

Investigation of the use of Raman Spectroscopy for Non-invasive Glucose Calculation in Blood

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By

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Abstract

Investigation of the use of Raman Spectroscopy for Non-invasive Glucose Calculation in Blood

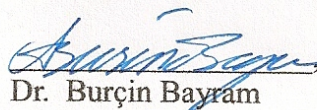
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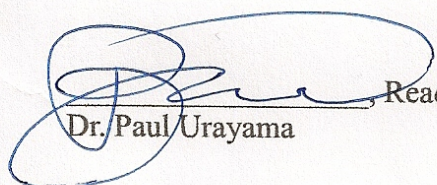
According to a study by the American Diabetes Association, 171 million people or 2.8% of people in the world suffered from diabetes. It is important to get more information about blood sugar levels which would allow them to better control their blood sugar levels and help reduce complications. This project was motivated by similar work recently studied by a MIT group. This project uses a simulation of Raman spectroscopy to calculate the glucose content of the simulated blood. Specifically, we back out how much glucose is in our simulated sample and quantitatively understand error within the analysis.

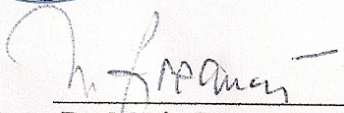
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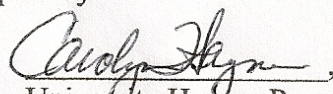
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1. Introduction

According to a study by the American Diabetes Association, 171 million people or 2.8% of people in the world suffered from diabetes. This number is expected to rise to 4.4% of the population by 2030 due to an increase in the number of seniors. [1] To measure blood glucose levels, a diabetic must prick their finger to extract blood. Depending on the type of diabetes, this has to be done once a week or up to six times a day. Each test strip can cost up to \$1.00 each. The estimated total annual cost of diabetes is \$132 billion. [2]

1.1 Non-invasive measurement

Measuring biological samples in a noninvasive manner has always been a goal of science. It is possible to measure the oxygen levels with pulse oximetry [3]. This method involves measuring the difference between the oxygen (which is made fluoresce with a light) at during and after a heartbeat. This is when there is a lot and very little blood in the blood vessels respectively. Many other measurements involve taking a sample. For example, measuring cholesterol levels requires a blood sample. This is not too much of an inconvenience because it is only done once a year. For some diseases such as diabetes, once a year is not sufficient. It would be a novel technique to accurately measure blood sugar levels using a non-invasive measuring tool.

More information about blood sugar levels would allow diabetics to better control their blood sugar levels which would help reduce complications. This is especially true for teenagers with diabetes who are notoriously bad at controlling blood sugar. Complications include diabetic comas, cardiovascular disease and, in untreated cases, diabetic ketoacidosis which can all result

in death. A non-invasive way to measure blood sugar would allow for the earlier diagnosis of diabetes through simple screenings at doctors' offices during check-ups. This could save a lot of healthcare costs because the earlier diagnosis would serve to mitigate the need for extended hospital stays.

A continuous non-invasive measurement would allow for an artificial external pancreas. Insulin pumps currently need user input about how much they eat and what their current blood sugar level is. With a continuous measurement of blood sugar, they would read the blood glucose levels and then automatically adjust insulin pump rates.

1.2 Introduction to Raman Spectroscopy

The frequency of a photon given off an atom is determined by the energy difference between the atomic energy levels. A photon of the right frequency will excite the atom into the higher energy level.

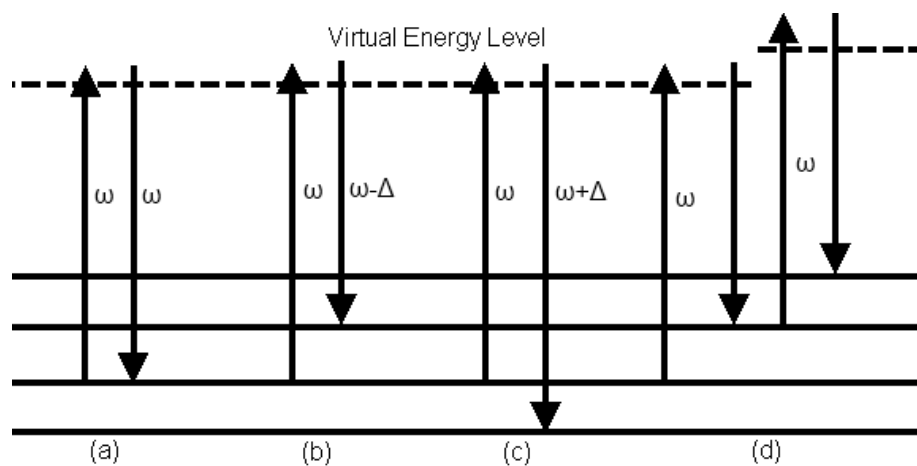


Figure 1. A diagram representing (a) Rayleigh scattering, (b) Raman Stokes scattering, (c) Raman anti-Stokes scattering, and (d) a two-photon Raman scattering process.

