4\). Lastly, genital tract trauma occurs when there is a cervical tear or vaginal laceration following delivery\(^2\).

1.1.1 Maternal Death and Morbidity

Postpartum hemorrhage (PPH), the global leading cause of both maternal death and morbidity, accounts for nearly 70,000 maternal deaths each year\(^1\). Apart from death, severe PPH can leave people with lifelong complications. However, a timely diagnosis and medical intervention can prevent over 90% of PPH cases\(^5\). Common treatments for moderate PPH include uterotonic drugs to induce contraction of the uterus, fluids to increase blood volume, and non-surgical ballons to or compression sutures to prevent blood loss\(^6\). For severe or emergency PPH, an obstetric hysterectomy is common\(^7\). Although this practice often saves the life of the mother, it irrevocably affects their fertility and mental wellbeing. In addition to causing these problems, PPH disproportionally affects low resource settings and racial minorities in the United States\(^8\). In
black mothers, the ~10% rate of PPH is significantly higher than in white mothers, for whom the rate is ~ 6%\textsuperscript{9}. In addition, black mothers are 2.5 times more likely to die due to pregnancy-related mortality\textsuperscript{8}.

1.1.2 Current Methods for Detecting and Monitoring Postpartum Hemorrhage

Currently, PPH is detected and monitored by estimating blood loss and monitoring vital signs. Doctors estimate blood loss volumes both visually and with quantitative aids. Visual estimation of blood loss often consists of looking at blood-soaked towels and pads to label PPH as mild, moderate, or severe (Figure 1.1, A)\textsuperscript{10,11}. However, in many clinical settings, visual assessment of blood loss is prone to underestimation, especially at higher volumes, and it often fails to detect internal bleeding. In addition to visual estimation, doctors quantify blood loss by weighing blood-soaked towels and pads or using a bag below the buttocks to catch blood expelled after birth (Figure 1.1, A)\textsuperscript{12,13}. Neither method of estimating blood loss provides real-time information and therefore delays early and accurate intervention.

Aside from estimating blood loss, doctors use vital signs to diagnose and monitor PPH (Figure 1.1, B)\textsuperscript{14,15}. During early PPH, patients experience sudden changes in equilibrium and the body uses compensatory mechanisms to keep the heart rate and blood pressure stable\textsuperscript{16}. Due to these compensation mechanisms, the vital signs of many patients show minimal changes in response to early hemorrhage. In many cases, vital signs do not begin to fluctuate until patients have lost significant amounts of blood, so vital sign monitoring can reliably identify only late stages of PPH\textsuperscript{17}. Unfortunately, late stage PPH often requires surgical intervention or blood transfusions, which are often unavailable in low resource settings\textsuperscript{7,18}. The current methods for
diagnosing and monitoring PPH are inadequate, and a more reliable monitoring tool and a method of earlier detection are required to allow timely treatment and provide safer outcomes.

In addition to early detection and diagnosis of PPH, it is also extremely important to monitor patients after the initiation of treatment for PPH. Treatments for PPH can present significant risks to the patient, depending on such factors as their weight, health, and medical history, so patients must be closely monitored following the introduction of treatment for PPH\textsuperscript{19}. However, this monitoring is typically limited to in-patient hospital care and the use of vital sign monitoring devices and is unavailable to mothers who gave birth at home, who have left the hospital, or who do not have monitoring devices\textsuperscript{17}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Schematic of the current PPH Detection Methods. A: Blood Loss Estimation and B: Vital Sign Monitoring}
\end{figure}

\subsection*{1.1.3 Hemodilution}

During PPH, the body tries to compensate for blood loss, and one compensation mechanism is hemodilution, in which water from the intravascular space is drawn into the circulation to increase blood volume, resulting in reduced hemoglobin concentration\textsuperscript{20}. If hemodilution is severe, it may compromise a patient’s ability to deliver oxygen throughout their body, creating a medical emergency. In addition, hemodilution caused by PPH directly affects
coagulation, and in severe cases of hemodilution, patients experience coagulopathies, where their blood clots inadequately or not at all\textsuperscript{21}. If coagulopathies are not treated early enough or not treated adequately, the patient will enter cardiovascular shock, multi-organ failure, and could die\textsuperscript{22}. Using hemodilution to monitor and detect PPH would provide an earlier diagnosis than vital sign monitoring devices and would give patients and providers time to employ effective intervention methods.

![Figure 1.2: Schematic of Hemodilution during Postpartum Hemorrhage](Image)

**1.2 Absorption Spectroscopy**

Absorption spectroscopy measures how much light is absorbed by a sample as a function of wavelength\textsuperscript{23}. One way to take an absorption measurement is to propagate broadband light through a sample, where the light is partially absorbed or transmitted, and then recorded. The absorption by the sample is determined by comparing a reference light spectrum, split off from the original beam, to the spectrum of the light detected after it passes through the sample\textsuperscript{24}. Absorption spectroscopy identifies properties of a sample and is largely governed by the Beer Lambert Law, which states that absorption is determined by a sample’s molar extinction coefficient multiplied by its molar concentration and by the pathlength of the light passing through the sample\textsuperscript{23}. Beer Lamber Law shows that absorption is directly proportional to the concentration of the sample and pathlength of light through the sample. However, the molar
extinction coefficient is unique to each biological absorber and measures how strongly a sample absorbs light at a particular wavelength\textsuperscript{24}. Characterizing the absorption spectra of biological absorbers is essential for understanding information about biological samples that can be used in many different engineering, chemical, and biological applications\textsuperscript{25}.

### 1.2.1 Current Biological Spectra

Biological absorption spectra have been widely used to develop optical diagnostics. Some of the most commonly used biological absorption spectra in optical devices are oxygenated hemoglobin, deoxygenated hemoglobin, water, melanin, and lipid (Figure 1.3). Researchers use the absorption spectra of these biological absorbers to better understand disease states and cardiac outputs. Although current biological spectra remain widely used in the biophotonics community, they have been limited to absorption values spanning the visible wavelengths (VIS) to the near-infrared wavelengths (NIR)\textsuperscript{26}. The visible wavelengths span from approximately 300 nm to approximately 800 nm and the near-infrared wavelengths span from approximately 800 nm to 1100 nm\textsuperscript{27}. Analyzation of the VIS to the NIR does not comprise how many biological absorbers react with different wavelengths of light past the NIR and into the SWIR. Without published SWIR spectra for different biological absorbers, clinical use of absorption spectroscopy will remain in the VIS and NIR regions, despite lower optical scattering in the SWIR. Utilizing SWIR spectra could improve light penetration depth and lower melanin absorption, which could reduce inaccurate optical diagnostic results for those with darkly pigmented skin. In addition, adding sources of biological contrast in the SWIR would potentially expand and improve upon optical diagnostics.
1.2.2 Clinical Use of Biological Spectra

Biological spectra can be used inform the development of non-invasive optical devices that utilize absorption at certain wavelengths. One example of a clinical tool based on absorption spectra spanning the VIS to the NIR is the pulse oximeter, which commonly uses LED wavelengths at 660 nm and 940 nm, corresponding to a high absorption of deoxygenated hemoglobin and oxygenated hemoglobin. Pulse oximeters use the absorption of light of oxygenated and deoxygenated hemoglobin to determine oxygen saturation in the body. However, the pulse oximeter has shown to produce inaccurate results in those with darkly pigmented skin due to a high presence of melanin absorption in the VIS. Utilizing SWIR wavelengths could provide an avenue for minimizing the effect of melanin on optical diagnostics.

1.3 Photoplethysmography
Photoplethysmography (PPG) is a non-invasive optical technique that measures blood volume changes and is normally used to detect heartbeats\textsuperscript{31,32}. To detect a PPG signal, light is directed into tissue and detected by a photodiode in either transmission or reflection mode (Figure 1.4, A). In both scenarios, the LED propagates light into the tissue, where some of it is absorbed. The light that is not absorbed by the tissue either transmits through the tissue or is scattered. In reflectance mode, a photodiode is placed adjacent to the LED, and some of the scattered light reflects back towards the photodiode, corresponding to blood volume changes from blood propagating from the heart as arterial pulse waves (Figure 1.4, B)\textsuperscript{31}. In transmission mode, the photodiode is placed on the other side of the tissue, directly across from the light source, where it detects light transmitting through the tissue (Figure 1.4, A). In a similar way to reflection mode, the light that is detected by the photodiode corresponds to blood volume changes from blood propagating from the heart and visually outputs an arterial pulse wave\textsuperscript{32}.

A) Transmission and Reflection Mode

B) Characteristic PPG Pulse Wave

\textbf{Figure 1.4:} A: Schematic of PPH Source-Detector Orientations for Transmission and Reflection Mode B: Characteristic PPG Pulse Wave

Scientists and engineers are able to use the structure of the PPG signal and the area under the PPG signal to understand information about the cardiovascular health of a patient\textsuperscript{31}. PPG
signals comprise a high frequency, pulsatile, “AC” component, which directly correlates to the heart pumping blood and arterial volumetric changes, and a low frequency, non-pulsatile “DC” component, also referred to as the DC offset, that directly correlates to underlying blood-tissue changes such as background tissue or venous blood (Figure 1.5). A typical PPG waveform contains two phases—systolic and diastolic—which respectively correspond to the rising and falling of the PPG signal. A PPG signal also has a characteristic structure that can help determine cardiac information, such as the subject’s cardiac output, arterial stiffness, age, and overall health. Many PPG sensors algorithms use signal features such as the systolic peak, diastolic peak, dicrotic notch, and pulse width, which can be used to deduce information about a patient. For example, young patients exhibit a pronounced dicrotic notch, which diminishes as they age.

