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In Vitro Observation of Optical Changes in Whole Blood Due to Injection of Glucose Solution

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A thesis submitted to the faculty of
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Master of Science

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ABSTRACT

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Significance: While the optical properties of blood have been extensively studied, the influence of glucose concentration on blood optics remains unquantified. This is information which could be used to develop a noninvasive blood glucose monitor.

Aim: This in vitro study investigated the optics of blood. Time-dependent effects and various methods of introducing glucose into blood samples were examined. The study aimed to quantify how alterations in glucose levels impacted blood optics across 14 distinct NIR wavelengths.

Approach: Blood in a cuvette was exposed to light, and the reflected and transmitted light was captured in integrating spheres. 14 distinct wavelengths from 1100 to 2400 nm in 100 nm steps were studied. Glucose concentration in the blood was altered by the addition of glucose solutions. Saline of the same volume and concentration as the glucose solution was added to a control sample of blood. Extraction of the scattering and absorption coefficients of these wavelengths at various levels of glucose was done using inverse adding doubling.

Results: Transmission at all 14 wavelengths increased as glucose solution was added to the blood. Reflection was impacted only at the wavelengths of 1100-1400 nm where it dropped slightly. Transmission decreased slightly with saline additions and reflection increased. Extraction of scattering and absorption coefficients revealed scattering decreased across all studied wavelengths with the addition of glucose and increased with the addition of saline. Absorption increased with both the addition of glucose and saline, but only at wavelengths above 1800 nm.

Conclusions: Increasing glucose concentration in blood causes changes in the optical properties of blood in vitro. These findings underline the potential for utilizing optical methods in assessing glucose concentrations within a physiological context.

Keywords: blood optics, blood scattering and absorption, in vitro measurements of blood, spectroscopy

ACKNOWLEDGMENTS

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Funding for this project came from Tula Health Inc. This research was possible because of their generosity in purchasing multiple pieces of equipment and other monetary helps they have provided.

I would like to acknowledge Dr. Bill Pitt for sharing his extensive knowledge on blood and the human body with us. Samples used in this research came from his lab and blood draws were organized by his students.

Finally, I would like to acknowledge Dr. Scott Prahl. Dr. Prahl is a widely recognized researcher in the field of tissue optics and also developed the IAD program used to analyze our data. Dr. Prahl also made himself available for meetings and responded to many emails. We are especially grateful for his insight and contributions to this project.

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Chapter 1

Introduction

The Beer-Lambert law (Eq. 1) is basic to the understanding of how light interacts with human tissues.

$$I_t = I_0 e^{-x(\mu_s+\mu_a)} \quad (1)$$

The law models the exponential decrease in intensity of a beam of light as it passes through a medium. I_0 is the initial intensity of the beam, I_t is the transmitted intensity of the beam, x is the distance the beam passes through the medium, and μ_s and μ_a are the scattering and absorption coefficients which describe the rate of intensity lost due to scattering and absorption per unit length.

The scattering and absorption coefficients, described in the Beer-Lambert law, are crucial optical properties for comprehending the interaction of light with biological tissues[1]. The coefficients are wavelength dependent, and they play a significant role in modeling the attenuation of light within tissues[1,2]. Biological tissues are composed of a matrix of solids, such as protein fibers, bone, or lipid bilayers, with a liquid medium interspersed[1]. Mismatched index of refraction between solid and liquid components of a tissue results in high scattering[1,3]. Thus, the way a tissue scatters light is due in large part to the mix and morphology of materials and how those materials contrast with each other[4]. Absorption, on the other hand, is dependent only

on the constituents of the tissue. In the infrared, tissue absorption is dominated by water's absorption spectrum[5].

Because blood is present throughout the body, the study of the absorption and scattering coefficients of human blood has been ongoing for several decades. Wavelengths studied have ranged from visible light to the near-infrared (NIR)[3,4,6–9]. Blood is composed of plasma, a protein-rich fluid in which all the other constituents are suspended; red blood cells (RBC), which carry oxygen or carbon dioxide through the body; white blood cells, which are a part of the immune system; and platelets, which clot the blood. The plasma and RBC have been found to be the most impactful on the optics of blood[10]. Mismatch between RBC and plasma indices of refraction have been hypothesized as a major factor in blood's scattering characteristics[3,10].

While RBC from healthy individuals have been observed to be optically similar from individual to individual[6,10], plasma color and opacity can vary widely among healthy individuals[11]. A large number of studies on the optics of blood use RBC suspended in saline as a blood analog[6,8,10,12]. While this method may be useful for understanding scattering phenomena, the absorbing properties of blood are mainly determined by the plasma and so removal of plasma drastically impacts the optics of blood[10].

Additional evidence suggests that RBCs are altered in their morphology by the removal of plasma and addition of PBS[13]. A study published in 2021 by Son *et al.* explored the impact of RBC shape as influenced by the solute which the RBC is suspended in. Refractive index tomography was used to observe changes in the morphology of RBC. It was found that PBS of the same osmolarity as plasma caused single RBCs to shrink. This result has implications for the impact of PBS on the scattering properties of blood.

Blood's scattering and absorption coefficients are dependent upon various chemicals in the blood. As in other biological tissues, water's absorption spectrum plays a large role in determining the absorption of NIR light in blood[4,8]. Water has low absorption in the visible spectrum and high absorption for much of the infrared spectrum. Relatively low absorption bands can be found in the NIR. These are known as the imaging windows and cover the ranges of 760 to 900 nm (the first NIR window), 1000 to 1700 nm (the second NIR window), and 2080 to 2340 nm (the third NIR window). Another chemical that is significant in the blood in these wavelengths is oxygen, which plays a noticeable role in the absorption of NIR light in blood[4]. This has been used to quantify the oxygen saturation of blood noninvasively and is the basis of the pulse oximeter.

The optics of blood have been long studied by a number of researchers[3,4,6–10,12]. A compilation of research on the scattering and absorption spectrums of blood was done by Bosschaart *et al.* and can be seen in Fig 1. Notably, the scattering coefficient (Fig 1a) varies widely from test to test, though the trends observed are universal. A decrease in scattering coefficient from approximately 800 nm on through the end of the spectrum is observed. The reduced scattering coefficient was also calculated and plotted (Fig 1c). The reduced scattering coefficient (μ_s') takes into account the effects of anisotropy (g). See Eq 2

$$\mu_s' = \mu_s(1 - g) \tag{2}$$

Absorption (Fig 1b) has more agreement between studies than scattering, though there is still variation. The absorption of blood is seen to follow closely the absorption spectrum of water after 1400 nm.

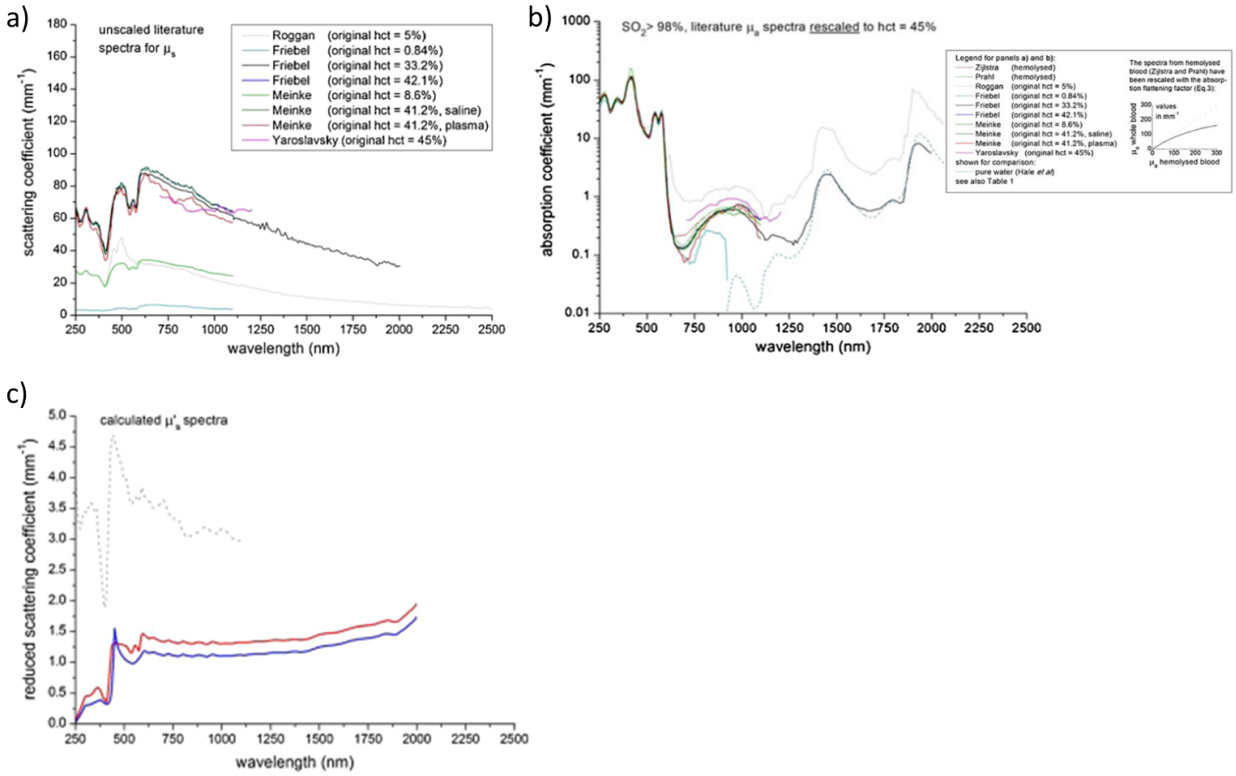


Figure 1 Compilation of values for the scattering, reduced scattering, and absorption coefficients of blood compiled by Bosschaart *et al.* It is observed that past the wavelength of 1500 nm, blood's absorption spectrum closely follows that of water. Scattering is seen to vary widely from experiment to experiment and decrease gradually from the wavelength of 750 nm on. [Figure from Bosschaart *et al.* 2014.]

While the optics of blood, water, and the impact of oxygen saturation on blood are represented in the literature, the impact of glucose concentration on the scattering and absorption coefficients of blood is sparse, despite the crucial role glucose plays in sustaining life. Though the direct effect of blood glucose concentration on the scattering and absorbing properties of blood have not been quantified, existing research implies that glucose does have an effect on the optics of blood[14,15]. Amerov *et al.* used bovine blood as an analog to human blood.

In their study, they observed how light transmitted through blood of various glucose concentrations. Adjustments to glucose concentration were made by incremental additions of powdered, dehydrated glucose[3]. Amerov *et al.* observed that the intensity of the transmitted beam decreased as glucose concentration increased. See Fig. 2. While Amerov *et al.* did not directly attempt to quantify the impact of glucose concentration on the scattering or absorption coefficients of blood, their findings do suggest that the optics are impacted by the addition of glucose into the blood.

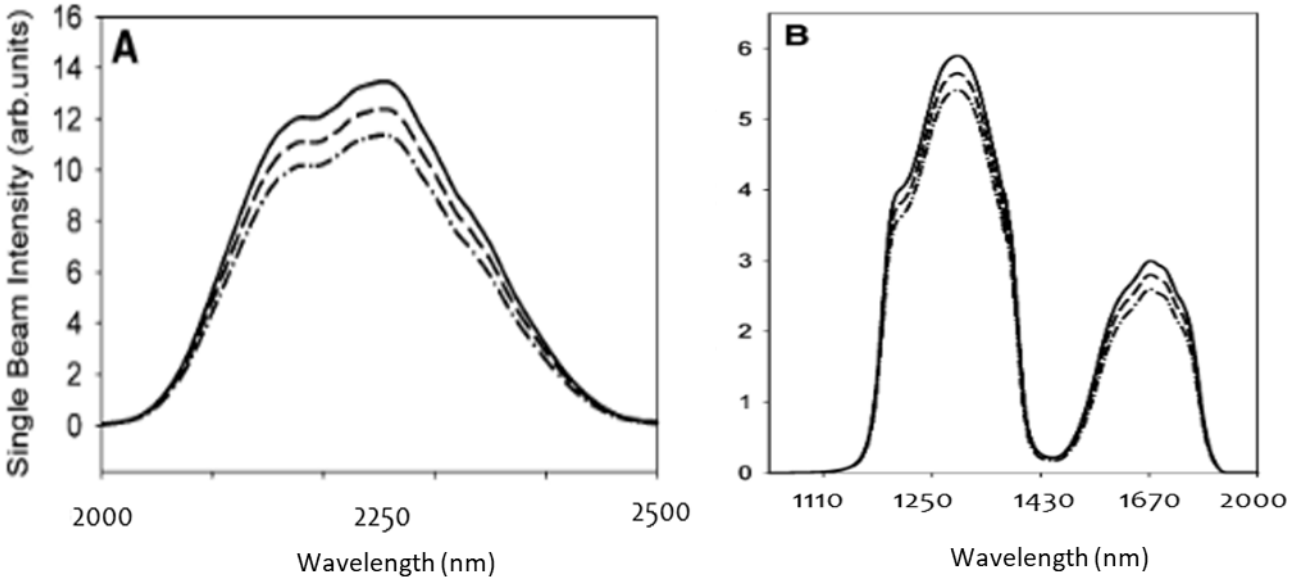


Figure 2 Bovine blood transmission measurements taken by Amerov *et al.* Glucose concentration was increased with each successive spectrum. 172 solid, 345 dash, 500 mg/dL dash-dot. Amerov *et al.*'s experiment shows a 10% decrease in transmission from the minimum to the maximum glucose concentration. [Figure from Amerov *et al.* 2005.]

Glucose is typically present in the blood at concentrations ranging from 70 to 140 mg/dL in healthy individuals. For those diagnosed with diabetes mellitus, however, blood glucose concentration can vary significantly, potentially leading to severe damage to nerves and organs throughout the body. Consequently, various monitoring techniques have been developed, all of

which require direct contact with the blood through pricks or probes. This can be uncomfortable for the patient and may limit how often measurements can be reasonably taken. Understanding the impact of glucose concentration on the scattering and absorbing properties of blood could be used to create a pulse oximeter-like device which could noninvasively monitor blood glucose levels.