Raman spectroscopy works by inelastic scattering of the photons hitting the molecule. The light raises the molecular energy level to a virtual energy level and then it decays to a various energy levels. If the frequency of the scattered light is shifted towards the red then it is called a Stokes Scattering. If the frequency of the scattered light is shifted towards the blue then it is called anti-Stokes scattering.

Each molecule has its own characteristic profile and so this allows one to differentiate the type of molecules under consideration.

2. Application of Raman Spectra Measurement to Glucose Content

Measuring the amount of scattered light can tell us how much of each type of molecule exists in our sample. Specifically, such a measurements lets us back out the amount of glucose in our sample. The Raman spectrum of glucose is shown in Fig. 2 [4].

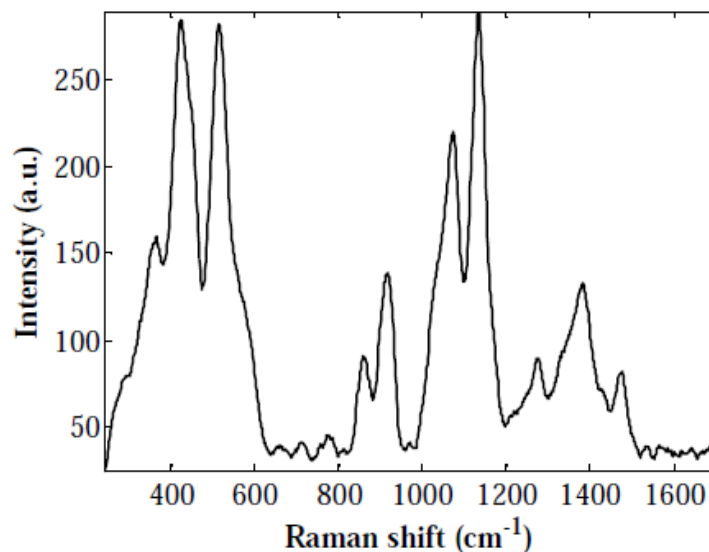


Figure 2: Raman spectrum of glucose water solution [4]

Fig. 2 shows the feasibility of Raman peaks in near infrared laser absorption. The

intensity of the Raman peaks is proportional to concentration of glucose in the beam path as well as intensity of laser. The frequency of the beam also affects the strength of the signals because some frequencies of glucose are more absorbing than others.

Near-infrared has three advantages to other frequencies. Due to its low energy it does not ionize molecules in the skin. For a system to be a practical continuous monitor, the risk of cancer needs to be mitigated. [4]

Second, infrared has a millimeter to centimeter penetration range due to reduced elastic scattering. This is because elastic scattering is less at longer wavelengths. Third, it has reduced background fluorescence. [4]

The measurement is made more difficult when measuring glucose in human skin because absorbers in the skin. Skin has many light absorbers including water, fat, hemoglobin, and skin melanin. It also fluoresces and proteins and lipids in the skin and blood will give off their own Raman signals. Finally skin is inhomogeneous which means some parts could have more blood and some parts less. This means that you have to be sure of where you're aiming at. [4] All of these factors complicate the measurement of glucose in humans.

2.1 Measuring Glucose

The intensity of the Raman peaks from glucose are directly proportional to the concentration of glucose. This relation is linear in the normal range for diabetics, 40 mg/dl (2.2 mM) to 300 mg/dl (16.7 mM) [5]. Fig. 3 shows how Raman peaks from glucose as a function of concentration measured in millimole. Millimole is the number of molecules of glucose per cubic meter.