![Figure 1.5: Schematic of AC and DC Components in PPG](image)

**1.3.1 Heart Rate Detection**

PPG signals are often used for heart rate detection in clinical and consumer health settings. When analyzing a PPG signal, the systolic peaks can be found using the fundamental
frequency of a signal, where the peaks are then counted in a fixed time window to extrapolate heart rate as beats per minute\textsuperscript{34}. Commercial devices such as smart watches or heart-rate monitors can detect PPG systolic peaks and other signal features, but face challenges such as sensitivity to motion artifacts and difficulty achieving good PPG signal quality\textsuperscript{35}.

In addition to heart rate detection, researchers can mine PPG signals to detect additional cardiovascular features. The first and second derivatives of PPG signals can help identify various PPG signal features by allowing for an easier visualization of inflection points when compared to the original signal (\textbf{Figure 1.6})\textsuperscript{34}. In a similar way, PPG signal feature extraction is becoming increasingly useful with machine learning and can help identify cardiac inadequacies such as arrhythmias like atrial fibrillation, which increases stroke risk significantly\textsuperscript{34,35}.

\textbf{Figure 1.6} A: Typical PPG Signal B: First Derivative of PPG Signal C: Second Derivative of PPG Signal\textsuperscript{34}

\textbf{1.3.2 Pulse Oximetry}
One of the most familiar devices using PPG signals is in the pulse-oximeter, which is used to non-invasively determine blood oxygen saturation levels in the blood stream. As discussed earlier, pulse oximeters usually use the wavelengths 660 nm and 940 nm, which correspond to strong light absorption by deoxygenated hemoglobin and oxygenated hemoglobin (Figure 1.3). To determine the oxygen saturation levels in the blood stream, pulse oximeters utilize the PPG signal outputs at a minimum of two wavelengths (typically two) in either transmission or reflection mode. As the PPG signals from both LEDs are recorded by the photodiode, the blood oxygen saturation is calculated using the ratio from the AC and DC components from the 660 nm LED (Red) and the 940 nm LED (Infrared or “IR”) (Equation 1.1). Over many years, pulse oximetry has proven to be a staple optical diagnostic that provides real-time information about blood oxygen saturation levels, and it has been incorporated into many commercial wearables to help alert people of possible medical conditions or emergencies.

\[
\text{SpO}_2 = \frac{AC_{\text{Red}}/DC_{\text{Red}}}{AC_{\text{IR}}/DC_{\text{IR}}}
\]

**Equation 1.1:** Formula for Oxygen Saturation Calculation in Pulse Oximetry

### 1.3.3 Skin Pigmentation Bias

Although pulse oximetry measures blood oxygen saturation non-invasively, recent research has revealed that high absorption of melanin in the VIS makes it prone to inaccuracy for darker skin tones. Compared to self-identified white patients, self-identified Black patients were three times more likely to have dangerously low oxygen levels that pulse oximeters did not detect. Because melanin strongly absorbs light in the VIS, blood oxygen saturation readings for those with high amounts of melanin in their skin can be falsely high (Figure 1.7). The skin
pigmentation bias in pulse oximetry has led to a racial bias and consequent inadequate medical treatment of countless Black patients, causing increased suffering, and erroneously dismissed symptoms. There is a clear need to provide equitable care for those with high levels of melanin in their skin, which will require technical changes to eliminate the skin-pigmentation bias of many optical diagnostics.

**Figure 1.7:** Accuracy of Pulse Oximetry in Measuring Arterial Oxygen Saturation, According to Race

1.3.4 Short-Wave Infrared

In the SWIR wavelength range, from approximately 1100 nm to 3000 nm, light is invisible to the human eye. The silicon detectors normally used in commercial optical devices are sensitive to light only at or below 1100 nm, making it difficult to see signals in the SWIR (Figure 1.8). Although absorption spectroscopy and many optical devices are hindered by their lack of sensitivity in the SWIR, new SWIR components have provided a way to minimize the
skin pigmentation bias. Detecting PPG signals in the SWIR requires a photodetector material that has a strong absorbance of SWIR photons, such as an InGaAs (indium gallium arsenide) detector\textsuperscript{38}. Although melanin is highly absorbing in the visible wavelengths, it has a very low absorption of light in the SWIR (Figure 1.3, Figure 2.4). SWIR light ranging from 1100 nm to approximately 1300 nm can penetrate further into tissue than VIS and NIR light due to decreased absorbance of hemoglobin and melanin as well as reduced tissue scattering, thus capable of detecting blood-volume changes deeper in the tissue\textsuperscript{39,40}. Around 1300nm, water absorption quickly rises with increases in wavelength, making it a primary absorber for use in SWIR optical devices beyond 1300 nm. SWIR components have the potential to detect PPG signals across varying skin types and enable non-invasive, racially equitable optical devices.

![Responsivity of Different Photodetector Materials as a Function of Wavelength](image)

**Figure 1.8** Responsivity of Different Photodetector Materials as a Function of Wavelength\textsuperscript{41}

1.4 Previous Studies

1.4.1 Hemoglobin Estimation Using PPG Signals

Previous research has laid the foundation for building non-invasive wearable devices that utilize PPG signals to estimate hemoglobin concentration\textsuperscript{42}. The signals have been evaluated at
wavelengths consistent with the high absorption of hemoglobin, and their characteristic features have been extracted to measure hemoglobin values\textsuperscript{43}. In addition, PPG-derived hemoglobin levels have been compared to ground truth hemoglobin levels measured from blood draws, showing feasibility of using the signals to quantify hemoglobin levels\textsuperscript{44}. Some of the most promising data on using PPG signals to extract hemoglobin levels has come from the peak amplitudes of PPG signals\textsuperscript{43}.

In addition to estimating hemoglobin levels, PPG signals in the SWIR have also been evaluated for determining the water content in tissue and estimating tissue hydration levels, especially through wearable devices. Although showing a lower SNR than the PPG signals used to evaluate hemoglobin levels, SWIR PPG signals have been reported to estimate hydration levels at 1450nm with detectable systolic peaks\textsuperscript{45}.

1.4.2 SWIR PPG Signals

SWIR LEDs and detectors have been applied in detecting PPG signals and non-invasively evaluating cardiac system performance. In zebra fish tissue, SWIR PPG signal detection was compared to VIS and NIR PPG signal detection\textsuperscript{46}. The SWIR hardware components provided stable and periodic PPG signals but had lower signal-to-noise ratios (SNRs) than the VIS and NIR signals. SWIR signals can adequately provide cardiac information such as the heart rate, and they have been used to map blood vessels beneath skin pigment in zebra fish. This mapping simultaneously demonstrated pigmentation’s transparency in SWIR range and its lack of transparency in the VIS/NIR ranges. In addition, using an InGaAs photodiode, SWIR PPG signals have been evaluated at wavelengths from 1070 nm to 1650 nm and showed the highest SNR in PPG signals ranging from 1070 nm to 1450 nm. The detection of high-SNR PPG signals in the SWIR and the absorption spectrum of water strongly motivates the
use of a 1300 nm LED for low-cost, portable, and continuous optical PPG measurement of water concentration in blood-volume levels\textsuperscript{47}.

1.5 Thesis Overview: Motivation and Aims

This thesis is motivated by the unmet need to develop an equitable device for continuous detection and monitoring of hemodilution during labor and postpartum delivery that helps prevent adverse events associated with PPH. The end goal for this device will be alerting healthcare teams when blood products are needed to treat PPH and will help to minimize global suffering and deaths from PPH. A primary obstacle in this work is the lack of SWIR systems and published spectra to provide ground truth for discovery. Thus, this thesis is a demonstration of feasibility and a proof of concept for a wearable hemodilution sensor for PPH. In addition to minimizing negative impacts of PPH on maternal health, this work will reduce or even eliminate the skin pigmentation bias in many light-based diagnostics. SWIR hardware components will enable PPG measurements at wavelengths where melanin has relatively low absorption and thus won’t interfere as significantly as in the VIS-NIR range. To develop this device, biological absorbers spanning from the VIS to the SWIR ranges must be characterized in order to identify wavelengths that will provide indications of hemodilution with high specificity. Beyond our goal of hemodilution, the characterization of biological absorbers from the VIS to the SWIR will allow SWIR-based technologies to be more readily used for a variety of biomedical applications and could make patient diagnostics and management more equitable.

Chapter 2 elaborates on the characterization of biological absorbers spanning the VIS-NIR-SWIR and describes how light-tissue interactions impact optical absorption spectroscopy measurements. In addition, it details the materials and methods used for obtaining high SNR absorption spectra, including the optimization of system and sample parameters, and it discusses
special considerations for obtaining absorption spectra in the SWIR. Lastly, chapter 2 covers the spectral results of biological absorbers from the VIS-SWIR obtained in this thesis. Chapter 3 will discuss the motivation, design, and development of a custom SWIR PPG device to detect and monitor hemodilution during PPH. It will also discuss the materials and methods used to create the sensor and the post-processing of the PPG signals to extract the hemodilution ratio. It will then present experiments performed with the sensor and their results. Chapter 4, the final chapter, will summarize the findings from our work and its contributions to the fields of maternal health and biphotonics, in addition to contributing to the development of equitable optical devices. To wrap up, the limitations of this work will be discussed, and opportunities for future work will be recommended.
Chapter 2: Characterization of Biological Absorbers, from the Visible to the Short-Wave Infrared

2.1 Introduction

Biological absorbers are the backbone of many light-based diagnostics and provide a non-invasive way to understand patient pathophysiology. However, in order to understand how biological absorbers inform the development of light-based diagnostics, it is important to consider the absorption spectra of each biological absorber as a function of wavelength. When conducting absorption-based diagnostics, absorption spectra govern the selection of LEDs, lasers, and detectors, and also allow researchers to predict how an absorber will impact a sample’s interaction with light. Some of the most widely referenced spectra include water, oxygenated and deoxygenated hemoglobin, melanin, and lipids (Figure 1.3). Technical advances in size, cost, power consumption, and wearable consumer electronics have driven increasing use of light-based diagnostics, but they still have intrinsic drawbacks, such as inaccurate readings from people with darkly pigmented skin whose high melanin content is highly absorbing. In addition, protocols that describe methods for collecting VIS-SWIR absorption spectra are lacking. This chapter will discuss the characterization of biological absorbers spanning the VIS-NIR-SWIR ranges and the methods used to obtain these spectra.