Scattering and absorption coefficients cannot be measured directly but can be extracted using an inverse adding doubling (IAD) simulation[6,1]. Data for this simulation must be collected by placing integrating spheres at the front and back of a sample to measure the percent of light transmitted and reflected. From these measurements, IAD can make an appropriate approximation of the scattering and absorption coefficients for a sample[16,17]. This technique will be discussed further in the methods section.

The goal of our experiment was to quantify the impact of glucose concentration on the optics of human blood. Changes to glucose concentration were made by hypertonic and isotonic injections of glucose solution to the blood. We chose to use whole blood in order to probe more closely the state of blood in the body. The desire of this experiment was to unveil the effects of glucose on the optical properties of whole blood. Because of this, the plasma was not removed and the blood was kept in its original state.

In order to better understand the results of our experiment, blood's aging out of the body was characterized. It was found that transmission of light through blood decreased by as much as 10% in the first fifty minutes of the experiment, after which, the signal became steady. Reflection was found to remain steady through the duration of the experiment. Methods of introducing glucose to the blood were also studied. It was found that hypertonic glucose solution injected into blood caused non-permanent spikes in transmission and drops in reflection at

1250 nm. Isotonic glucose solution injected into blood caused permanent increases in transmission and drops in reflection at the same wavelength.

14 wavelengths ranging from 1100 to 2400 nm in 100 nm steps were studied as isotonic glucose injections designed to raise the blood glucose concentration by 400 mg/dL were added twice. It was observed that isotonic glucose additions raised transmission through the blood for all 14 wavelengths while isotonic phosphate buffered saline (PBS) added in the same volume had little to no effect on transmission. Reflection was also studied. It was found that while glucose injections did not impact reflection significantly, saline added to the blood increased reflection. These results were used as inputs to IAD and scattering and absorption coefficients were extracted. It was found that glucose added to blood as an isotonic solution lowers scattering for all wavelengths studied and lowers absorption at the higher wavelengths.

Chapter 2

Methods Section

2.1 Blood preparation and handling

Fresh human blood with the addition of EDTA as an anticoagulant was used for the study. No further additions were made to the blood prior to the experiment. The blood was collected under protocols approved by Brigham Young University IRB #X-2021-135. Laboratory processes were started within forty minutes of the draw. Two 10 mL aliquots were placed in 3D printed polymer containers. These blood reservoirs were partially submerged in a bath held at 37℃. The reservoirs were positioned so that the top of the blood level was beneath the level of the water in the temperature-controlled bath. The wall of the reservoir had a thickness of 0.5 mm to allow for good thermal contact between the blood and the temperature-controlled bath. The reservoirs were kept open at the top to allow oxygen diffusion into the blood for oxygen saturation. The reservoir design included an opening at the bottom with a 2 mm diameter tube which led above the surface of the bath. A cuvette and pump were attached to the reservoir's rigid tube by a flexible silicone tube. See Fig. 3.

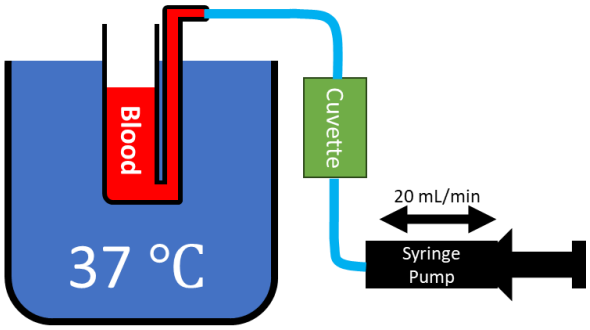


Figure 3 Blood reservoir partially submerged in a temperature-controlled bath. The blood from the reservoir was drawn out and through a cuvette by a syringe pump moving the blood at a velocity of 20 mL/min.

Blood was drawn out of the reservoir and through a cuvette by a syringe pump and returned through the same path. The cuvette was composed of a 3D printed polymer conduit with opposing glass windows 1 mm apart. The beam passed through these windows for the optical measurements to be taken. See Fig. 4. The flow rate was 20 mL/min and the shear rate was 109 s^{-1} in the window and 255 s^{-1} in the tubing. Cuvette and tubing hold 2.5 mL of blood. 5 mL was infused/withdrawn from the syringe to ensure good mixing.

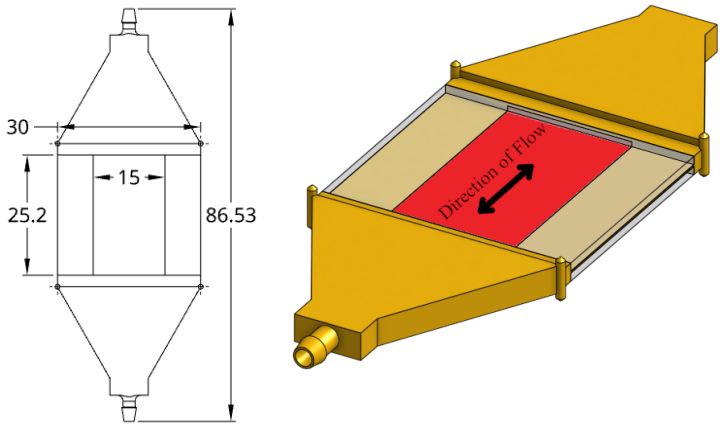


Figure 4 Top down and isometric views of cuvette. Units in millimeters. The body of the cuvette was 3D printed polymer with microscope slide windows on either side to encapsulate the blood. The space between windows and beam length through the blood was 1 mm.

2.2 Experiment and Control

To assure that the effects observed in the blood were due to glucose additions and not variation between individuals or other uncontrolled effects, two blood aliquots were studied simultaneously, one as a glucose experiment and the other as a control. Blood samples came from the same individual and were drawn at the same time. Identical cuvettes and blood reservoirs were used. The syringe pump held both syringes and subjected them to the same infuse/withdraw program.

Solutions of glucose of hypertonic (3,000 mOsmol/L) and near isotonic (300 mOsmol/L) concentrations were added to the experiment aliquot. Isotonic phosphate buffered saline (PBS) was added to the control aliquot in the same volume and at the same time as the glucose additions to the experiment. This was done in part to ensure that the changes in hematocrit induced by the addition of glucose solution was matched in the control.

2.3 Optics and Photonics

Light for the experiment came from a mercury arc lamp (HBO 100 w/2). A point source was approximated by an aperture and the light was collimated by lenses. The collimated light was then focused down to a point, chopped at a frequency of 2 kHz for lock-in detection, and passed through a linear variable filter (LVF) to select a 20 nm band of wavelengths. The LVF can be positioned to select the desired band. After the LVF, the beam was recollimated and split approximately 50/50 for the experiment and control branches. Each branch consisted of two integrating spheres with a cuvette placed between them as described by Pickering *et al*[16].

Intensity of light reflected by the blood was measured by photodiodes in the spheres before the sample on either branch. Intensity of light transmitted was measured by integrating spheres placed behind the sample on the beam path. A more detailed explanation of the use of integrating spheres for extracting scattering and absorption coefficients from blood can be found in Prahl[17].

An optical chopper ran at 2 kHz and served as the reference for all four lock-in amplifiers (Stanford Research 830). Lock-in amplifiers measuring reflection data were used in current mode. The amplifiers measuring transmission data were used in voltage mode and a preamplifier was used to amplify the signal 10^6 V/A. This choice was based on instrumentation availability and operation. Diagrams of the optical set up can be found in Fig. 5.

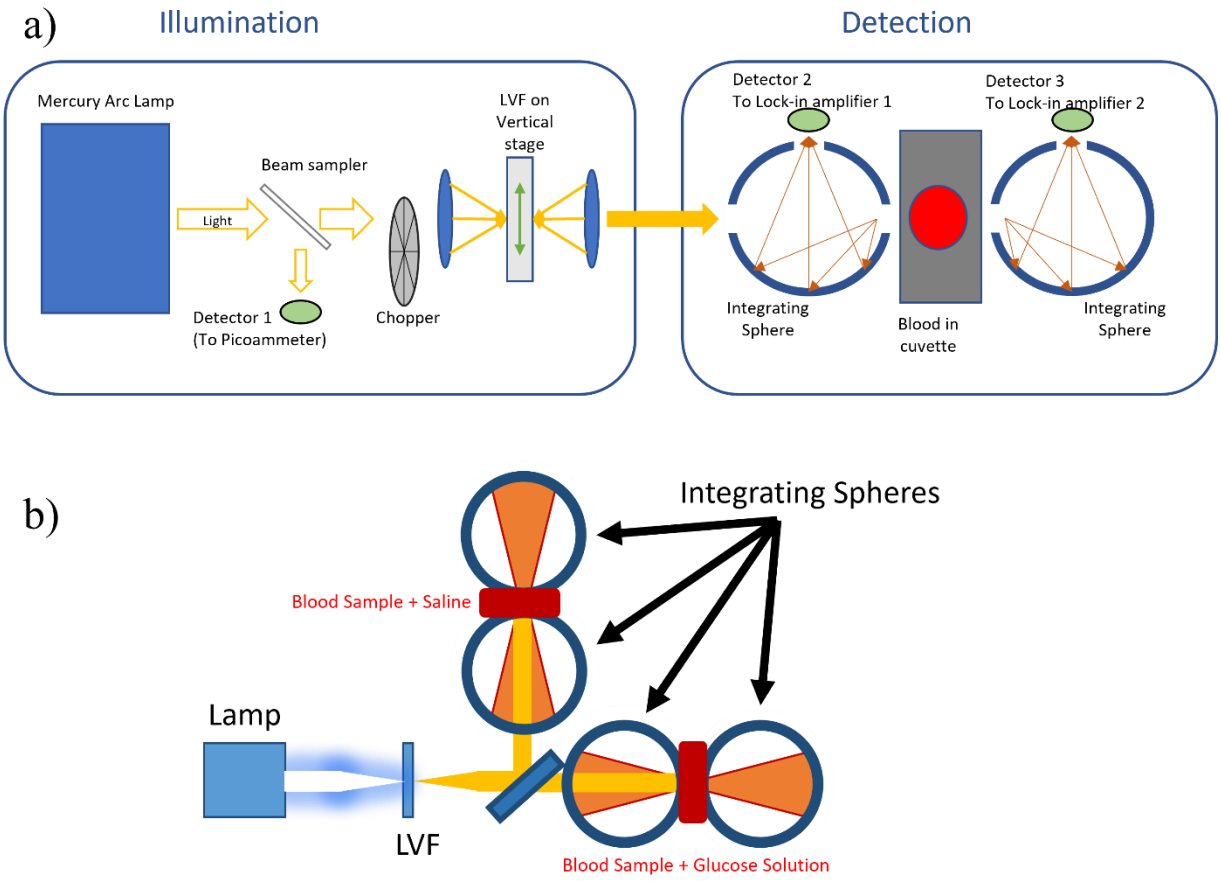


Figure 5 Diagrams of the optical set up. a) Diagram of the double integrating sphere configuration on the Detection side, and a diagram of the beam conditioning on the Illumination side. b) Diagram showing the experiment and control branches of the experiment.

2.4 Experiment Design

Information on the optics of blood aging outside of the body and the best practices for introducing glucose to the blood were not readily available in the literature but were deemed essential for our investigation on the impact of glucose on the optics of blood. Experimental set up (see Fig. 5) for measuring the effects of blood aging and comparison of two methods for

introducing glucose to the blood are described in this section. Also found in this section is a methodology for measuring the impact of glucose on the optics of blood at 14 wavelengths.

2.4.1 Blood Aging Test

Total diffuse reflection and transmission were measured as blood was flowed through the cuvette. Neither glucose nor saline was injected into the blood throughout the duration of the experiment. The LVF was set to permit 1250 ± 10 nm light. The experiment started 45 minutes after the blood draw and continued for another 200 minutes.

2.4.2 Addition of Hypertonic and Isotonic Glucose Solutions

A glucose in deionized water solution with an osmotic concentration of 3,000 mOsmol/L was prepared. 300 μL of solution was injected twice into the 10 mL aliquot of blood, once at t = 43 min and again at t = 79 min. Saline was added to the control blood at t = 43 and t = 79 minutes to match the additions of the glucose solution into the experiment. The saline injections matched the volume of the glucose injections but were at an osmotic concentration of 300 mOsmol/L. The LVF was set to permit 1250 ± 10 nm light and data was collected.

A replication of the experiment with a near isotonic glucose solution was completed. Glucose at an osmotic concentration of 300 mOsmol/L was prepared. 300 mOsmol/L was chosen as an approximation of the osmotic concentration of the blood. 3 mL of the glucose solution was injected into the blood twice, both times designed to rise the blood glucose concentration by 1200 mg/dL. A calibrating addition of 0.75 mL was added before the experiment to confirm the

rise in glucose concentration was as calculated. Parallel to the experiment, the control received 0.75 mL of saline and 3 mL of saline twice.

Pathological increases in glucose concentration were chosen to amplify the effect far above the noise in the measurement.

2.4.3 +400 mg/dL Fourteen Wavelength Survey

Blood was exposed to 14 wavelengths of light spaced 100 nm apart and ranging from 1100 to 2400 nm. Data was taken sequentially from each wavelength in ascending order. The LVF was held stationary for 1.5 seconds, or approximately five lock-in amplifier integration constants, before data was sampled. The cycle was repeated through the duration of the experiment.

At the start of the experiment, unaltered blood was pumped through both experimental and control channels as the 14 measurements were taken. After 40 cycles of 14 wavelengths were completed, 1 mL of isotonic glucose solution was added to the experiment and the same volume of saline was added to the control. Another 40 cycles were completed, and the same addition was made, and the final 40 cycles were completed.

Reflection and transmission data at the three glucose levels from the experiment and control were averaged. The fraction of the beam transmitted and reflected was calculated. The mean was calculated from between 35 and 40 data points per wavelength.