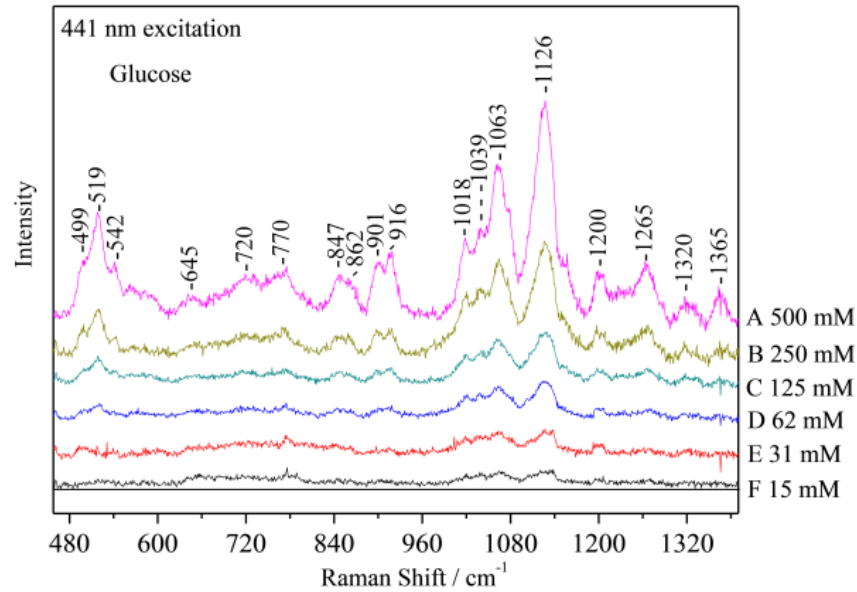


Figure 3: This graph shows the Raman peaks changing in size purely by changing glucose concentration. 15 mM corresponds to 270 mg/dl and 500 mM corresponds to 9000 mg/dl. [5]

According to Fig. 3, the intensity of the Raman shifts increases as concentration increases.

Normal blood glucose concentration is from 80 mg/dl to 180 mg/dl. Low blood sugar is anything below 80 mg/dl. Low enough blood sugar can cause a person to faint and perhaps die if left untreated. High blood sugar is 180 mg/dl and above. High enough blood sugar can lead to diabetic ketoacidosis as well as diabetic coma.

2.2 Absorption

Absorption is governed by Beer's law,

$$I(z) = I_0 \exp(-a(\omega)z) \quad [\text{eq 1}]$$

where a is the frequency dependent absorption coefficient, ω is the incident frequency, z is the distance, and I and I_0 are the intensity and initial intensity respectively. Skin components' absorption ranges from 0.005-3000 /cm. [4,6] The laser frequency is based on where water, hemoglobin, melanin and fat absorb the least. This forms a so-called absorption “window.” See Fig. 4.

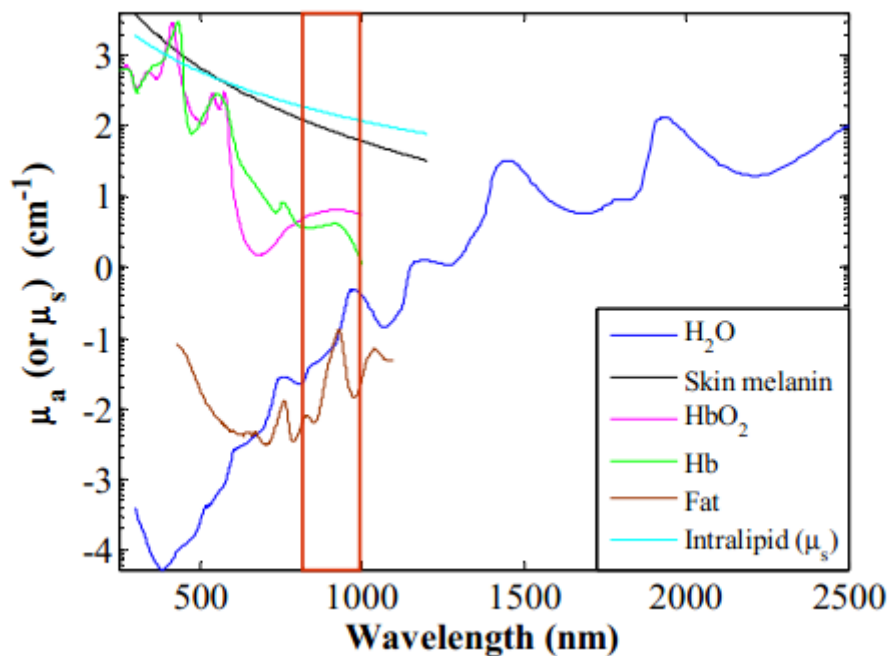


Figure 4: this figure demonstrates the absorption characteristics of components of skin. From [4,8]

2.3 Fluorescence Measurement

Fluorescence caused by the plethora of molecules in skin such as by the proteins and lipids in the skin. Fluorescence causes an increase spectrometer shot noise. [4] The fluorescence can be modeled as a fifth order polynomial however removing it from the spectrum can be

difficult due to the introduction of spectral artifacts. [4]