2.1.1 Optical Properties of Light and Tissue Interactions

When light illuminates tissue, the tissue will either scatter the light, absorb it, or pass through without any absorption or scattering (ballistic transmission) depending on the tissue’s composition and the light’s wavelength. The tissue’s ability to scatter photons is measured by its scattering coefficient, while its ability to absorb light is measured by its
absorption coefficient. These fundamental properties determine light-tissue interactions and are important in when trying to predict how light will behave in a certain material such as tissue, or when developing tissue-mimicking phantoms for testing optical diagnostics. In addition to tissue-mimicking phantoms, scattering and absorption of light play important parts in all optical diagnostics and spectroscopy. As discussed earlier, PPG blood volume signals are based on the time-varying absorption of light that occurs when the volume of blood in vessels increases and decreases during the cardiac cycle. By conducting PPG at carefully chosen wavelengths, one can ascertain the relative proportion of blood components, such as oxygenated hemoglobin, deoxygenation hemoglobin, or water, and how they are changing over time.

2.1.2 The Beer Lambert Law

The Beer Lambert Law relates the concentration of a substance to its optical absorptivity and the pathlength of the light passing through the sample. The optical absorptivity of a sample can also be represented as the relationship between the incident light ($I_0$), or light intensity before passing through a sample, and the transmitted light ($I$), or the light intensity after passing through a sample. The absorptivity of a sample is logarithmically related to its transmittance, where zero absorption corresponds to complete transmittance. The Beer-Lambert Law states that absorbance is linearly related to concentration, the optical pathlength, and the molar extinction coefficient, which is specific to each sample and measures how strongly the sample absorbs at a particular wavelength. If the molar extinction coefficient is known along with the light pathlength, absorption spectra allow researchers to determine the concentrations of the sample and in the case of a mixture of chemicals, the ability to identify and quantify the particular absorbers that are present. However, molar extinction coefficients in the SWIR are poorly documented, making it difficult to predict light-tissue interactions at these wavelengths.
Characterizing biological absorbers and their molar extinction coefficients in the SWIR will advance our understanding and development of SWIR-based optical applications.

\[ A = \varepsilon LC \]

** Equation 2.1: **Beer-Lambert Law Governing Equation of Absorption (A), Molar Extinction Coefficient (\( \varepsilon \)), Pathlength (L), and Concentration (C)

\[ A = \log_{10}(I_0/I) \]

** Equation 2.2: **Beer-Lambert Law Governing Equation of Absorption (A), Incident Light (\( I_0 \)), and Transmitted Light (I)

### 2.1.3 Significance and Motivations

Although biological absorbers have known spectra and molar extinction coefficients in the VIS and NIR, much less is known about how they interact with light in the SWIR. The advancements of InGaAs detectors have made it possible to characterize biological absorbers in the SWIR and use SWIR components in spectroscopy and optical diagnostics. This thesis is motivated by the technical and ethical need to better characterize biological absorbers in the SWIR, which could allow for the development of SWIR optical devices that eliminate the skin pigmentation bias that compromises the accuracy of most commercially available optical devices.

### 2.2 Materials and Methods

#### 2.2.1 Spectrophotometer System

The Olis Cary 14 System is a UV/VIS/NIR spectrophotometer with a spectral range of 185 nm to 2600 nm and a photometric accuracy of ± 0.0005 absorption units (AU). This system
has both a deuterium lamp, with an illumination range of 190 nm to 370 nm, and a tungsten lamp, with a range of 320 nm to 2600 nm. For high SNR detection across a large spectral range, the Olis 14 system has one silicon and one InGaAs detector, enabling absorption measurements that span from the VIS to the SWIR\(^{38}\). As seen in Figure 2.1, the Olis Cary 14 system calculates the absorption values of a sample by comparing the intensity of the light transmitted through the sample to the intensity of the same incident light directed towards a reference sample, which is normally the solvent that the sample of interest is dissolved in\(^{51,52}\).

In operation, broadband-light from one of the lamps is directed through a monochromator by lenses and mirrors to a prism that refracts the light. After the prism, the light is reflected by a series of mirrors and guided through a series of slits, where it finally reaches a grating, which separates the light into its component wavelengths. Finally, the light reflects off a mirror and is passed through a slit where it exits the monochromator. After exiting the monochromator, a beam splitter, powered by a motor spinning at 30 Hz, alternates the light beam between the sample and reference, sending half of the light to the sample and half of the light to the reference. The light pulses sent to the sample and reference are out of phase with one another, ensuring that the phototube receives only light from the sample or reference at one time\(^{53}\).

In addition to its high sensitivity, spanning from the VIS to the SWIR, the Olis Cary 14 system helpfully calculates a flat baseline for absorption measurements. The system scans a spectrum without a sample present, using only the reference, or solvent, in both the sample and the reference slots. The Olis then stores the absorption value of the reference-only (blank) spectrum and subtracts it as the baseline background for new sample raw data. The baseline
measurements achieve better than 0.001 AU baseline flatness\textsuperscript{53}.

**Figure 2.1**: Optical Diagram of the Olis Cary 14 System\textsuperscript{52}

### 2.2.2 Optimization of Olis Cary 14 System Parameters

To achieve accurate, high-SNR absorption measurements of biological absorbers, it is important to optimize the different Olis Cary 14 system parameters. The data collection options include absorbance, transmittance, reflectance, single beam, and raw signals\textsuperscript{53}. Here, we will focus on the absorbance system, which reports the sample and reference signals as the log of the reference over the sample, reported in AU. The system also has scan and assay modes for data collection. This work employs the data scan mode, in which the data is recorded as a function of wavelength, rather than recording a single wavelength as a function of time. Although the scan mode in the Olis system offers several options, this work uses the constant bandwidth mode, which adjusts the amount of light directed towards the sample by widening or narrowing slits. The bandwidth chosen for each sample depends on the sample preparation, but higher bandwidths illuminate the sample and reference with more light, while lower bandwidths provide
less light. Other options that can be changed for each sample are the numbers of datapoints per measurement and the number of reads per datum. The number of datapoints per measurement defines the step size in wavelengths across a spectrum, where higher datapoints per measurement will give higher spectral resolution at the expense of slower read times. Similarly, reads per datum is the average number of data reads needed to collect one data point, and a higher number gives higher SNR measurements, at the expense of slower read times \(^{53}\).

If a previously published spectrum is available, it is helpful to view it and decide what wavelength range to measure using the Olis. It is recommended to try and replicate a previous spectrum in the VIS and NIR before taking new SWIR measurements. After this, the first step is to check the “live mode” display that shows the current absorption of the sample and the reference at a specified wavelength within the selected range. Using a previously published spectrum (if available) will allow for the selection of a wavelength at an absorption peak, which is a good place to implement the “live mode” button. The Olis system provides the highest SNR measurements when the absorption units are between 1 and 5, with 0 being no absorption, and 5 being saturation. Therefore, using the “live mode” at an absorption peak will give a good idea of how the system parameters should center around the sample. For example, if the live mode shows saturation, the sample is too highly absorbing with these parameters and needs to be adjusted accordingly. Adjustments to both the sample (such as diluting if the sample photodetector does not see any transmitted light) and the system parameters can optimize absorbance readings. Once the system parameters such as bandwidth, reads per datum, and number of increments have been established, a baseline measurement must be taken with the solvent in both the reference and sample slots. After taking the baseline measurement and
selecting apply baseline, the sample cuvette can be placed in the sample holder and absorbance measurements can be taken.

To capture an absorption spectrum, it is recommended to take more than one measurement and optimize the sample and system parameters for the selected wavelength range. For example, 800 nm is a common wavelength for changing from the silicon detector to the InGaAs. If a measurement spans the detector change wavelength, the system introduces error into the absorption reading at the change wavelength. Thus, it is better to take two separate measurements with overlapping wavelength ranges from the different detectors and stitch them together during post-processing. Likely, samples will exhibit large absorption changes at different wavelengths, and this will require multiple absorption measurements with different sample concentrations and system parameters to provide a full, high SNR absorbance spectrum.

2.2.3 Considerations for Absorption Spectra in the SWIR

Unlike absorbance measurements in the VIS and NIR, absorbance measurements in the SWIR require special considerations. As discussed previously, high SNR SWIR measurements require an InGaAs detector, which has higher sensitivity in the SWIR than silicon detectors (Figure 1.8)\textsuperscript{38}. In addition, SWIR absorption spectroscopy measurements require quartz cuvettes for the sample and reference liquids, not the glass or plastic cuvettes normally used for absorption spectroscopy in the VIS and NIR regions. Quartz cuvettes have a higher transmission range than glass in the SWIR, making them more suitable for spectra spanning from the VIS to the SWIR. Lastly, due to the high absorption of water in the SWIR, in SWIR measurements where water is the reference and solvent, deuterium oxide (D\textsubscript{2}O), also referred to as heavy water or deuterated water, must be used instead of water (H\textsubscript{2}O). Otherwise, water’s absorption
spectrum could dominate the absorbance values, making it difficult to capture a true absorbance spectrum of the sample (Figure 2.2)\textsuperscript{54}.