2.5 Simulation and Calculations

Adding doubling is a numerical technique for solving the radiative transport equation. Scattering and absorption coefficients of a material are used to calculate transmission and reflection through a sample of a given size. Inverse adding doubling is a method by which the scattering and absorption coefficients are guessed and the calculated transmission and reflection values are compared with the measured experimental values and the scattering and absorption coefficients are updated and the process is repeated until the calculated transmission and reflection match the experimentally measured transmission and reflection to a predetermined tolerance. Scattering and absorption coefficients used by the simulation to calculate the accepted reflection and transmission are assumed to represent the actual scattering and absorption coefficients of the sample. For this research an IAD code written by Scott Prahl was used. Documentation and a more complete explanation can be found in Prahl's paper[17].

Metadata required for the use of Prahl's IAD included dimensions of the integrating spheres and the sample and cuvette, the measured total diffuse reflection and transmission, and an assumed anisotropy value (g) of 0.96 which was obtained from a literature review of blood optical properties completed by Bosschaart *et al*[6].

Chapter 3

Results

3.1 Blood Aging

It was observed that transmission through blood decreased approximately 10% in the first 50 minutes after the measurement started and then remained constant for the duration of the measurements. Reflection remained mostly unchanged except for a rise of a couple of percent over the 200 minutes the blood was studied. See Fig 6.

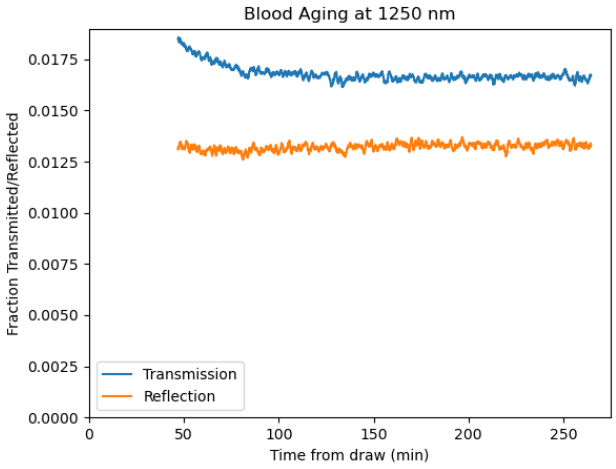


Figure 6 Optical aging of blood as seen by reflection and transmission measurements. It was observed that transmission of light through the blood dropped approximately 10% in the first fifty minutes after the measurements began and in the first 100 minutes after the draw. Reflection rose 2%.

This finding necessitated the use of a control as described in the methods section, which was employed in part to help distinguish blood aging effects from changes in the blood glucose level.

3.2 Glucose Introduction Method

3.2.1 Hypertonic

Hypertonic glucose solution was injected into the experiment sample of blood symulteniously with isotonic saline injections into a control sample of blood. Transmission and reflection of 1250 nm light was recorded. Figure 4 shows the data from the experiment divided by the data from the control. The injections were calculated to raise the concentration of glucose in the blood each time by 1200 mg/dL. At both additions it was observed that the transmission dropped and the reflection increased. The value then underwent decay towards the original value for both transmission and reflection. The changes induced by the addition decayed back to the original transmission and reflection values after about 10 minutes.

The decay experienced by the blood outlasted the calculated time for concentration in the line to reach less than 1% of the equilibrium value. The cuvette and tubing hold 2.5 mL and the pump infuses and withdraws 5 mL at a rate of 20 mL/min. Assuming turbulant mixing in the

reservoir, the time to reach 1% of equilibrium is under two minutes. This implies that there is some mechanism in the blood by which the experiment is changing relative to the control and that change is reversing in about ten minutes.

3.2.2 Isotonic

Following isotonic injections, the transmission was observed to increase. Unlike the hypertonic additions, the change remained constant. Reflection at 1250 nm dropped when glucose was added as an isotonic solution. Similar to transmission, the change was sustained. This suggests a different physiological response to isotonic glucose additions versus hypertonic glucose additions. See Fig. 7.

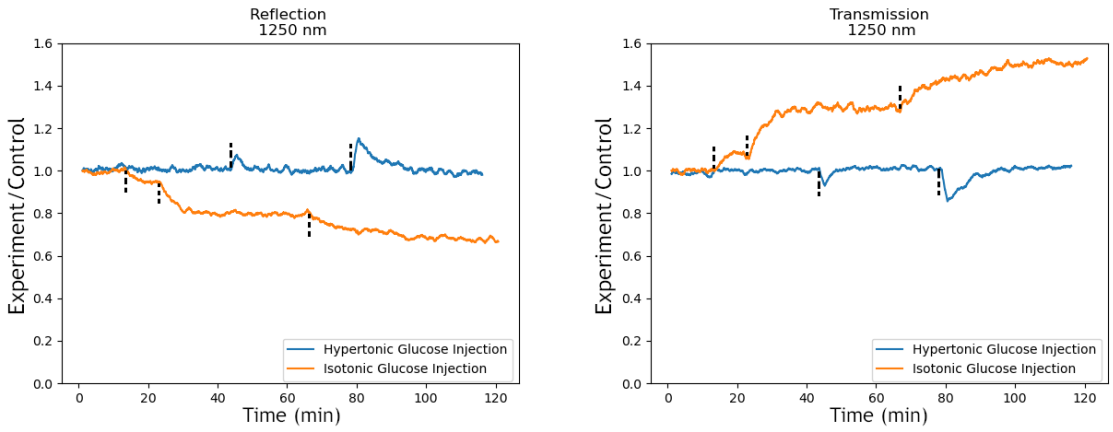


Figure 7 Reflection and transmission for hypertonic glucose solution additions and isotonic glucose solution additions. Additions indicated by vertical dotted lines. The first isotonic glucose injection was a calibrating injection, and the subsequent two injections were designed to raise glucose by 1200 mg/dL.

3.2.3 Summary

Differences recorded between the hypertonic and isotonic glucose introduction methods suggests a different physiological response from the blood to each. The difference may be in part attributed to changes in volume of the RBCs due to hypertonic injection and no significant change in volume due to isotonic injection. The mechanism which causes the change from hypertonic injections to be transitory and the change from isotonic injections to be stable is not understood and requires further research which was beyond the scope of this study.

3.3 +400 mg/dL Experiment

Considering the noise on the hypertonic/isotonic experiments, it was decided that changes in glucose level representing normal physiological levels would be difficult to observe and that detecting those of a pathological level would be more feasible. Raising the blood glucose level by 400 mg/dL twice by additions of isotonic glucose solution was chosen as the glucose parameter for the test.

Increasing the blood glucose concentration by 400 mg/dL resulted in an observed increase in light transmission through the blood at all 14 wavelengths (1100 – 2400 nm in 100 nm steps). When saline was added to the blood at the same volume, no significant effect on transmission was observed except a slight decrease for wavelengths less than 1800 nm. A graphical representation of this at 2300 nm for sample 7 can be found in Fig. 8. A complete representation of the 14 wavelengths for Sample 7 can be found in the Appendix Fig. 13. Data on all other samples used in this study can be found in the BYU_Blood_Optics_Repo.

Fig. 8 shows an example data set at 2300 nm for a sample 7 (chosen because it was representative of the full dataset) and includes both transmission and reflection results for the glucose and saline channels separately, unlike the representation of isotonic/hypertonic glucose additions. This difference in representation was chosen so that changes made to the blood by the saline additions could be viewed separately from the changes made to the blood by the glucose additions.

A distinct upward step in transmission corresponding to the addition of glucose was observed. This was similar to the experiment involving a 1200 mg/dL increase in glucose concentration. The transmission change caused by the glucose addition remained stable throughout the remainder of the experiment. Saline addition to the control blood did not result in a noticeable change in transmission. Aging of the blood was evident in both the experiment and the control and the signals matched between the two experiments until the addition of glucose solution into one and the addition of saline into the other.

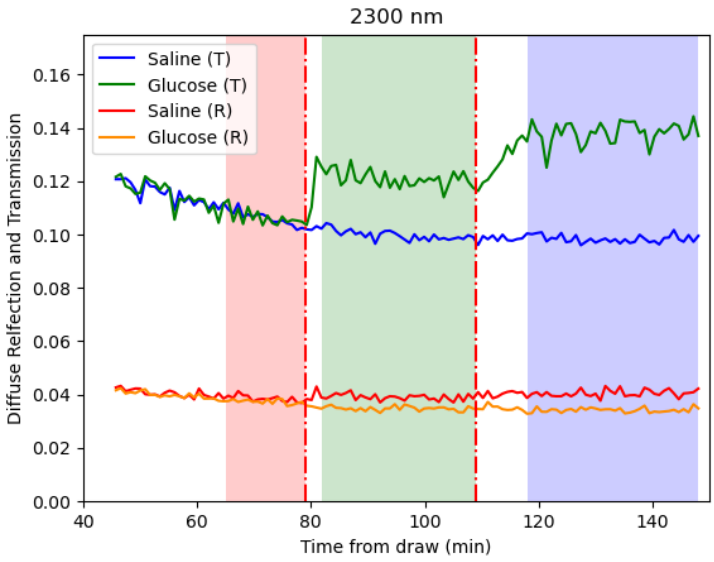


Figure 8 Transmission and reflection in time at 2300 nm for sample 7. Diffuse transmission and reflection of 2300 nm on blood measured for 110 minutes. 1 mL

injections were made at the dotted lines. Glucose injections were designed to raise the glucose concentration of the experiment blood by 400 mg/dL. Saline injections were also made at the dotted lines. Red, green, and blue shaded areas show data averaged for further analysis.

Data was collected at 14 wavelengths ranging from 1100 to 2400 nm in 100 nm steps (see Fig. 13). Across all wavelengths transmission increased with glucose additions and remained largely unchanged with saline additions. Analysis of the reflection data revealed contrasting outcomes compared to the transmission data. After glucose injections, no significant impact on the overall diffuse reflection was observed, except at wavelengths of 1100, 1200, and 1300 nm, where a decrease was recorded (See Fig. 13 a, b, c). This finding aligns with the results observed in the hypertonic/isotonic injection experiments where a decrease in reflection was also observed at 1250 nm. Findings suggest there is some impact on reflection due to the addition of glucose on the lower wavelengths studied, but not on higher wavelengths. Reflection increased uniformly across wavelengths with saline additions. For detailed reflection data, refer to Figure 6.

3.4 Variation Among Samples

For analysis of the blood optics, a mean value was calculated from the point at which the transmission stabilized until either the next glucose addition or until measurements ended. These time periods are shown in figure 6 by the red, green, and blue color bands. Data was collected from all eleven blood draws.

From sample to sample, initial total diffuse transmission varied by as much as fifty percent and reflection by as much as twenty percent. Plasma was not removed for these experiments and

hematocrit was not altered except by the addition of fluids for the experiment or control. It is likely that variation observed among the experiments was the result of donor to donor variation. Despite variation from individual to individual, trends due to the addition of glucose or saline were seen across all experiments. Glucose additions universally increased transmission. Saline additions universally increased reflection. Though human blood will differ from one individual to another optically, data collected indicates that the impact of glucose is the same among all blood samples. See Fig. 8 for a variation comparison at 2300 nm and Fig. A2 for all 14 wavelengths.

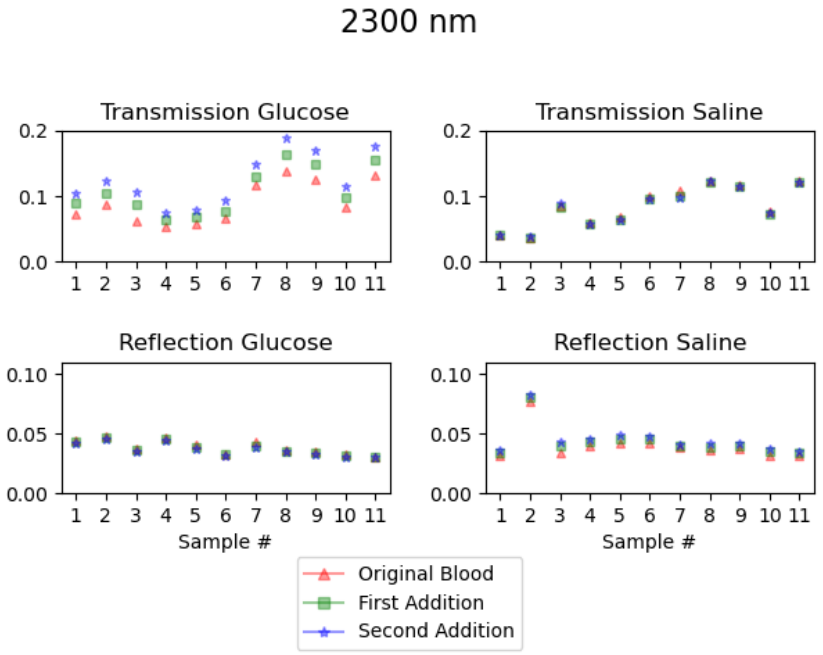


Figure 9 Transmission and reflection for all 11 samples at 2300 nm. Using the means from the time data, these plots were compiled representing the eleven samples at 2300 nm. It can be observed that reflection and transmission varied largely from individual to individual but trends in the transmission experiments and trends in the reflection control were matched across all samples.

Fig. 9 shows the increase in transmission for glucose added. Increases are shown to be steady among all samples, despite the initial difference in starting value.