2.4 Accuracy of Test Strips

Test strips are the standard way to measure blood sugar in diabetes patients. One Touch Ultra Blue Test strips mix a small blood sample with special chemicals that produce a small current in proportion to the glucose concentration. This current is measured to give a value for blood sugar level. The meters can measure glucose from 20 mg/dl to 600 mg/dl. [7]

The One Touch insert reports that meters “should agree within 15 mg/dl of a laboratory method when concentration is lower than 75 mg/dl and within 20% of a laboratory method when glucose concentration is 75 mg/dl or higher.” [7]

Their results from 100 subjects show that 100% of their values fall within 15 mg/dl when glucose concentrations are less than 75 mg/dl. 48.8% fall within 5 mg/dl and 84.5% fall within 10 mg/dl. For above 75 mg/dl, 38.0% fall within 5% of the laboratory test, 68% fall within 10%, 88.2% fall within 15%, and 95.7% fall within 20%.

To create a non-invasive device, the device should have comparable statistics. That is, the standard deviation should be less than 6.6% because three standard deviations usually cover 96%.

2.5 Clarke Error Grid

A Clarke error grid allows one to measure the effectiveness of new glucose measurement method by comparing it with a reference method. In a plot of the new device vs. a reference method, the plot is subdivided into five regions. Each regions represents a quality [2,8]. Region

A represents all the new device values that are within 20% of the reference method value. Region B represents values that are not within 20% but are not bad for the patient. Region C values lead to unnecessary treatment of high or low blood sugar. For example, the device says the patient has low blood sugar when they actually have blood sugar in a normal range. Figure 5 shows a Clarke Error grid [9].

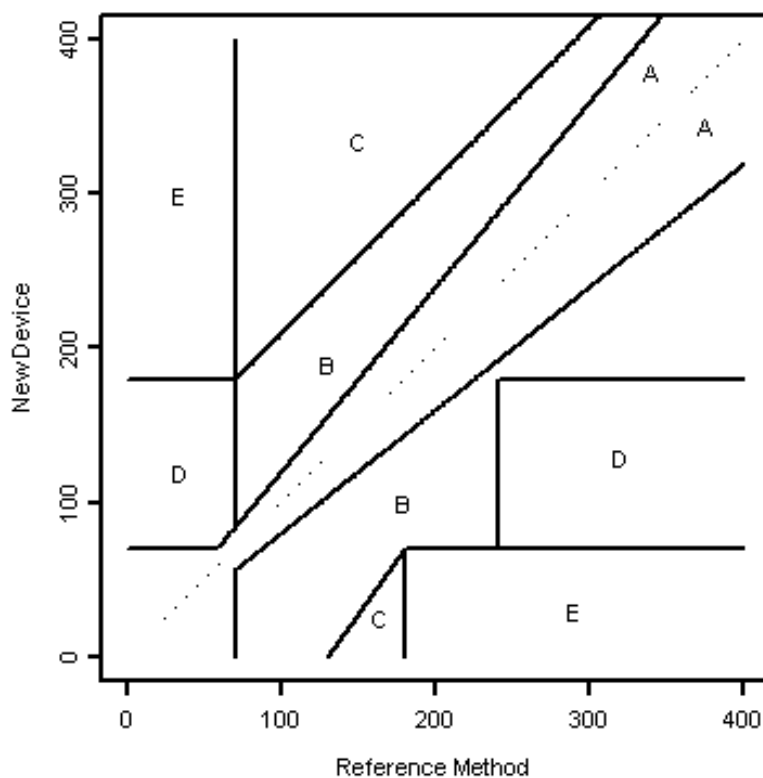


Figure 5: A Clarke error grid.

Region D values are values that fail to detect high or low blood sugar. This could potentially be life threatening for the patient and should therefore be avoided. Region E is even worse because it represents values where the new device predicts low blood sugar but the patient

actually has high blood sugar, and vice versa. This would lead the patient, for example, to treat themselves for high blood sugar by injecting insulin but they would only worsen their low blood sugar. This could lead to death.

2.6 Non-invasive Methods of Measuring Glucose

Several methods have been proposed to varying degrees of accuracy. The different methods are developed to overcome problems of differing fluorescence between individuals, to get better signal to noise ratios with the glucose, and, in some cases, to provide a reliable testing method. These methods are pulse glucometry [3], stimulated Raman spectroscopy [10], two wavelength excitation [11], and surface enhanced spectroscopy [2,12]. A new method, which still needs to be developed and tested on people, relies on giving the patient a tattoo of carbon nanotubes [13].

Pulse glucometry measures the Raman spectrum continuously and keeps track of the patient's pulse at the same time [3]. As the heart beats, the blood level fluctuates. This gives two measurements. One where a lot of blood is measured and one where very little blood is measured. This difference gives one the spectrum from only the blood and effectively filters out the fluorescence from the skin. This technique is similar to pulse oximetry which is used to measure blood oxygen levels.