![Graphs showing absorbance spectra of water and deuterated water](image)

**Figure 2.2:** Water Absorbance Spectrum versus Deuterated Water Absorbance Spectrum\textsuperscript{54}

### 2.2.4 Absorber Sample Preparation

In addition to optimizing the system parameters, it is also important to use an absorber-specific preparation to optimize each sample and ensure the absorber is in solution. Some procedures apply to all absorbers, such as removing scattering from the sample and optimizing concentrations based on the intended wavelength range of a measurement. Many biological absorbers are procured in a powdered form that must be dissolved in a solvent. However, dissolved powders commonly exhibit too much scattering, distorting absorption measurements. One remedy is sonication, which disrupts the molecular interactions between the powder and the liquid solvent, causing them to separate and mix, effectively decreasing the scattering of the sample\textsuperscript{55}. Another common optimization technique is to change the biological absorber’s concentration for different spectral ranges, depending on its predicted absorption in a certain
wavelength range. If a sample is very highly absorbing in one wavelength range, a lower concentration will decrease the amount of light that it absorbs there. However, this technique works best if a biological absorber has a previously published spectrum, or if one has been established through trial and error. Other absorber-specific optimization techniques that will be discussed in this thesis include oxygenated hemoglobin, deoxygenated hemoglobin, melanin, water, and corn oil. Note that all biological absorber spectra presented in this thesis will be scaled on a log y axis.

2.2.5 Calibration and Multi-Region Stitching of Spectra

To achieve a full absorption spectrum on the Olis Cary 14 system, taking multiple absorption measurements can optimize both the sample preparation and system parameters. After multiple measurements are taken with different concentrations and/or system parameters, the varying absorption values will require post-processing to create a full spectrum. Calibrating the absorption curves to achieve a full spectrum requires a spectrum or measurement to serve as the ground truth to which other measurements will be normalized. A wavelength at a characteristic peak or trough is chosen from the ground truth spectrum and its indices are recorded. The other spectrum must then be normalized to this wavelength according to its own indices at the selected peak or trough wavelength. This will “match” the spectra to each other and provide a normalized absorption transition from one measurement to the next. However, overlap of the spectra could result, so the spectra must be “cut” and stitched together at an easy transition point. Transition points are most often found in linear regions where the spectra line up perfectly to provide a seamless transition of the curves.
2.3 Results

2.3.1 VIS-NIR-SWIR Absorption Spectrum of Water
Water, a primary biological absorber, is a key consideration in creating a wearable SWIR PPG sensor to detect hemodilution during PPH. To establish water’s full absorption spectrum, tap water was used as the sample and deuterated water was used as the reference and solvent. To begin, deuterated water in two quartz cuvettes was placed in the reference and sample cuvette slots in the Olis. Two baseline measurements were taken, one using the silicon detector and the other using the InGaAs detector. A quartz cuvette containing all tap water then replaced the deuterated water cuvette in the sample holder. An absorption measurement was then taken over the VIS to the partial-NIR region. For the NIR to SWIR measurement, it was noted that water had a previously published spectrum showing very high absorption in the SWIR, so the concentration of water was lowered by diluting with deuterated water to prevent saturation. A second measurement was then taken using the InGaAs detector. These two measurements were then normalized to one another and stitched together, resulting in the full water absorbance spectrum seen in Figure 2.3. The water absorbance spectrum shows relatively low absorption in the VIS, which increases at longer wavelengths. Characteristic absorption peaks can be seen at approximately 750 nm, 970 nm, 1200 nm, and saturation of the signal can be seen from 1380 nm to 1600 nm.
2.3.2 VIS-NIR-SWIR Absorption Spectrum of Melanin

The biological absorber melanin must be fully characterized to understand how it affects optical diagnostics and how skin pigmentation impacts data from light-based devices such as pulse-oximeters and smart watches. To capture melanin’s full absorption spectrum, powdered melanin was used as the sample and dimethyl sulfoxide (DMSO) as the solvent and reference. DMSO was chosen as the solvent for melanin because of the presence of sulfonate groups in its chemical signature, which are also present in the chemical signature of melanin, allowing it to dissolve easily in DMSO. Baseline measurements were taken with DMSO in both the sample and the reference quartz cuvette slots in the Olis. To determine absorption in the VIS to NIR, multiple baseline measurements were taken with the silicon detector due to the large changes in melanin absorption over this wavelength range. Similarly, multiple baseline measurements were
taken with the InGaAs detector due to large changes in melanin absorption over the NIR to SWIR wavelength range.

After baseline measurements, sample measurements could be taken. Previously published melanin spectra have shown very high absorption in the VIS and NIR regions and very low absorption in the SWIR, prompting an absorber-specific preparation with a low concentration of melanin for VIS and NIR measurements and a high concentration for NIR to SWIR measurements. To ensure optimum absorption and decreased scattering, samples were sonicated for 10 minutes before absorption measurements were taken. Figure 2.4, the resulting melanin spectrum, shows significant absorption in the VIS range that decreases to very low absorption in the SWIR.

![Melanin Absorbance Spectrum](image)

**Figure 2.5:** Melanin Absorbance Spectrum

### 2.3.3 VIS-NIR-SWIR Absorption Spectrum of Lipid: Corn Oil
Lipid, another primary biological absorber, offers insight into how fat affects tissues’ absorptive properties. In this study, we focused on characterizing corn oil, but in future studies we would like to characterize cholesterol for more tissue-specific absorptive data. Air with no cuvette had previously been determined to be the best reference for corn oil, and air with no cuvette was also used for the baseline measurements\textsuperscript{57}. For the baseline measurements and sample measurements, the wavelength ranges were again split into two regions, one ranging from 300 nm to approximately 900 nm utilizing the silicon detector, and one ranging from approximately 900 nm to 1600 nm utilizing the InGaAs detector. Multiple measurements were taken in both the VIS to NIR wavelength range and NIR to SWIR wavelength for spectrum optimization. The full spectrum had unwanted noise at approximately 980 nm that was removed using the Olis stitching post-processing tool. The resulting spectrum can be seen in Figure 2.5. The spectrum shows relatively high absorption in the VIS and absorption at approximately 930 nm, 1240 nm, and 1420 nm, and shows high correlation to a previously published corn oil absorption spectrum\textsuperscript{57}. 
Characterizing oxygenated hemoglobin is arguably one of the most important feats in biomedical optical spectroscopy and therefore in optical diagnostics, but oxygenated hemoglobin’s absorption spectrum in the SWIR remains poorly understood. We developed a novel protocol, detailed in the appendix, to determine oxygenated hemoglobin’s absorptive properties in the SWIR. However, first we verified that the absorption spectrum of oxygenated hemoglobin in the VIS to NIR region was correct when compared to previously published spectra. The oxygenated hemoglobin absorption spectrum from 300 nm to 1000 nm was compared to Dr. Scott Prahl’s published absorption spectrum, which can be seen in Figure 2.7. Two different methods verified oxygenated hemoglobin’s spectrum in the VIS to NIR, the first using whole defibrinated sheep’s blood reconstituted in water, and the second using lyophilized...
whole defibrinated sheep’s blood reconstituted in deuterated water. The preparation of multiple tubes of oxygenated whole defibrinated sheep’s blood is described in the appendix.

One of the most challenging aspects of determining the absorption spectrum of oxygenated hemoglobin in the SWIR is removing the water in blood, which is very highly absorbing in the SWIR. To do this, defibrinated sheep’s blood was lyophilized, a low-temperature dehydration process that freeze dries a substance. Lyophilization begins with a frozen sample, a low amount of pressure, and a small amount of heat to sublimate water. After the water solidifies, the temperature is raised slightly, causing the water to evaporate and dry the substance. The resulting oxygenated hemoglobin spectrum is a combination of the whole defibrinated sheep’s blood and the lyophilized whole defibrinated sheep’s blood. However, as seen in Figure 2.7, the spectrum clearly shows a strong correlation with Dr. Prahl’s absorption spectrum of oxygenated hemoglobin. The new oxygenated hemoglobin absorptive data in the SWIR shows low absorption spanning from 1200 nm to 1600 nm.
2.3.5 VIS-NIR-SWIR Absorption Spectrum of Deoxygenated Hemoglobin

Characterizing the absorption spectrum of deoxygenated hemoglobin has also been extremely important in optical spectroscopy and optical diagnostics. In a similar way to oxygenated hemoglobin, deoxygenated hemoglobin absorption has been widely used and understood when spanning the VIS to the NIR, but there is little to no knowledge of how deoxygenated hemoglobin absorbs in the SWIR. In this study, novel processes for determining the absorption spectrum of deoxygenated hemoglobin were developed, especially to provide data in the SWIR. Both deoxygenation processes were outlined with use of whole defibrinated sheep’s blood and lyophilized whole defibrinated sheep’s blood. In the first process, argon, which has atomic number 18 and a density of 1.784 g/L, is bubbled into the blood solution to
remove oxygen, which has an atomic number of 8 and density of 1.429 g/L. Since argon is heavier than oxygen, bubbling argon into the solution pushes the oxygen out of the sample and effectively deoxygenates it\textsuperscript{60}. In the second process, sodium dithionite induces dissociation of oxygen from hemoglobin, creating deoxygenated hemoglobin and maintaining structural integrity of the hemoglobin sample\textsuperscript{61}. The resulting deoxygenated hemoglobin spectrum is a combination of deoxygenation processes including use of whole defibrinated sheep’s blood and lyophilized whole defibrinated sheep’s blood. As seen in Figure 2.8, this data was compared to Scott Prahl’s deoxygenated hemoglobin tabulated values as a ground truth\textsuperscript{58}.