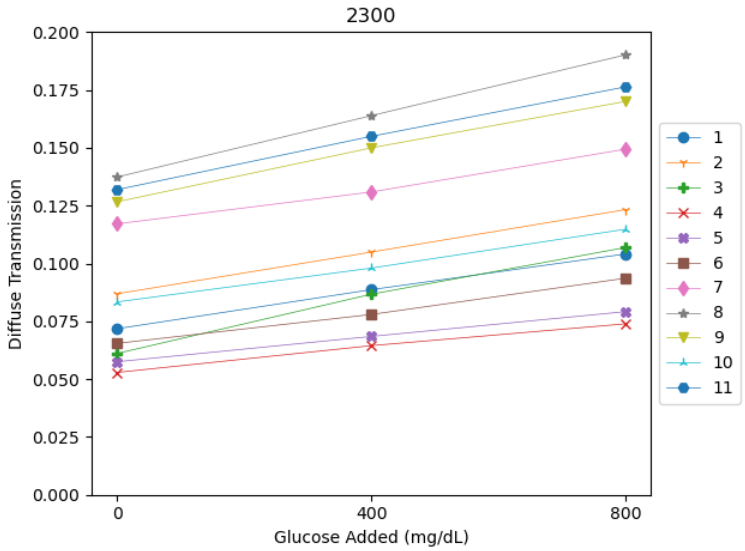


Figure 10 A representation of all samples and how transmission changed with increases in glucose. It can be observed that the amount of increase in transmission was similar among all samples despite their different starting values.

3.5 Extraction of Scattering and Absorption Coefficients in Blood with Isotonic Glucose and Saline Additions

As can be seen in Fig. 10, unaltered control and experiment samples were not always in agreement. Though difference in the optics between samples is expected, differences in value between two aliquots from the same sample should be the same. These discrepancies showed up even before the addition of saline or glucose. The discrepancy was attributed to poor calibrations, which resulted in a difference in reflection or transmission value between aliquots of the same sample. Across all wavelengths, it was found that the unaltered sample measurements of samples

4 and 7-11 varied less than 20%. A single dataset was compiled from the samples with the least variation and averaged. A visual representation of the dataset can be seen in Fig. 11.

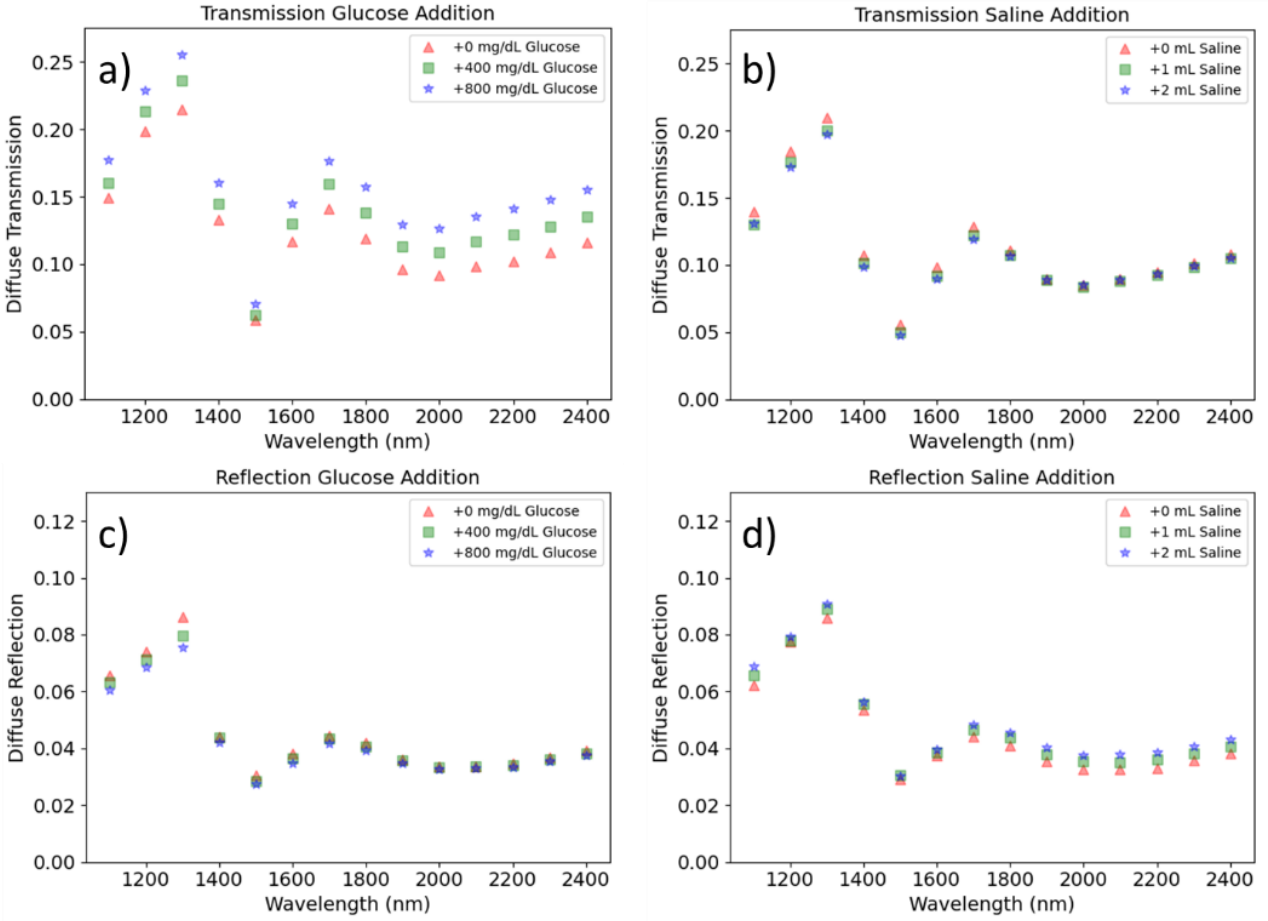


Figure 11 The mean of samples 4 and 7-11 which were chosen for low variation between unaltered blood measurements at control and experiment aliquots. Each glucose value and wavelength represented distinctly.

Trends seen in single samples are observed in the compiled dataset as well. Transmission increases with glucose concentration while reflection is minimally affected. Saline addition has some small impact on the transmission, slightly decreasing the value across all wavelengths, and has the effect of increasing reflection. A possible explanation for changes caused by saline addition are changes in the cell's morphology as observed by Son *et al.*

The data set of all averaged data was used as input into Dr. Prahl's IAD program as outlined in the methods section. The resulting calculated μ_a and μ_s` values are displayed in Fig. 12. What can be seen is that scattering is significantly impacted by the additions of glucose in a way that differs from saline addition. Glucose additions lower the scattering coefficient across all wavelengths studied. Saline additions had the effect of raising the scattering coefficient, the opposite effect of glucose. The absorption coefficients recorded did not see the same trends as scattering. There was a change in the absorption coefficient with glucose addition above 1800 nm, but this change is also observed with saline additions.

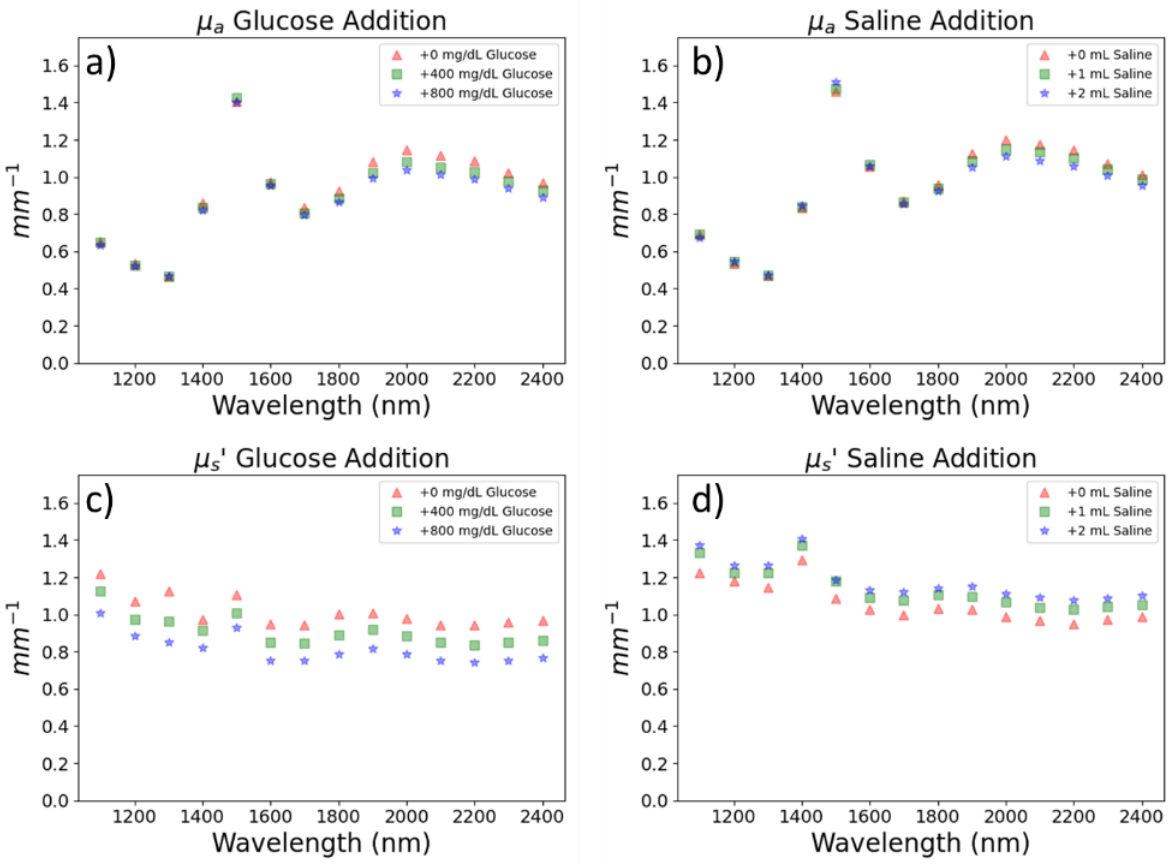


Figure 12 Extraction of μ_a and μ_s` from the mean of the previous data.

The underlying mechanism controlling optical changes seen in the blood remains unclear. Decreases in the absorption coefficient can be explained as resulting in the replacement of plasma with water. Meinke *et al.* claimed that the absorption coefficient of blood is most impacted by the plasma and not the RBCs[10]. With both the glucose solution and the saline solution additions, the plasma is being diluted. Changes in scattering are harder to assign causes to. Amerov *et al.* proposed that a relative change in index of refraction between the RBCs and the plasma account for changes in the scattering coefficient[14]. It is possible that other, glucose induced, physiological responses such as RBC swelling or increased or decreased aggregation could prove to influence scattering as well. Work done by Son *et al.* suggests that the cells are changing morphology due to saline's influence which is likely to have an impact on the scattering of the RBCs. More research needs to be done to narrow down the possibilities.

Chapter 4 Conclusions

4.1 Blood Aging

After removal from the body and with the addition of EDTA as an anticoagulant, blood changes in its optical properties over time. Our experiment revealed that the transmission rate decreased by around ten percent within the initial fifty minutes from the start of the experiment. At the same time, the reflectivity of the blood remained unchanged. This experiment was done on whole blood, unlike many other blood optics experiments where the plasma has been removed. Whether RBC in saline experience the same effect was not within the scope of this experiment.

4.2 Hypertonic/Isotonic

The results from hypertonic glucose injections varied widely from the effects observed from isotonic injections of glucose, even when the molar mass of glucose added in either experiment was the same. The results of isotonic glucose additions were long lasting as opposed to those from hypertonic glucose, which decayed to the original values within ten minutes.

This information is relevant to further glucose in blood *in vitro* experiments. It may also shed some light on previous experiments like Amerov *et al.* where glucose powder was added to blood, effectively a hypertonic glucose addition.

4.3 Discussion on the use of Saline as a control

Volumes of glucose solution added to blood were matched by the same volume of isotonic PBS added to the control blood. This was done to better isolate the effects of glucose on the blood and not the additions of fluid to the blood. It was observed that saline added to the blood in the same volume as the glucose solution had an opposite effect to the glucose. This could imply that the effect of glucose on blood is larger than observed. More study needs to be done on saline as a control and other controls should be tried before this conclusion is accepted.

4.4 Effects of isotonic glucose injections on blood optics

Injecting glucose into the blood by additions of isotonic glucose solutions increases transmission for all 14 wavelengths studied in this experiment. Reflection is largely unaffected by isotonic additions of glucose except at the lower wavelengths (1100-1400 nm) where it appears to decrease the reflection. PBS additions to blood had differing results than those of glucose. Reflection universally increased while transmission of light through blood was unaffected.

The results of our experiment inserted into IAD suggest that the scattering coefficient is decreased across all studied wavelengths by the presence of glucose and that the absorption

coefficient is unaffected except at wavelengths above 1400 nm. Saline has similar effects on the absorption coefficient as glucose, suggesting the null hypothesis for an impact of glucose on absorption, but has an opposite though lesser effect on the scattering coefficient.

4.5 Comparison with Published Work

Results of our study differ from those recorded by Amerov *et al.*, where it was observed that increases in glucose concentration decreased transmission of light through blood. It is important to note the similarities and differences between our experiment and Amerov *et al.*'s. The most stark of the differences between the two experiments was the use of bovine blood in Amerov *et al.*'s experiment and human blood used in this study. A 2020 forensic study found that human blood differentiated from that of other mammals such as cows by IR spectroscopy at wavelengths as low as 2500 nm[17]. The study did not investigate lower wavelengths such as those observed in this study, but it can be seen that bovine blood differs optically from human blood. That discrepancy alone could account for the differences observed in transmission as glucose was added to human and bovine blood. Other differences exist. In Amerov *et al.*'s paper it is unclear for how long the blood had aged after it was collected from an abattoir. In our study we saw how length of time from draw can impact the optics of the blood. The decrease in transmission observed by Amerov *et al.*'s may have been due to blood aging as we saw similar effects in human blood. Also worth considering is that glucose addition in our experiment was done by isotonic injections where Amerov *et al.* added dehydrated, powdered glucose to the blood. This addition method would have been closest to our hypertonic addition experiment which did see an increase in transmission, though that change was not sustained.

Ultimately, differences between our experiment and Amerov *et al.*'s might be too great for direct comparison.

A comparison was made between extracted reduced scattering and absorption coefficients for unaltered blood in this study and those found in Bosschaart *et al.* The absorption coefficient measured in this study was found to be in agreement with that of Bosschaart *et al.*'s from the wavelengths of 1100 to 1800 nm. Absorption of blood reported by Bosschaart from her review rises to almost an order of magnitude greater than the data collected in this study, peaking at approximately 1950 nm. This peak corresponds to a water absorption peak. Data gathered in Bosschaart *et al.*'s review came from studies where plasma of blood was replaced by saline. It is possible that the discrepancy between this study and Bosschaart *et al.*'s is due to the plasma having not been removed from the blood. Plasma contains proteins, lipids, and many other complex molecules that saline would not. The presence of these biological compounds could reduce the water absorption peak at 1950 nm. See Fig 13.

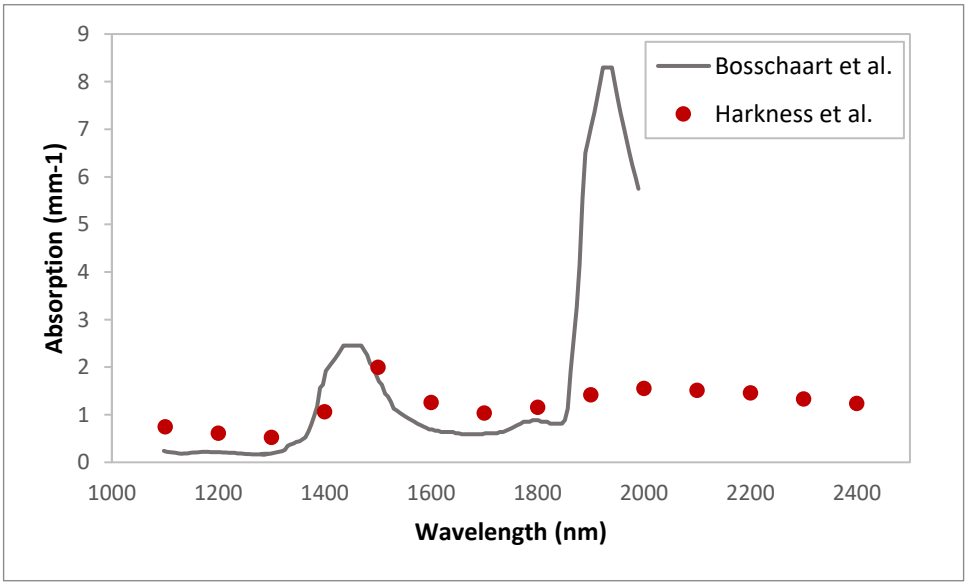


Figure 13 Comparison of absorption coefficients for unaltered blood from this study and a compilation value from Bosschaart *et al.* General agreement between the values

can be seen from the wavelengths of 1100 to 1800 nm. [Data from Bosschaart *et al* 2014.]

Greater differences appear when comparing the reduced scattering coefficients. Scattering observed in our study had greater variation between wavelengths and also was consistently lower than that of the Bosschaart *et al.* review. It is worth considering in this study how the addition of saline to blood resulted in an increase of scattering. Blood in the Bosschaart *et al.* review had plasma replaced with saline. This could be one explanation for the differences seen. See Fig 14.

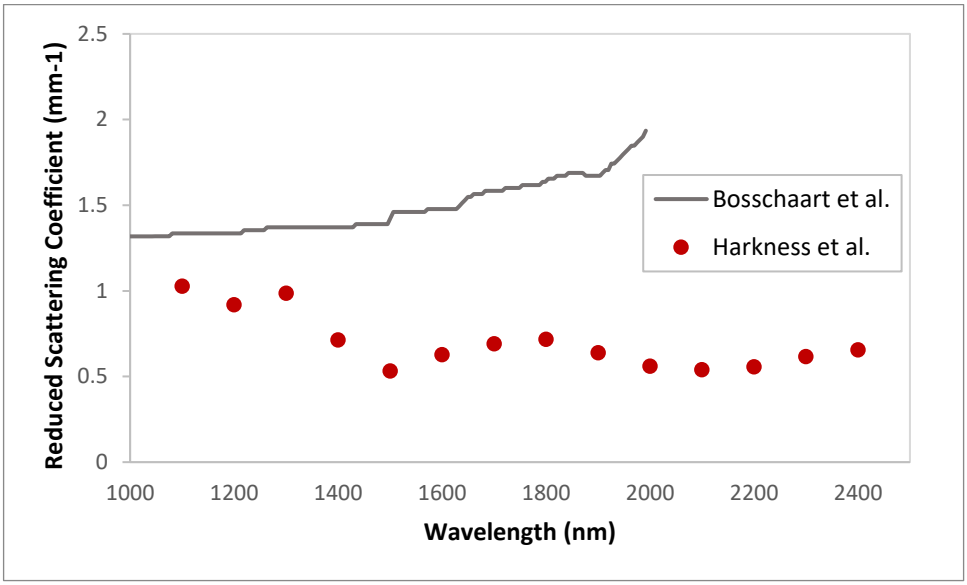


Figure 14 Comparison of reduced scattering coefficients for unaltered blood from this study and a compilation value from Bosschaart *et al.* An explanation for low agreement in values is the presence of plasma in our experiment and the lack of plasma in Bosschaart *et al.* [Data from Bosschaart *et al* 2014.]

Differences between the scattering and absorption coefficients obtained in this study and those compiled by Bosschaart *et al.* might be explainable by the phenomenon studied by Son *et al*. Son *et al.* observed that PBS in direct contact with RBCs caused a decrease in their volume and change in shape[13]. This is likely to have an impact on the optics of the blood. While blood used in this study was altered only by the addition of an anticoagulant, blood in many of the

studies compiled by Bosschaart *et al.* had plasma removed and PBS inserted. More study on the difference in optics of RBC in plasma and in PBS is needed.

4.6 Impact

To develop a noninvasive blood glucose monitor, we need to conduct computer modeling to comprehend how light interacts with the skin, blood, and other biological tissues. The data collected from this study is crucial for modeling, as it helps us understand how scattering decreases with higher glucose concentrations. Additionally, an important insight from this research is that each individual's blood has a unique scattering and absorption coefficient. Monitoring glucose levels will require tracking changes in this coefficient.

4.7 Further Work

It was observed that glucose added to blood in an isotonic solution changes the scattering coefficient in a way that the same volume of isotonic saline added does not. Further work that could be done include experiments to determine the mechanism by which glucose changes the optics of the blood. Experiments such as adding inverse glucose, which is not accepted by RBCs, could shed light on what changes are happening in the blood to cause the observed changes.

As stated earlier, the merits of saline as a control in this experiment could be investigated. It might be found that plasma from the same individual used as a control might yield different results from saline. It may also be in the interest of future researchers on the project to remove the plasma from blood to bring this experiment into greater conformity with other research done

in this field. There may be insights into the mechanism by doing this as well as blood without plasma may not respond in the same way as what was observed in this study.

Another crucial study to be performed would be to decrease the glucose additions to the point where the changes in glucose concentration in the blood match those that happen physiologically. It would be more informative for the development of glucose detection techniques *in vivo.*

Additional directions for future work done to take could be observing visible wavelengths of light. With the optical system that was created it would be relatively easy to switch out the IR LVF for one which filters in the visible wavelengths. Behavior observed in SWIR may or may not continue in visible.

Miscellaneous

5.1 Code, Data, and Materials Availability

Data collected for this experiment can be found on the BYU_Blood_Optics_Repo.

Two figures used in this paper were taken from the work of Bosschaart *et al.* and are used under Creative Commons Attribution 4.0 International License which permits unrestricted use, distribution, and reproduction in any medium.

One figure used in this paper was taken from the work of Amerov *et al.* and are used Creative Commons Attribution 4.0 International License which permits unrestricted use, distribution, and reproduction in any medium. Sizing changes were made to this figure as well as the conversion of wavenumber to wavelength in nanometers.

5.2 Conflicts of Interest

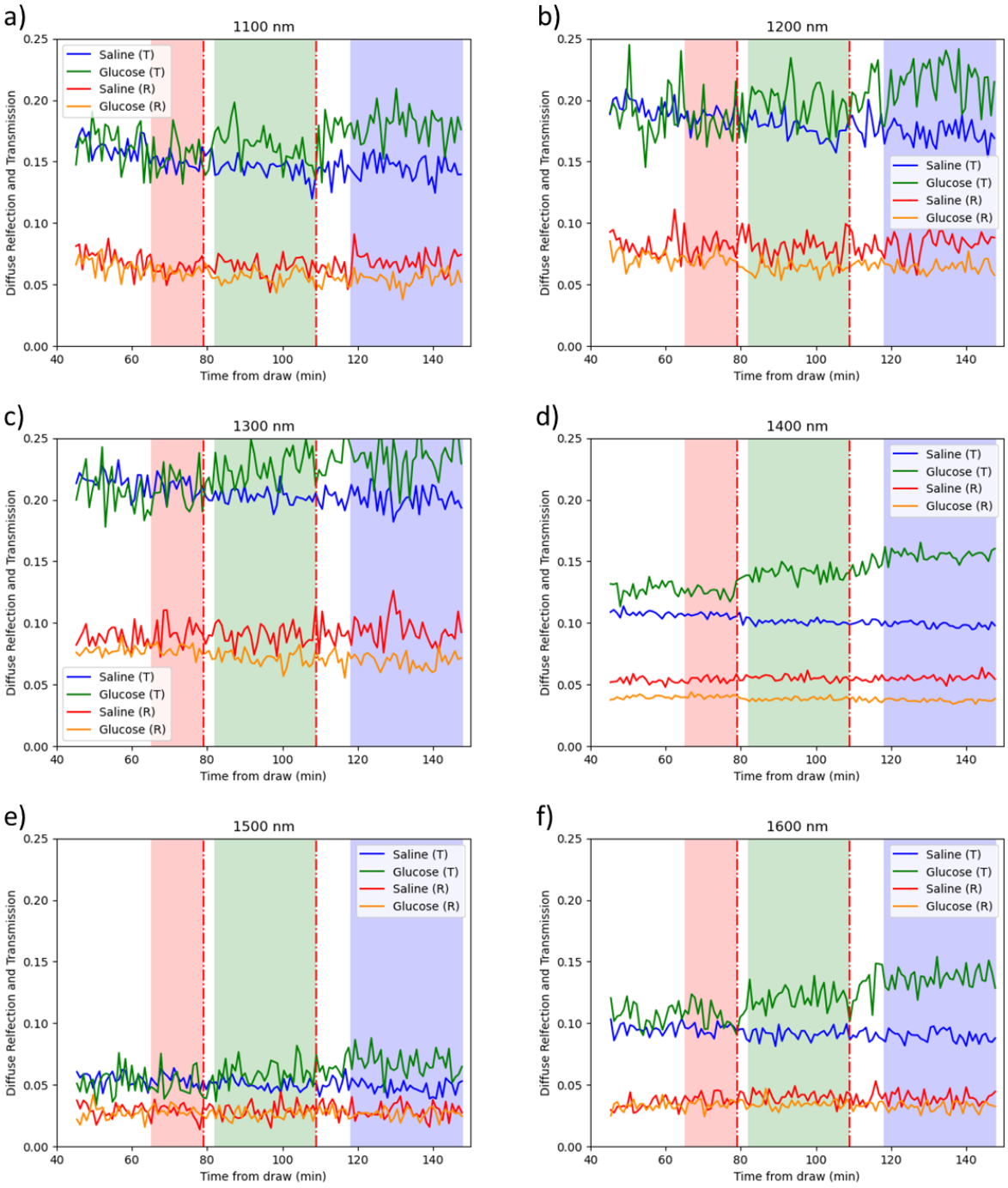
The authors of this paper declare no conflicts of interest.

5.3 Acknowledgements/Funding Sources

Funding for this project was provided by TULA Health, Inc.

Appendix

14 wavelengths were studied in the +400 mg/dL glucose increase experiments. As it was impossible to represent data collected on all of these wavelengths in the text, a single representative wavelength was selected and the full set can be found here.


a)
1100 nm
b)
1200 nm
c)
1300 nm
d)
1400 nm
e)
1500 nm
f)
1600 nm
Saline (T)
Glucose (T)
Saline (R)
Glucose (R)
Diffuse Relfection and Transmission
Time from draw (min)