![Deoxygenated Hemoglobin Absorbance Spectrum versus Prahl](image)

**Figure 2.8:** Deoxygenated Hemoglobin Absorbance Spectrum versus Prahl\textsuperscript{58}

### 2.4 Discussion and Future Work

Absorption spectroscopy is used in many different facets of biomedical engineering, but little is known about biological absorbers from the VIS to the SWIR. This work sought to
contribute to the biophotonics community by investigating the absorption spectra of important biological absorbers—water, melanin, oxygenated and deoxygenated hemoglobin, and corn oil—at wavelengths from the VIS to the SWIR. As the results above show, the SWIR spectra of these biological absorbers show a wide range of absorption values, that are highly specific to each absorber. Water has very high absorption peaks in the SWIR, making it a clear choice for PPG measurement estimations of water content in blood. Both oxygenated and deoxygenated hemoglobin have low absorption in the SWIR but high absorption in the VIS and some of the NIR region. Melanin also has very little absorption in the SWIR, while corn oil shows absorption peaks throughout the NIR to the SWIR.

Future work in absorption spectroscopy will include investigating the discrepancies between the obtained deoxygenated hemoglobin spectrum and Dr. Prahl’s’ spectrum. This effort will include recording absorption measurements of deoxygenated hemoglobin using sodium dithionite and bubbling with argon, testing human blood samples, and verifying the fraction of oxygenated Hb, deoxygenated Hb, methemoglobin, and carboxyhemoglobin using a clinical co-oximeter. In addition, multiple replicates of all of the biological absorbers will confirm their spectra and absorption coefficients. Future work will also include the creation of a library of VIS to SWIR spectra that includes common biological absorbers and fluorophores. In addition to recording spectra, this library will also include detailed steps on preparation of the sample and optimization of the system parameters.
Chapter 3: Development of a Custom Short-Wave Infrared Photoplethysmography Device

3.1 Introduction

3.1.1 Considerations for SWIR PPG

Most PPG signals in optical devices such as smart watches and pulse oximeters are detected with silicon detectors and VIS/NIR LEDs. SWIR detectors, LEDs, and other components are rarely used because there are few published SWIR absorption spectra and few commercially available SWIR-compatible components. One such SWIR-component, discussed previously here, is the InGaAs detector, which has high sensitivity at SWIR wavelengths and can be integrated into a circuit with the use of a custom transimpedance amplifier. When using an InGaAs detector, researchers must consider its SNR, especially the sensitivity to both read noise and shot noise. Read noise is normal noise in every sample measurement that is produced when a photodiode produces an electronic signal, and shot noise is statistical noise that follows a Poisson distribution proportionate to the square root of the light intensity upon the detector. InGaAs detectors have higher readout noise due to their inability to detect low-light signals when compared to silicon detectors.

3.1.2 Significance and Motivations

This work is motivated by a need to detect and monitor hemodilution during PPH, the global leading cause of maternal death and morbidity. Early detection can prevent 90% of deaths from PPH, but current PPH detection methods are inadequate and delay the timely introduction of treatment. Current detection methods include blood loss estimation, which can be inaccurate and does not account for internal bleeding, vital sign monitoring, which delays detection due
bodily compensation mechanisms that keep vital signs stable\textsuperscript{11,15}. The inaccurate estimations of blood loss and the time lag in vital sign changes hinder early decisions to employ blood products or other treatment options\textsuperscript{14}.

The strong absorption of light by water and hemoglobin at distinct wavelengths motivates the use of an optical PPG sensor, which would allow for real-time tracking of PPG signal changes and features related to the amount of hemoglobin and water in blood. This sensor would provide doctors with an inexpensive and non-invasive way to detect PPH early, giving ample time to administer treatment to the mother. This work aims to use SWIR components with a custom PPG circuit for SWIR compatibility. Using SWIR wavelengths can greatly reduce inaccurate optical device readings from people with darkly pigmented skin, resulting in more equitable medical treatment.

3.2 Materials and Methods

3.2.1 LED and Photodiode Selection

To use PPG signals to estimate hemoglobin and water concentrations in the SWIR, we selected two LEDs. The first LED, a 12 mW Thorlabs 910E, emits at 910 nm, a wavelength where hemoglobin is strongly absorbing. The second LED, a 2.0 mW Thorlabs 1300E, emits at 1300 nm, where water is strongly absorbing (Figure 2.3). In addition to the LEDs, a Thorlabs FDGA05 InGaAs photodiode was chosen as the SWIR-compatible detector. The FDGA05 is highly sensitive to both pulsed and continuous wave light from 800 nm to 1700 nm. It has a responsivity of 0.95 A/W, an active area of 0.196 mm\textsuperscript{2}, and a dark current of 6 nA at 5 V.

3.2.2 Custom Analog Front End Circuit

To integrate the FDGA05 InGaAs detector, we constructed a custom analog front end circuit that includes a transimpedance amplifier for current to voltage integration, and high and
low pass filters remove as much noise and DC offset as possible\textsuperscript{64}. The transimpedance amplifier converts the small current generated by the photodiode into a voltage large enough to provide an ample signal. To provide the most stability to the photodiode and the highest voltage output to the circuit, the transimpedance amplifier was optimized with a feedback capacitor, feedback resistor, and the specifications of an ST Microelectronics TSV7722 operational amplifier (op amp), which has high bandwidth and low offset voltage, and can operate from 1.8 V to 5.5 V\textsuperscript{64,65}. This op amp works well with low-side current measurements and features a low input bias current, which enables high-accuracy measurements with photodiodes, motivating its use in this circuit. In addition to the op amp, the feedback capacitor in the transimpedance amplifier was sized to handle the photodiode junction capacitance, the operational amplifier differential input capacitance, and the operational amplifier common mode input capacitance. Using the specifications from the FDGA05 photodiode and the TSV7722 operational amplifier, it was found that the transimpedance amplifier was optimized using a 1pF capacitor. Finally, the transimpedance amplifier was optimized using a 1MΩ feedback resistor selected based on the junction capacitance.

The transimpedance amplifier is followed by a third order bandpass filter. As the order of filters increases, the slope of the frequency response also increases, allowing PPG signals to maintain highly specific feature extraction, while also providing sufficient filtering of both high frequency noise and removing DC offset\textsuperscript{66}. For this circuit, 3\textsuperscript{rd} order active filters were chosen as a tradeoff between complexity and specific filtering responses. The bandpass filter first starts with an active high pass filter that removes the DC offset, the mean amplitude displacement from zero. In electronics, DC offset is due to a positive or negative DC voltage output that increases the signal mean. In PPG, the DC offset is due to detection of non-arterial signals such as venous
blood or tissue. In addition to the high pass filter, the circuit also uses an active low pass filter which removes high-frequency noise in signals. In this circuit, the of the filter had a pass band of 0.21 - 7.4 Hz. After bandpass filtering, the signal is sent to a programmable gain amplifier (PGA) that stabilizes and amplifies the signal to ensure high signal quality throughout measurements and across different patients. This MCP6S22 PGA (Microchip Technology Inc., Chandler, AZ) can be configured for gains from +1V/V to +32 V/V, and it has a serial peripheral interface (SPI) for easy integration into software applications \(^{67}\).

**Figure 3.1:** Block Diagram of Analog Front End Circuit to Measure the Hemodilution Ratio

### 3.2.3 Feature Extraction

PPG signals indicate changes in arterial/arteriole blood volume as blood is cyclically pumped from the heart to the rest of the body. PPG signals can be taken at multiple locations in the body, although the location will affect the PPG signal morphology and quality. Most PPG signals are acquired from the fingertip, which contains arteries sensitive to vasoconstriction (a narrowing of blood vessels that decreases peripheral blood flow) \(^{68}\). Vasoconstriction can reduce PPG signal amplitude and result in low SNR. In addition, PPG signals are sensitive to motion artifacts, so a location with robust perfusion and low motion artifacts provides the highest quality
PPG signals with minimal noise. PPG signals are typically discretized and then exported for post-processing to for additional signal processing and feature extraction such as the pulse-wave amplitude for each heartbeat.

Characteristic PPG signals can convey cardiovascular cues, such as age and health. For this work, PPG signal features were analyzed only to quantify the relative concentration of water and hemoglobin changes in blood. The systolic amplitude or systolic peak of a PPG signal is a measure of the blood volume changes that occur in arterial blood flow. A decrease in the amplitude of systolic peaks can be associated with a decrease in absorption, which directly relates to the change in blood vessel volume during the cardiac cycle (such as vasoconstriction which reduces blood volume changes) and concentration of the blood component of interest within the blood, both of which occur during PPH. However, both channels (910 nm-hemoglobin & 1300 nm-water) will detect the same blood volume change caused by increased or decreased perfusion, therefore the differences observed between the channels will be due to changes in blood component concentrations.

PPG signals must be carefully filtered to accurately determine their systolic peaks and extract the pulse-wave amplitude. To accomplish this, a 3rd order low pass Butterworth filter, which can maintain a flat filter response and has low computational complexity, removes the high frequency noise from the signal. After high-frequency noise removal, the envelope function in MATLAB is used to baseline the signal to zero and remove any additional low frequency baseline drift. The envelope function returns two continuous curves that touch the upper and lower peaks of the PPG signal, where the curves are determined by spline interpolation over local maxima separated by a selected number of samples. Once the envelope returns the upper and lower peaks of the signal, the lower bound is subtracted, effectively baselining the signal to
begin at 0 on the y axis. Once the signal has been filtered and baselined, the systolic peaks can be found using the findpeaks.m function, which returns a vector of the amplitude and locations of the systolic peaks in PPG signals (Figure 3.2)
3.2.4 Hemodilution Ratio

During PPH, excessive bleeding occurs, and the body uses compensation mechanisms to prioritize blood flow to vital organs. One compensation mechanism causes hemodilution, where water is drawn in from the extravascular space to increase blood volume\textsuperscript{20}. Hemodilution, expressed as a ratio of PPG signals, estimates the amount of blood and water in the blood and can be used to effectively detect and monitor PPH. The hemodilution ratio uses the 910 nm PPG signals and divides them by the 1300 nm PPG signals to give a real-time determination of how hemoglobin concentration is changing in the blood during PPH. In addition to detecting and monitoring PPH, the hemodilution ratio also can monitor the success of PPH treatment, which normally includes the introduction of fluids and blood products to prevent death and morbidity. The calculation of the hemodilution ratio using the 910 nm and 1300 nm PPG signals is visually
represented in Figure 3.3.