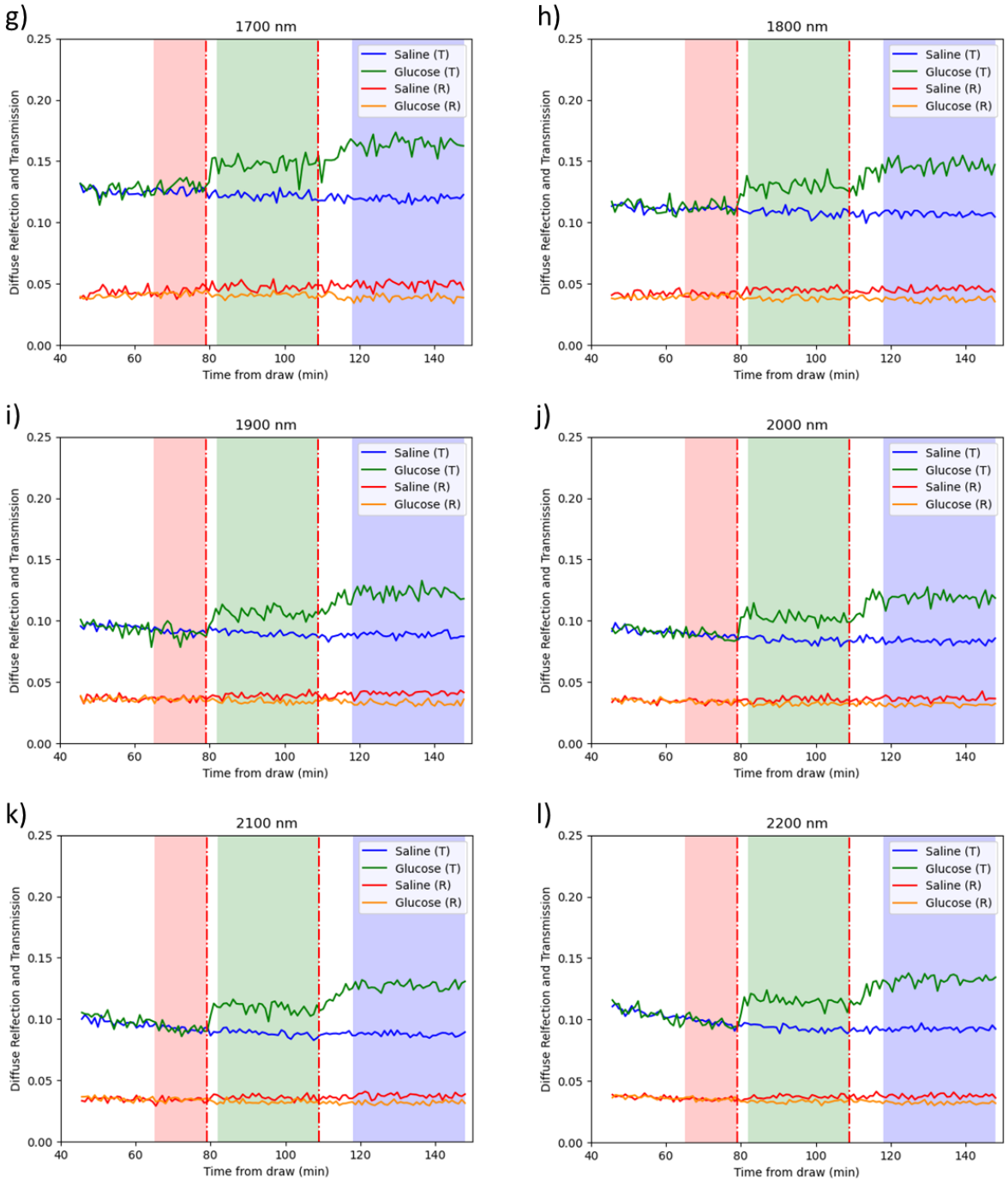
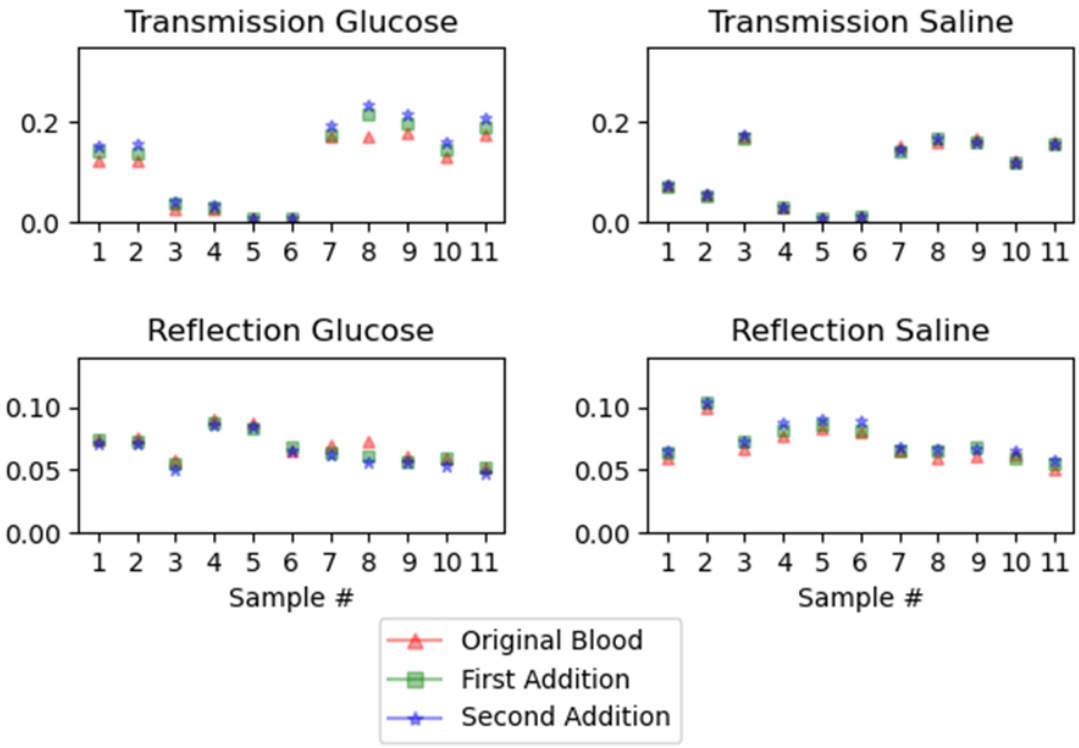
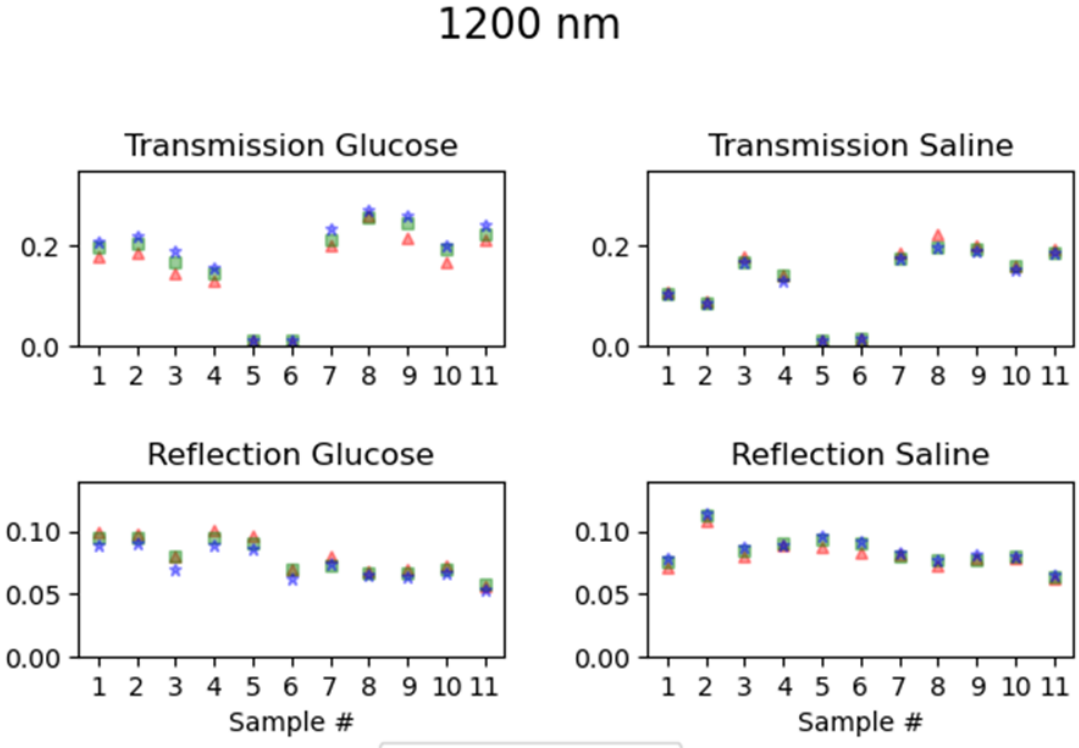


Figure 15 Diffuse transmission and reflection of all 14 wavelengths studied from sample 7. 1 mL injections were made at the dotted lines. Glucose injections were designed to raise the glucose concentration of the experiment blood by 400 mg/dL. Saline injections were also made at the dotted lines. Red, green, and blue shaded areas show data averaged for further analysis.

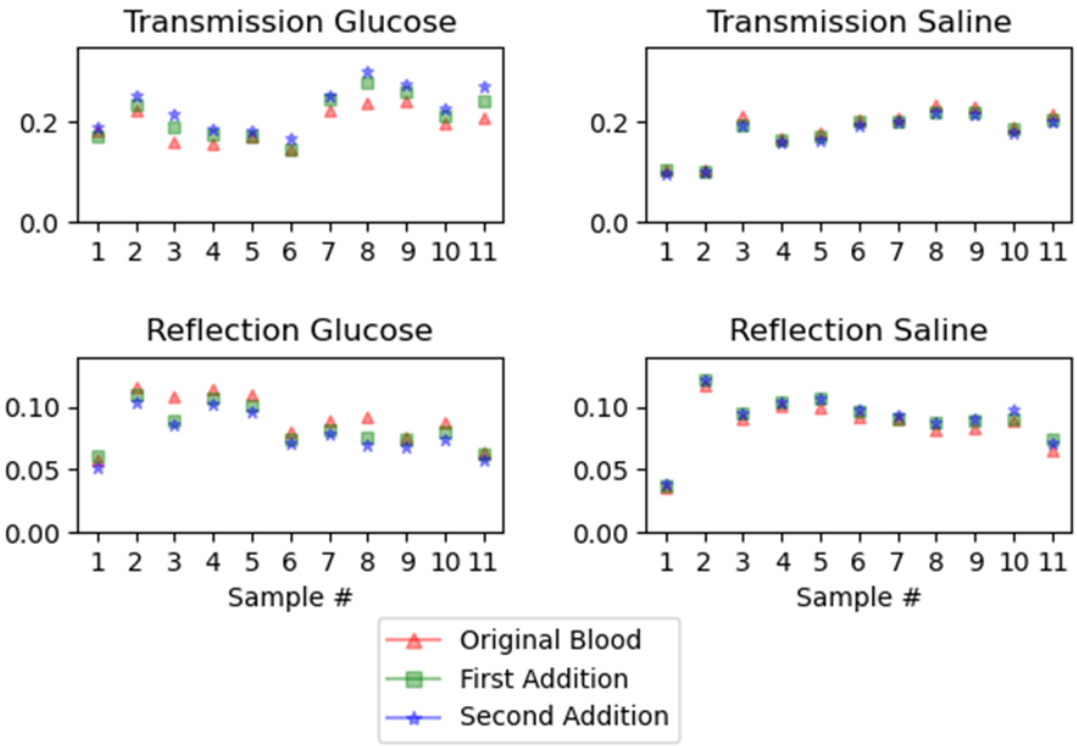
a)


1100 nm
Transmission Glucose
Transmission Saline
Reflection Glucose
Reflection Saline
Sample #
Sample #
Original Blood
First Addition
Second Addition

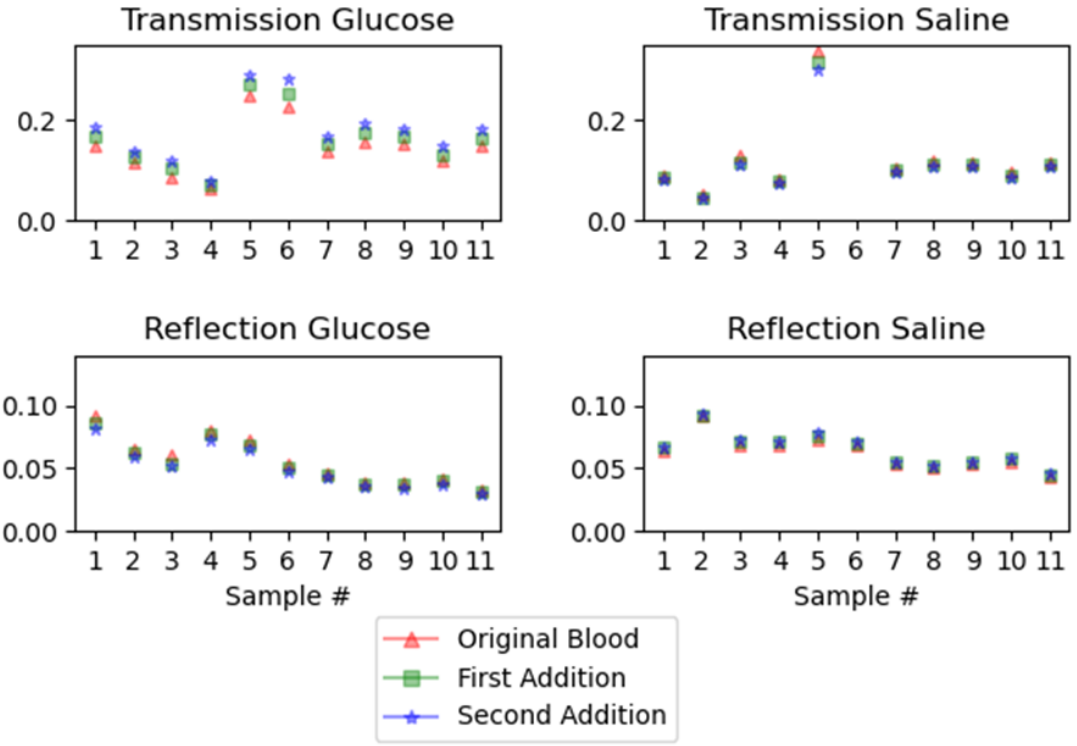
b)


1200 nm
Transmission Glucose
Transmission Saline
Reflection Glucose
Reflection Saline
Sample #
Sample #
Original Blood
First Addition
Second Addition

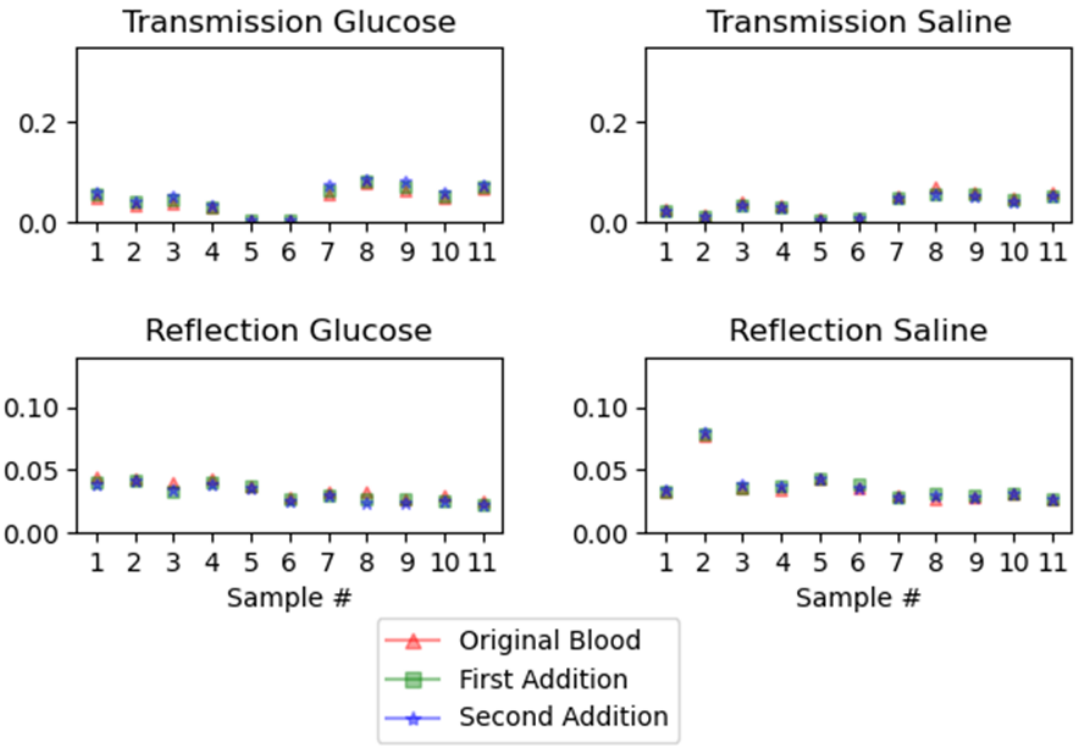
c)


1300 nm
Transmission Glucose
Transmission Saline
Reflection Glucose
Reflection Saline
0.0
0.2
0.00
0.05
0.10
1 2 3 4 5 6 7 8 9 10 11
Sample #
Original Blood
First Addition
Second Addition

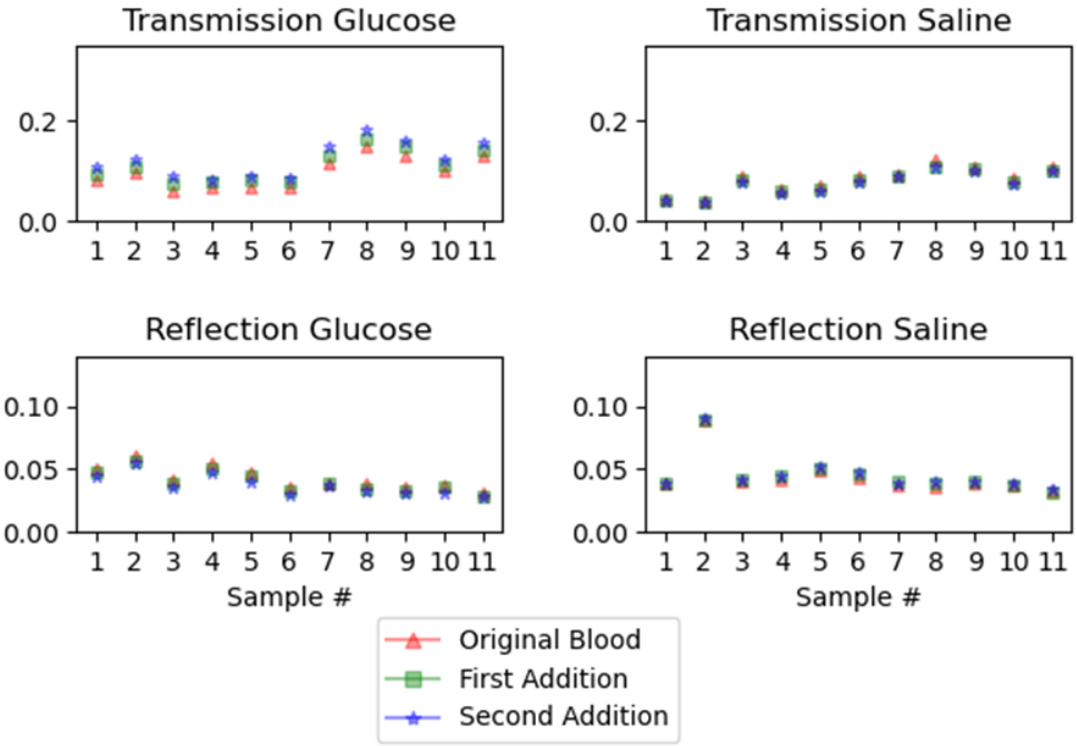
d)


1400 nm
Transmission Glucose
Transmission Saline
Reflection Glucose
Reflection Saline
0.0
0.2
0.00
0.05
0.10
1 2 3 4 5 6 7 8 9 10 11
Sample #
Original Blood
First Addition
Second Addition

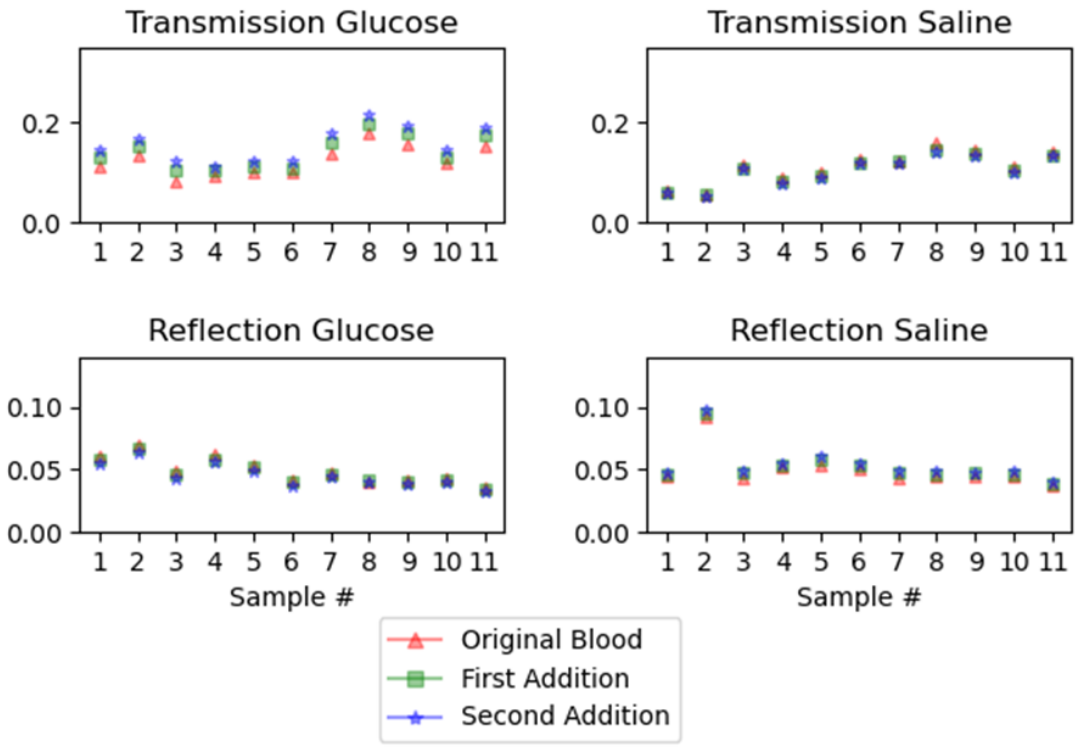
e)


1500 nm
Transmission Glucose
Transmission Saline
Reflection Glucose
Reflection Saline
0.2
0.0
0.10
0.05
0.00
1 2 3 4 5 6 7 8 9 10 11
Sample #
Original Blood
First Addition
Second Addition

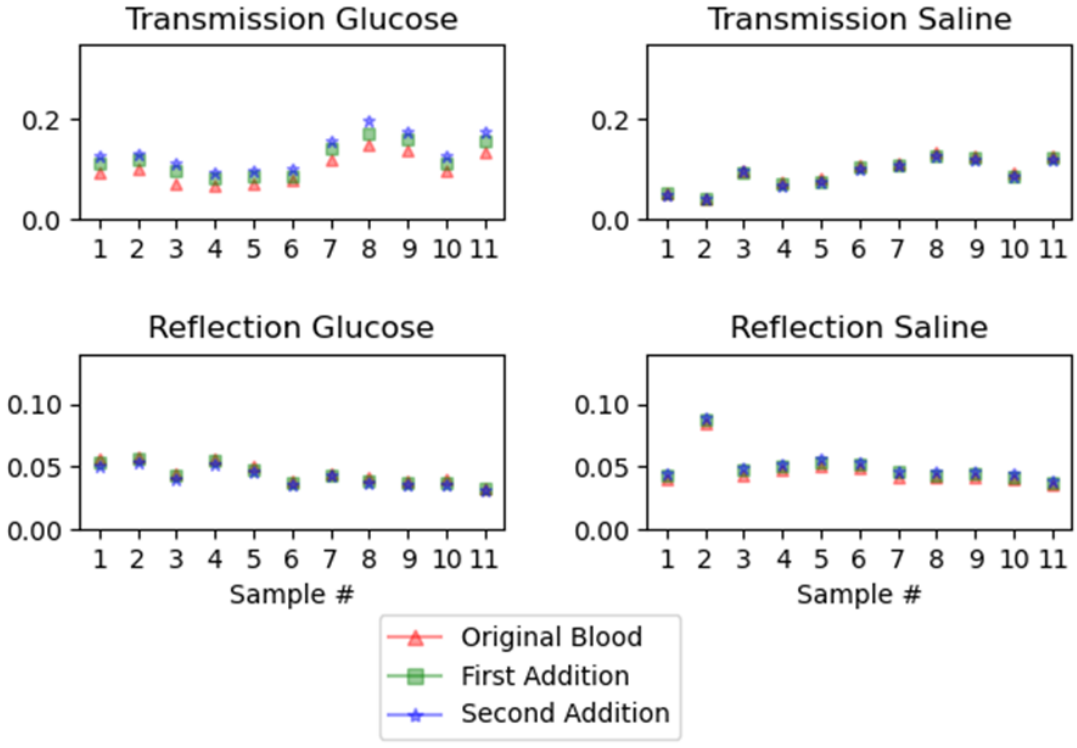
f)


1600 nm
Transmission Glucose
Transmission Saline
Reflection Glucose
Reflection Saline
0.2
0.0
0.10
0.05
0.00
1 2 3 4 5 6 7 8 9 10 11
Sample #
Original Blood
First Addition
Second Addition

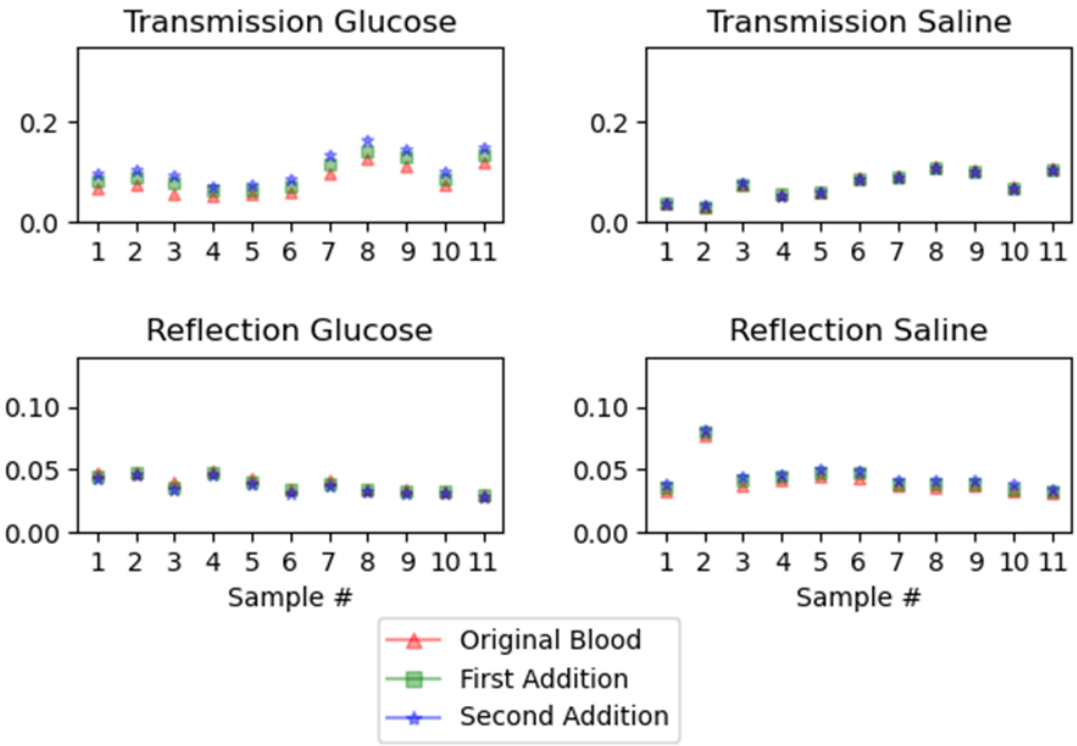
g)


1700 nm
Transmission Glucose
Transmission Saline
Reflection Glucose
Reflection Saline
0.0
0.2
0.00
0.05
0.10
1 2 3 4 5 6 7 8 9 10 11
Sample #
Original Blood
First Addition
Second Addition