![Figure 3.3: Schematic of Hemodilution Ratio Calculation](image)

**3.3 Results**

**3.3.1 Detection of SWIR PPG signals**

The detection of SWIR PPG signals is motivated both by the need to detect and monitor blood and water concentrations during PPH and the need to create devices that use optical components in wavelength regions where melanin has a low absorption of light. As stated previously, the selected wavelengths for a wearable SWIR PPG sensor for detecting and monitoring hemodilution during PPH were chosen at 910 nm, corresponding to a high absorption of hemoglobin, and at 1300 nm, corresponding to a high absorption of water. Absorption spectroscopic measurements, seen in Chapter 2, confirmed that water and hemoglobin are the primary absorbers at 910 nm and 1300 nm, in addition to confirming that melanin absorbs little light in the SWIR.
Although previous studies have shown that PPG signals are detectable at SWIR wavelengths, little is known about specific PPG signal quality at 1300 nm. To gather information on SWIR PPG signal quality at 1300 nm, we performed human volunteer studies. In this work, PPG signal measurements were recorded on the fingertip in reflection mode on one subject with darkly pigmented skin and one subject with lightly pigmented skin (Figure 3.4). Although high quality PPG signals at 910 nm have been well documented, PPG signals at this wavelength were also recorded in the same human volunteers for comparison with the SWIR 1300 nm PPG signals. As seen in Figure 3.5, this study confirms that high quality PPG signals at both 910 nm and 1300 nm were readily detectable in the subject with high amounts of melanin in their skin, which significantly de-risked our pursuit of developing a SWIR PPG device.

A: Lightly Pigmented Skin

B: Darkly Pigmented Skin

Figure 3.4: Lightly Pigmented Skin (A) Darkly Pigmented Skin (B) from Human Volunteer Study
3.3.2 Phantom Study

A study using a tissue mimicking flow phantom with a subsurface 2mm diameter channel for blood flow was performed to determine the performance of the SWIR PPG prototype device in detecting and monitoring hemodilution. The tissue mimicking phantom was made up of a flexible resin that mimics light absorption in tissue, mixed with titanium dioxide to mimic how light is scattered in tissue. The phantom study was performed in reflection mode, where the LEDs and detectors were placed on the same side of the tissue-mimicking phantom. The phantom study was performed with varying amounts of hemoglobin and water, mimicking hemoglobin and water concentration changes that occur during a hemorrhage. A hand-operated syringe pump produced a pulsatile volume change through the channel, similar to volume changes in blood vessels. The channel was blocked at one end, so the human-applied pressure from pushing and pulling the syringe caused pulsatile, volumetric changes in the elastic tube,
which were detected via the PPG signals. The solution was prepared with a mixture of whole defibrinated sheep’s blood and water to create 4 distinct hemoglobin and water concentration changes. The resulting hemoglobin concentrations ranged from 1.15 g/dL to 4.6 g/dL. Future studies will include hemoglobin changes that more accurately represent human hemoglobin concentrations during hemorrhage. After the blood mixtures were prepared, PPG waveforms were recorded at both 910 nm and 1300 nm illumination wavelengths using an Arduino Uno and were exported for post processing (Figure 3.6). The hemodilution ratio was calculated using the same approach as outlined in section 3.2.3: Feature Extraction. The hemodilution ratio of the phantom study showed a strong linear correlation between hemoglobin concentration and the calculated hemodilution ratio with an r-squared value of 0.99.