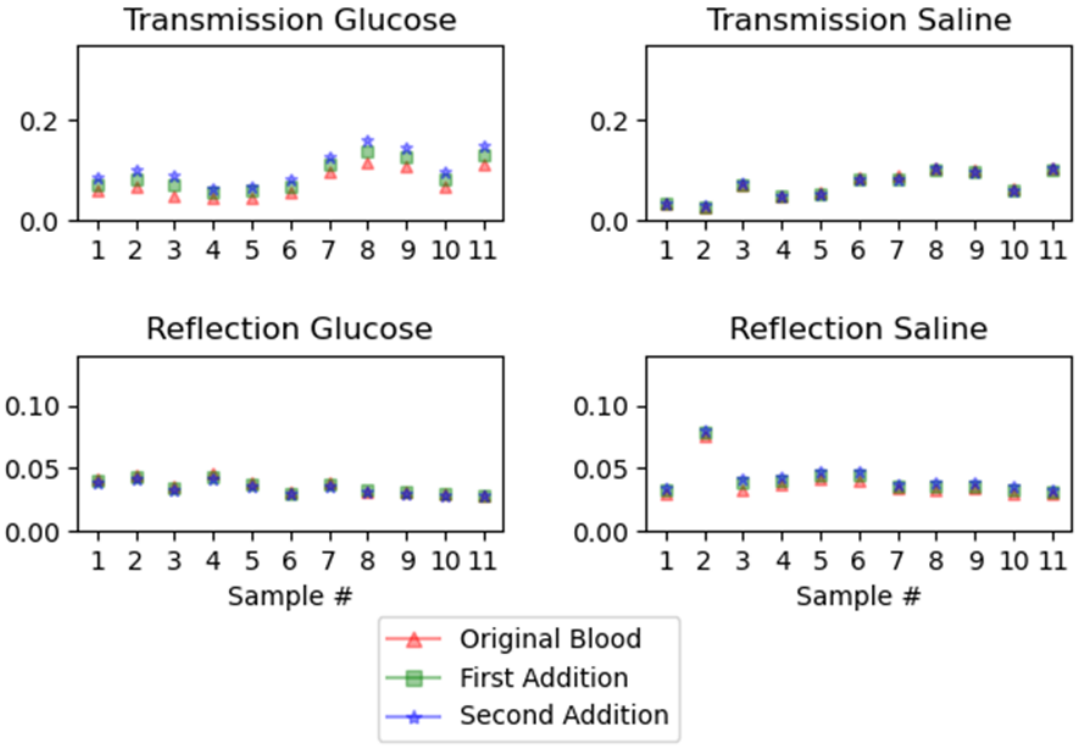
h)


1800 nm
Transmission Glucose
Transmission Saline
Reflection Glucose
Reflection Saline
0.0
0.2
0.00
0.05
0.10
1 2 3 4 5 6 7 8 9 10 11
Sample #
Original Blood
First Addition
Second Addition

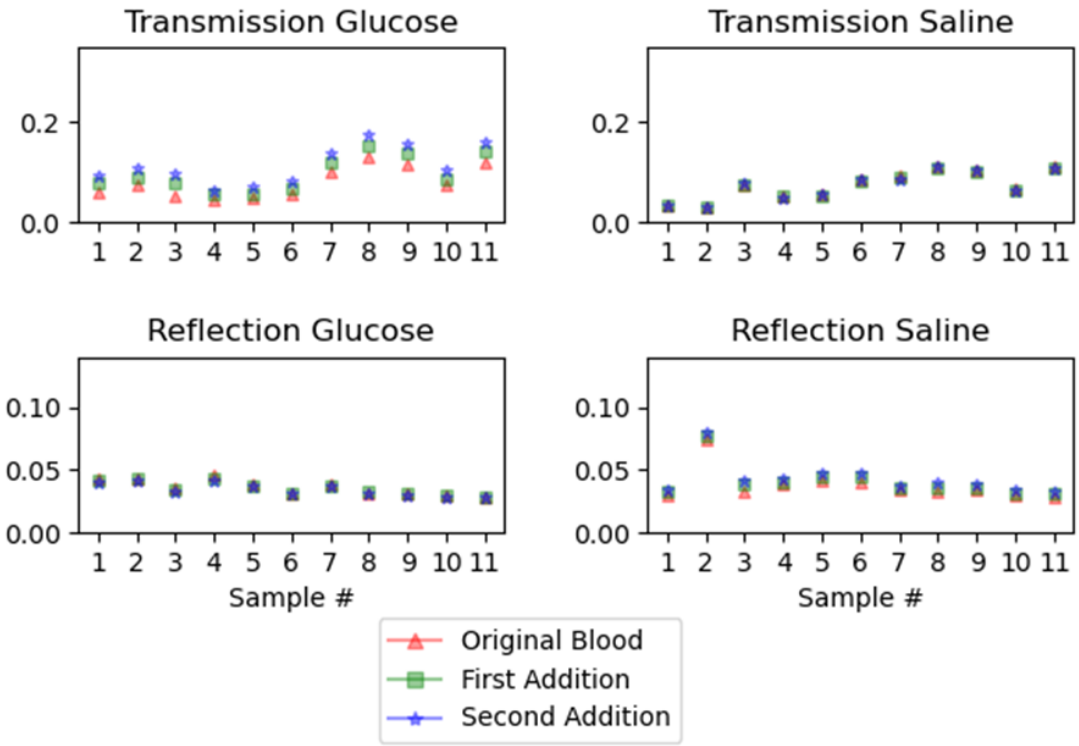
i)


1900 nm
Transmission Glucose
Transmission Saline
Reflection Glucose
Reflection Saline
0.0
0.2
0.00
0.05
0.10
1 2 3 4 5 6 7 8 9 10 11
Sample #
Original Blood
First Addition
Second Addition

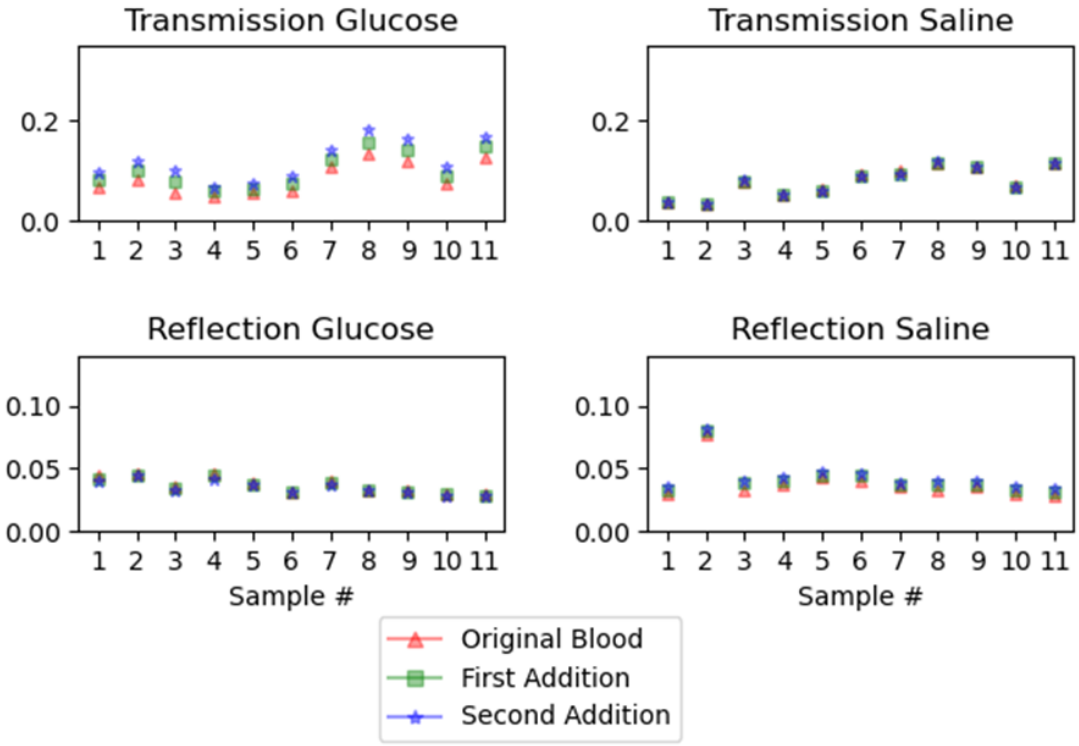
j)


2000 nm
Transmission Glucose
Transmission Saline
Reflection Glucose
Reflection Saline
0.0
0.2
0.00
0.05
0.10
1 2 3 4 5 6 7 8 9 10 11
Sample #
Original Blood
First Addition
Second Addition

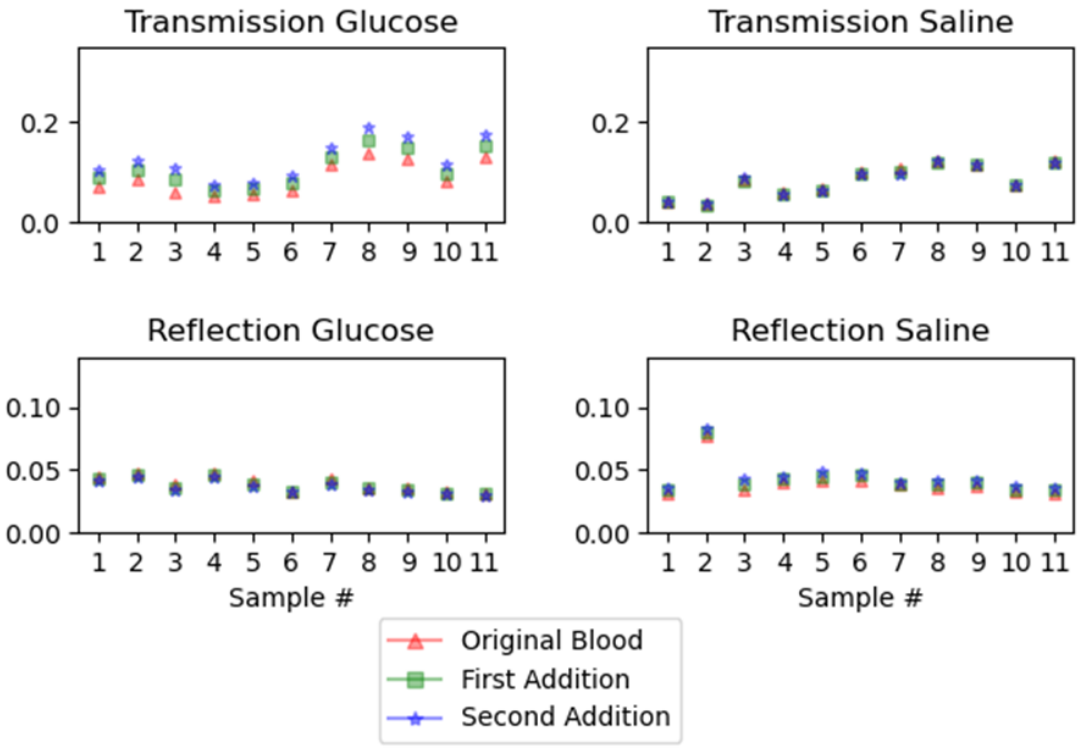
k) 2100 nm



l) 2200 nm



m)


2300 nm
Transmission Glucose
Transmission Saline
Reflection Glucose
Reflection Saline
0.0
0.2
0.00
0.05
0.10
1 2 3 4 5 6 7 8 9 10 11
Sample #
Original Blood
First Addition
Second Addition

n)

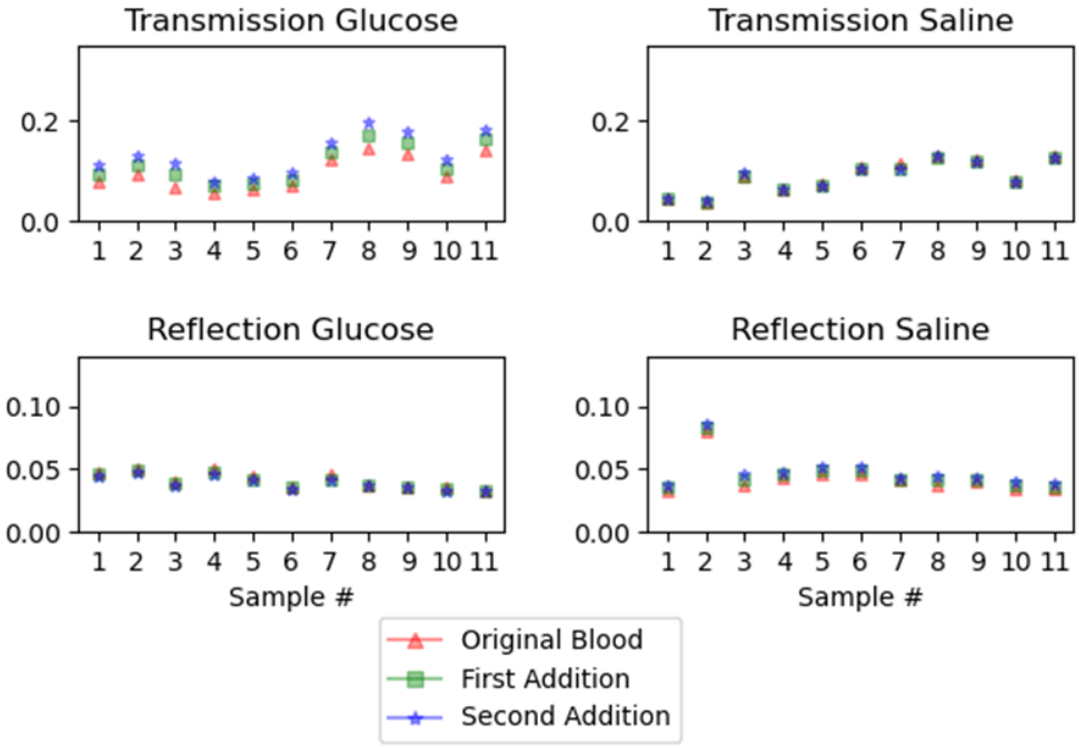

2400 nm
Transmission Glucose
Transmission Saline
Reflection Glucose
Reflection Saline
0.0
0.2
0.00
0.05
0.10
1 2 3 4 5 6 7 8 9 10 11
Sample #
Original Blood
First Addition
Second Addition

Figure 16 Using the means from the time data, these plots were compiled representing the eleven samples at 2300 nm. It can be observed that reflection and transmission varied largely from individual to individual but trends in the transmission experiments and trends in the reflection control were matched across all samples.

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