Figure 3.6: Schematic of Phantom Study with Varying Hemoglobin and Water Concentrations

```none

highest hemoglobin concentration

phantom hole expansion and contraction creates PPG signals

lowest hemoglobin concentration

stopper creates closed system

910 nm LED

InGaAs

1300 nm LED
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Figure 3.7: Hemodilution Calculated from PPG Signal Amplitude Ratio from 910 nm and 1300 nm Channels Versus Hemoglobin Concentrations of Pulsed Blood and Water in the Phantom

3.3.3 Swine Studies

Due to the similarities in swine and human cardiovascular structure and function, swine were used in animal studies to confirm the feasibility of the prototype device in detecting and monitoring hemodilution during PPH. Two swine studies were performed, and both consisted of two serial phases: blood withdrawal and saline infusion. Swine were anesthetized by trained veterinary staff, monitored during the course of the study, and euthanized upon completion. During the first phase, which mimicked bleeding during hemorrhage, blood was withdrawn from the swine left femoral artery (Figure 3.8) using a catheter in increments of 60 mL every 5 minutes until they reached a mean arterial pressure (MAP) of 40 mm Hg, at which point blood withdrawal was ceased, to retain cardiovascular function.
Figure 3.8: Schematic of Sensor Placements on Swine 010 and Swine 011 During Hemorrhage and Resuscitation

In the second phase, to mimic the fluid resuscitation administered during PPH, swine were infused with 60 mL of saline every 5 minutes until reaching half of the blood volume removed. The number of blood draws and saline infusions varied with each swine and was dependent on how fast the swine reached a MAP of 40 mm Hg. During blood draws and saline infusions, hemoglobin concentration was measured using a blood-gas analyzer and a hematocrit count. The blood gas analyzer gave approximate hemoglobin concentrations that varied and was taken out...
of commission shortly after the swine studies. To account for the possible error from the machine, interpolated values are shown which are likely a more accurate representation of a dropping hemoglobin and hematocrit count during a hemorrhage. Interpolated values are shown from swine 010 in Figure 3.9 and Figure 3.10, and the true values are shown in Figure 3.11. Similarly, the interpolated hematocrit count is shown for swine 011 in Figure 3.12 and Figure 3.13, and the true hematocrit count from the blood gas analyzer is shown in Figure 3.14. The estimated hemoglobin concentrations were compared to the prototype device’s approximation of the hemodilution ratio. For both studies, a Diligent Analog Discovery 2, a 14-bit 2 channel oscilloscope, was used to record PPG signals from the 910 nm and 1300 nm LEDs.

During the first swine study (swine 010), the prototype SWIR PPG sensor was placed on the chest (Figure 3.8). The sensor included two FDGA05 photodiodes, one recording PPG signals from a single 910 nm LED and one recording PPG signals from a single 1300 nm LED. PPG signals were recorded for 20 seconds at a sampling rate of 400 Hz approximately every minute. After the study, the hemodilution ratio was calculated using the PPG signal amplitudes at systolic peaks. The hemodilution ratio of swine 010 showed a linear correlation to hemoglobin concentration during the blood withdrawal phase of the experiment with an r-squared value of 0.8723. However, after saline infusions began, and thereafter until the infusions were ended, the system no longer showed a linear correlation between hemoglobin concentration and hemodilution ratio.
**Figure 3.9:** Swine 010 Calculated Hemodilution Ratio and Interpolated Hemoglobin Concentration During Blood Withdrawal
Figure 3.10: Swine 010 Hemodilution Ratio and Interpolated Hemoglobin Concentration During Blood Withdrawal and Saline Infusion
In the second swine study (swine 011), the prototype sensor was placed on the neck of the swine, rather than the chest, to prevent breathing motion artifacts caused by the periodic movement of the swine’s diaphragm. In addition, three LEDs were utilized for each wavelength to increase the SNR in the recorded signals (Figure 3.8). PPG signals were recorded with the same sampling settings and the hemodilution ratio was calculated using the PPG signal amplitudes at systolic peaks. The hemodilution ratio of swine 011 showed a linear correlation to hemoglobin concentration during the blood withdrawal phase of the study and during part of the saline infusion phase with an r-squared value of 0.8089. However, during the saline infusion phase, the sensor detecting PPG signals from the 1300 nm illumination wavelength was
repositioned after losing contact with the swine. This caused a distortion in the calculated hemodilution ratio, which no longer showed a linear correlation, as seen in Figure 3.12.

**Swine 011 Calculated Hemodilution Ratio During Blood Withdrawal and Partial Saline Infusion**

![Swine 011 Calculated Hemodilution Ratio During Blood Withdrawal and Partial Saline Infusion](image)

**Figure 3.12:** Swine 011 Calculated Hemodilution Ratio and Interpolated Hematocrit Percentage During Blood Withdrawal and Partial Saline Infusion
Figure 3.13: Swine 011 Hemodilution Ratio and Interpolated Hematocrit Percentage During Blood Withdrawal and Saline Infusion Before and After 1300 nm Detector Repositioning
Figure 3.14: Swine 011 Hemodilution Ratio and True Hematocrit Percentage During Blood Withdrawal and Saline Infusion Before and After 1300 nm Detector Repositioning

3.4 Discussion and Future Work

The human volunteer studies showed that high SNR PPG signals can be detected in subjects with high amounts of melanin at both 910 nm and 1300 nm. Future human volunteer studies could be performed with a larger range of skin tones to better understand how VIS, NIR, and SWIR PPG signal detection changes with different amounts of melanin in skin. Future studies could also include a colorimeter to measure the melanin content in the human volunteer’s skin\(^7\). In addition to adding a colorimeter and a range of skin tones, future studies should further optimize NIR and SWIR LED wavelengths that correspond to hemoglobin and water. While the LEDs chosen for these studies present a good option for off the shelf, low-cost use,
investigations with other SWIR LEDs may allow additional contrast to small changes in hemoglobin concentration and help to achieve the highest SNR PPG measurements possible for all skin types.

The phantom study showed a linear correlation between hemoglobin concentration and the hemodilution ratio calculated from the PPG signal amplitudes at systolic peaks. Although this study showed initial feasibility, additional studies are needed. The phantom study was performed using a human-powered pulsatile syringe pump to create PPG signals inside the tissue-mimicking phantom. Although PPG signals were created, human error could have skewed the PPG signal shape and amplitude. In addition, the tissue-mimicking phantom was made of a semi-flexible resin that cracked during use, leaking blood and water throughout the course of the study. These leakages could have decreased the pressure inside the phantom and decreased the PPG amplitude, skewing the results. Further, a broader range of hemoglobin studies should be tested in the future to assess performance across the range of physiologically relevant hemoglobin concentrations (4-15 g/dL).

In addition to optimizing the phantom study, future studies must improve the post-processing feature extraction to better estimate the hemoglobin and water contents. Current post-processing methods detect the PPG signal amplitudes at systolic peaks to estimate hemoglobin and water concentrations. However, the PPG signal amplitudes at systolic peaks do not account for the entire area under the PPG signal, called the AC/pulsatile component, which gives a better estimation of optical absorption. Future studies should calculate the area under the curve to better estimate hemoglobin and water concentrations, which would yield a more accurate hemodilution ratio.
The prototype device’s performance in swine 010 showed initial feasibility of the device in detecting and monitoring hemoglobin concentration changes during the hemorrhage portion (blood withdrawal) of the study. The blood withdrawal portion showed a linear correlation between a decrease in swine hemoglobin concentration and the calculated hemodilution ratio. However, when the swine was infused with saline, the device failed to show a correlation between the dropping hemoglobin concentration and the hemodilution ratio, and instead seemed to follow the overall perfusion of the swine rather than hemoglobin concentration. This occurrence could be due to low SNR of the PPG signals from either the 910 nm LED, the 1300 nm LED, or both illumination wavelengths. Future studies could include more LEDs and better filtering techniques to increase the SNR. The failure to show a linear correlation between hemoglobin concentration and hemodilution ratio during saline infusions could also be due to separation of the detector from the swine’s skin, skewing the PPG signal amplitudes at the systolic peaks. Future studies should firmly place the photodiode against the swine’s skin to ensure detection of changes in arterial blood volume. The second swine study, swine 011, showed a linear correlation between the dropping hemoglobin concentration and a dropping hemodilution ratio during the blood withdrawal phase and part of the saline infusion phase. As marked on Figure 3.12, the sensor detecting PPG signals from the 1300 nm illumination wavelength was repositioned when it lost contact with the swine skin. This movement skewed the PPG signal amplitudes and resulted in a non-linear correlation between the hemoglobin concentration and hemodilution ratio for that portion of the study, which continued until the study was completed. Before the detector repositioning, the PPG signal amplitude calculations showed only slight variation, demonstrating overall initial feasibility for detecting and monitoring hemodilution during PPH. In the swine studies, two separate PPG sensors, one
dedicated to 910 nm and the other decided to 1300 nm, were placed on the swine so that a higher analog to digital converter (ADC) resolution could be obtained using our Analog Discovery oscilloscope. For two channel PPG, these systems should ideally be using the same detector so that the same volume of tissue is interrogated throughout the experiment. Future work will integrate a 16-bit ADC.

In addition to remediying the issues noted above, future phantom studies will include tissue mimicking phantoms that contain a “skin” layer with varied concentrations of India ink, mimicking different amounts of melanin in the skin (Figure 3.13). Future studies will also include an automated pulsatile pump to generate PPG-mimicking signals and remove human error. This will include the use of a 16-bit ADC chip that will be integrated into the circuit to provide high-SNR PPG signals. The current device utilizes the Arduino Uno, a 10-bit system, or the Analog Discovery 2, a 14-bit system. The addition of the 16-bit ADC chip will allow for measurements in the same vessel and will provide much better resolution than the current system. In addition to resolution changes to the circuit, the prototype device will be refined. During this thesis study, the prototype’s analog front end was on a breadboard with long wires connecting to the LEDs and photodiodes. Future work will miniaturize the circuit to a PCB format, which will make it much easier to use in phantom, swine, and human studies. In addition to miniaturization, future work will create a fully wireless wearable system with surface mount LEDs and detectors, able to detect PPG signals in both transmission and reflection modes. Finally, future studies will investigate the inclusion of additional illumination wavelengths, which could detect SWIR-based blood oxygen saturation levels and hemodilution levels, creating a wearable SWIR pulse oximeter and PPH detector/monitor.
Figure 3.15: India Ink Absorbance Spectrum
Chapter 4: Conclusions and Broader Implications

4.1 Summary of Findings

Postpartum hemorrhage is the leading cause of maternal death and morbidity, but 90% of PPH-induced deaths are preventable with early detection and early introduction of treatment. During PPH, bodily compensation mechanisms cause hemodilution, in which the loss of blood draws in excess water from the extravascular space to increase blood volume. To combat PPH, a prototype wearable SWIR PPG sensor was developed to detect and monitor hemodilution. In human volunteer studies, a phantom study, and swine studies, the device showed feasibility for detecting and monitoring hemodilution during PPH. In addition, this device also demonstrated feasibility for detecting PPG signals in human subjects with darkly pigmented skin. However, future studies and developments are needed to fully demonstrate the feasibility of the device in detecting and monitoring hemodilution during PPH.

In addition, the contribution of biological absorption spectra from the VIS-SWIR especially impacts the development of optical devices. The absorption spectra of biological absorbers spanning from the VIS to the NIR are well documented, but less is known about the transition from the VIS-NIR to the SWIR. In this work, the VIS-NIR-SWIR absorption spectra of water, melanin, corn oil, and oxygenated and deoxygenated hemoglobin were measured using the Olis Cary 14 system. Another obstacle is the scant documentation on the process of preparing for and recording biological absorber spectra for absorption measurements. The work herein details the processes for optimizing both the sample and spectrophotometric parameters to record biological absorber spectra.

4.2 Contributions to Women’s Health
Once fully developed, this device will positively contribute to women’s health, which has been historically underfunded and under-researched. A wearable device that detects and monitors hemodilution during PPH could save myriad people who suffer due to PPH; it could provide an early alert to doctors, allowing them as much time as possible to introduce blood products or fluids, or employ other lifesaving procedures. In addition to detecting and monitoring PPH in a hospital, the development of a wearable version of this device would allow patients to take the device home. The wearable device could alert people to a hemorrhage and advise them to seek immediate medical treatment, thus reducing the suffering and death due to undetected PPH. In addition to its wearability, this device can be inexpensive, which will allow it to be distributed globally, thus helping minimize the disproportionate impacts of PPH morbidity and mortality on low resource populations.

**4.3 Contributions to Biophotonics**

The development of this device also positively contributes to the biophotonics community, especially in regard to custom SWIR optical sensor designs. The prototype SWIR PPG sensor shows that optical devices can detect high SNR PPG signals with SWIR components like an InGaAs detector and SWIR LEDs. In addition to optical sensor design, this thesis also significantly contributes to advancements in SWIR absorption spectroscopy. Although absorption spectra are used ubiquitously in biophotonics, few recorded absorption spectra span the VIS-NIR-SWIR wavelengths. Aside from the absorption spectra seen in Figures 2.3-2.8, this thesis also contributes to the overall knowledge and detailed processes of prepping samples and optimizing both sample and system parameters for best-practice absorption spectra recordings.

**4.4 Implications for Creating Equitable Optical Devices**
This work has potential for creating more equitable optical devices by minimizing the impact of melanin absorption on optical recordings. Figure 2.4 shows that melanin is strongly absorbing in the VIS range but has low absorption in the SWIR, motivating the use of SWIR components like the FDGA05 InGaAs detector and 1300 nm LED. This work features a custom SWIR PPG prototype device that captures high SNR SWIR PPG signals with minimal distortion from melanin absorption. In addition, the absorption spectra shown in Figures 2.3-2.8 would allow for the development of other SWIR-based optical devices, which could minimize melanin-based racial disparities in maternal healthcare.

4.5 Limitations

During the phantom study, pulsatile PPG signals were created manually with a syringe driving fluid into a tissue-mimicking phantom, and inevitable human error skewed the hemodilution ratio calculations and made it difficult to maintain a constant PPG signal amplitude. In addition, cracks in the tissue mimicking phantoms lessened the pressure inside and leaked blood and water onto the device. These errors likely contributed larger than expected variability and distorted the PPG signal morphology.

In the swine studies, the prototype device attached to swine 010 detected motion artifacts from swine diaphragm movement and captured only low SNR PPG signals during the infusion portion of the study, which caused a non-linear correlation between the calculated hemodilution ratios and the approximate hemoglobin concentrations. In addition, during the study on swine 011, the sensor associated with the 1300 nm illumination wavelength was moved part-way through the saline infusion portion of the study, distorting the PPG signal amplitudes at systolic peaks and therefore the calculated hemodilution ratio. The result was in a non-linear correlation between the approximate hemoglobin concentrations and the hemodilution ratio. In addition,
these sensors were placed at different locations on the swine, therefore measuring different vessels and blood volume changes. This limited the detection of blood volume changes in the same vessel and could have skewed the hemodilution ratio results.

In addition to these limitations, absorption spectroscopy measurements also posed challenges. The most significant limitation was the failure to capture a deoxygenated hemoglobin absorption spectrum that matched Dr. Prahl’s tabulated values. Possible causes include scattering from sodium dithionite particles, or oxygen lingering in the sample, potential differences between human and sheep blood such as different proportions methemoglobin and carboxyhemoglobin across species, all of which would distort the absorption spectrum.

4.6 Future Work

This thesis outlines the beginning stages of development for a custom prototype SWIR PPG sensor to detect and monitor hemodilution during PPH. This device impacts women’s health, biophotonics, and the creation of equitable optical devices, but further advancements must be made to ensure feasibility of the device. Future work will develop the device into miniaturized, low-cost, wireless, wearable system as a watch, finger clip, or necklace. This will allow for constant monitoring of hemodilution and will also allow for distribution of the device to lower-resource settings, which might not be able to afford common optical devices. In addition to wearability, future work will also investigate multiple different illumination wavelengths. Investigating multiple wavelengths will not only ensure that 1300 nm is the optimum wavelength for determining water concentration in the body, but it could also allow future monitoring of other blood volume changes such as glucose, fat, and oxygen saturation. Besides changes to the device, future work will include more phantom and swine studies. Phantom studies will use a more robust and automated pulsatile pump, rather than a human-
powered syringe pump. These improvements will allow for more clear determinations on feasibility of the device for detecting and monitoring of hemodilution. In addition to phantom tests, the device will also detect and monitor hemodilution during PPH on pregnant patients with varying amounts of melanin in their tissues. The pregnant patient studies will show a clear determination of feasibility for the device to detect and monitor hemodilution during PPH.
Equations

Equation 1.1: Formula for Oxygen Saturation Calculation from PPG in Pulse Oximetry
\[ \text{SpO}_2 = \frac{(AC_{Red}/DC_{Red})}{(AC_{IR}/DC_{IR})} \]

Equation 2.1: Beer-Lambert Law Governing Equation of Absorption (A), Molar Extinction Coefficient (\( \varepsilon \)), Pathlength (L), Concentration (C), \( A = \varepsilon L C \)

Equation 2.2: Absorption (A) Equation, Incident Light (\( I_0 \)), Transmitted Light (I), \( A = \log_{10}(I_0/I) \)
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Appendix

1. Preparation of Water Sample:

1.1 Fill quartz cuvette with deuterated water for the reference.

1.2 Fill quartz cuvette with water from the sink to take spectroscopy measurements of the sample.

2. Preparation of Powdered Melanin Sample:

2.1 Put powdered melanin into centrifuge tube and add dimethyl sulfoxide (DMSO) as the solvent.

2.2. Sonicate the tube for 10 minutes to ensure the powdered melanin dissolves in the DMSO.

2.3 Once dissolved, transfer to quartz cuvette for spectroscopy measurements.

Note: Melanin absorption has a wide range spanning from the VIS to the SWIR and will require concentration changes to ensure high SNR measurements. The VIS will require the lowest concentration of melanin due to its high molar extinction coefficient in this range to obtain a high SNR spectroscopy measurement, while the SWIR will require the highest concentration of melanin to obtain a high SNR spectroscopy measurement. The concentrations will start from 1.5mL/mg and increase in the SWIR.

3. Preparation of Corn Oil Sample:

3.1 Add pure corn oil to a quartz cuvette for spectroscopy measurements.

Note: No cuvette or solution will be used as the reference. The reference will be open air.

4. Preparation of Oxygenated Hemoglobin Sample with Whole Defibrinated Sheep’s Blood:
4.1 To isolate the red blood cells and remove as much water from the blood as possible, first pipette 2 ml of whole defibrinated sheep’s blood (Quad Five—610) into each microcentrifuge tube, and centrifuge at 5000 revolutions per minute (rpm) for 10 minutes. Approximately 4 microcentrifuge tubes are recommended.

4.2. After centrifugation, remove the tubes from the microcentrifuge, then remove the supernatant, which makes up the liquid and debris above the pellet, from the centrifuged blood using a pipette and discard it. The red blood cell pellet will pool at the bottom of the microcentrifuge tube.

Figure 3.16: Pellet and Supernatant from Centrifugation

4.3. Reconstitute the pellet with approximately 1500 μL of deuterated water (Millipore Sigma--1.13366) and re-centrifuge for 5000 rpm for 10 minutes.

4.4. Remove the tubes from the centrifuge, then remove the supernatant using a pipette and discard it: What remains is the pellet.

4.5. Re-constitute the pellet in a larger volume of deuterated water, such as 8000 μL, to lyse the cells. However, this volume could change depending on the desired sample concentration.

4.6. Remove the solution from the tube with a blunt tip syringe needle and syringe. Carefully remove the blunt tip syringe needle and discard in a sharps container. Then connect the syringe to a 0.22 μm syringe filter and push the syringe contents through the filter into a 10 mm
pathlength quartz cuvette (Thorlabs--CV10Q35EP). This will remove optical scattering from the sample by filtering out the cellular organelles and membranes.

4.7. Tightly secure an air-tight stopper over the cuvette to prevent water vapor from the air contaminating the sample.

5. Preparation of Oxygenated Hemoglobin Sample with Lyophilized Whole Defibrinated Sheep’s Blood:

5.1. Freeze approximately 2 mL of whole defibrinated sheep’s blood overnight in –80 °C freezer in 4 different sterile and sealable conical tubes/large centrifuge tubes. Cover the tube caps with parafilm to ensure an airtight seal

5.2. After the samples have been frozen overnight, take samples out of freezer and remove lid. Cover openings with two layers of Kim wipe and secure tightly with rubber bands. Poke ~8 small holes in the tightly secured Kim wipe for ventilation in the lyophilizer. The quicker you can work this process, the less likely the sample is to melt and the better it will perform in the lyophilizer.

5.3. Place samples in the lyophilizer and lyophilize for ~3-4 days to remove all water from the sample.

5.4. After 3-4 days, take the samples out of lyophilizer and recap them. Place in –80 degrees C freezer for storage. As determined by trial and error and using previously published oxygenated hemoglobin spectra as a guide, add an appropriate amount of lyophilized blood powder to deuterated water in a centrifuge tube and tightly secure the lid. The molar extinction coefficient of lyophilized blood in deuterated water will vary depending on the selected wavelength range.
5.5. Sonicate the blood solution for 10 minutes to ensure that the sample dissolves in the solvent. Work quickly after the sonication because deuterated water quickly reverts to H2O when exposed to humidity/water and even small amounts of H2O will overwhelm the absorption spectrum.

5.6. Remove the solution from the centrifuge tube with a blunt tip syringe needle and syringe. Remove the syringe needle and connect the syringe to a 0.22 μm syringe filter. Push the blood inside the syringe into the syringe filter and direct the filtered sample into a quartz cuvette for spectroscopy measurements.

5.7. Tightly secure an air-tight stopper over the cuvette to preserve the deuterated water for a correct spectroscopy reading.

6. Preparing Deoxygenated Hemoglobin Sample with Argon Bubbling\textsuperscript{60,71}

6.1 Follow either steps 4.1-4.5 or steps 5.1-5.5 for initial hemoglobin sample preparation.

6.2 Remove the solution with a syringe and force it through a 0.22 μm syringe filter into a long-neck quartz cuvette with a tight-fitting septa cap.

6.3 Place long neck cuvette in clamp and puncture septa cap with a small needle until 1 inch deep into cuvette. This will allow the release of oxygen gas that will be bubbled out of the solution. Attach a longer needle to tubes that connect to an argon gas tank. Puncture septa cap with longer needle until tip of needle reaches the bottom of the cuvette. This will be the source of argon bubbles entering and solution.

6.4. Bubble with argon for approximately 40 minutes.

6.5 Once deoxygenated with argon for approximately 40 minutes, remove needles and be sure to keep cuvette closed with septa caps to prevent re-oxygenation.
7. Preparing Deoxygenated Hemoglobin Sample with Sodium Dithionite:

7.1 Follow either steps 4.1-4.5 or steps 5.1-5.5 for initial hemoglobin sample preparation.

7.2 Add approximately 2.5 mg of sodium dithionite to the solution per gram of blood.

7.3 Sonicate for 10 minutes to ensure sodium dithionite dissolution in solvent.

7.4 Tightly secure a lid or stopper over the cuvette to preserve the deuterated water for a correct spectroscopy reading.

8. Obtaining High SNR Absorption Measurements

8.1 Prepare sample and reference with steps above and place in quartz cuvettes for VIS-NIR-SWIR measurements. Keep the sample and reference handy during this period of collection. Make sure lids on cuvettes are secure.

8.2 Turn on the spectrometer lamp and detector. Keep measurement space other than the spectrometer light as dark as possible as to not distort the absorption measurements.

8.3 Once you have determined the correct concentration and incident light of your sample, record chosen settings on each individual spectrometer and sample/reference parameters. Note: Chosen settings and parameters can include but are not limited to incident light power, exposure time, pathlength, concentration, etc.

8.4 Take an absorption measurement of the reference with a silicon detector from the VIS to the NIR and with an InGaAs detector from the NIR to the SWIR. Repeat with sample measurements this time.

Note: Keep all system parameters the same between the reference and sample measurements or this will skew the spectrum.

Example Pseudo-Code of Calibration and Multi-Region Stitching of Absorption Spectra:
VIS-NIR measurement with silicon detector from 300 nm – 850 nm
NIR-SWIR measurement with InGaAs detector from 800 nm – 1600 nm
Goal—stitch and normalize these spectra for full absorption spectrum

Overlapping wavelength range= from 800-850 nm
Selected wavelength for normalization: 830 nm
Absorption spectrum #1 is the VIS-NIR measurement and has an absorption value of 2 AU at 830 nm
Absorption spectrum #2 is the NIR-SWIR measurement and has an absorption value of 4 AU at 830 nm
Multiply all absorption values on #1 by the division of the absorption value of #1 at 830 nm by the absorption value of #2 at 830 nm (4 AU /2 AU) = 2 AU
Now the absorption value at 830 nm from absorption spectrum #1 is 4 AU
Cut absorption spectrum #1 at an overlapping wavelength like 830 nm and add it to absorption spectrum #2, but only from 831 nm on.
Resulting Spectrum is:
Normalized VIS-NIR measurement from 300 nm to 830 nm + NIR-SWIR measurement from 831 nm to 1600 nm.