A similar effect can be achieved by pressing down on the measurement area for a measurement. This produces a signal without much blood. This can be used to subtract from a signal with blood to get only the blood's signal [4]. Shih, et al. also report measurement using blood serum samples taken from subjects. These samples were measured unprocessed, unfiltered,

and filtered. Measurements of whole blood were also taken as well as simply measuring from the skin of subjects. A table of the accuracy of different measurement techniques is listed below.

Source		Error (mg/dl)	Reference
Yamakoshi, et al.	Method A	22.3	[3]
	Method B	21.9	
Shih, et al.	Serum: unprocessed	27	[4]
	Unfiltered	17	
	Filtered	6	
	Whole Blood	22	
	Tissue Modulation	22	

Table 1: a table of the various accuracies of the different methods for measuring blood glucose levels

According to the table, one can see that the error on using the Raman spectrum directly from people's skin is at about 22 mg/dl. On the other hand, removing the blood and then filtering it so that only the glucose and other molecules remain, can reach an error of 6 mg/dl.

3. Experiments

3.1 Optical Phantom

We set out to create realistic samples that simulate the optical properties of blood and skin tissue. These are called optical phantoms. These phantoms would allow for controlled experiments. It is possible to create realistic samples that simulate the optical properties of blood and skin tissue. These are called optical phantoms [14,15,16]. Using optical phantoms it is also possible to practice coaxing out the glucose Raman signal since one could know the original glucose concentration. It would give repeatable glucose levels and tunable variables such as the

thickness of the skin. Optical phantoms can be made from something as simple as milk mixed with water and a sample. Skin can sometimes be simulated with jello. [17] In the past, optical phantoms have been made from gelatin [14], absorbing dyes [15], and silicon dioxide [16] for the purposes of simulating human tissue and blood.

3.2 Glucose Hydrolysis

Optical phantoms are difficult to produce in the laboratory because glucose suffers from hydrolysis. This means that glucose decomposes into fructose, acetic acid, humic solid and gaseous products. Depending on temperature and acidity, glucose can reach 80% of the original levels in 10 min. [18]

3.3 Raman Spectrum

In order to understand the Raman scattering spectroscopy, we used liquid nitrogen excited with a 532 nm Nd:YAG pulse laser. An ocean optics spectrometer was set at 90 degrees from the laser beam, see Fig 6.

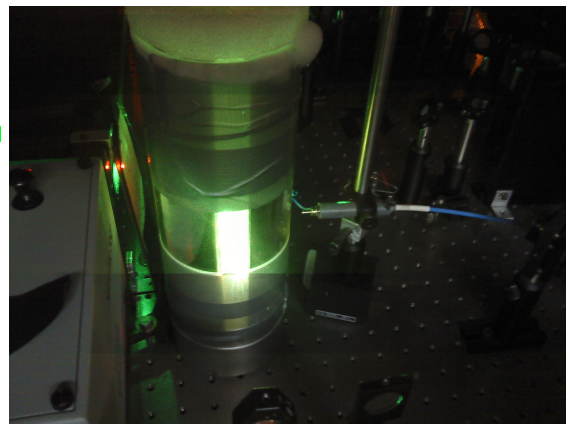
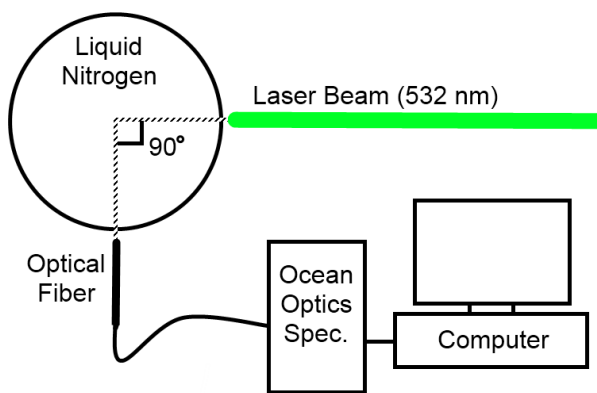


Figure 6: The experimental setup (left) and a picture of the setup from our laboratory (right). The

laser is coming in from the bottom of the figure and the fiber is on the right. The liquid nitrogen is glowing due to intense scattering.

Due to the simplicity of the N_2 molecule compared to glucose, the liquid nitrogen spectrum is very simple yet it provides a strong Raman signal for study.

Raman Scattering Spectrum for N2 (Pump 0 cm^{-1})

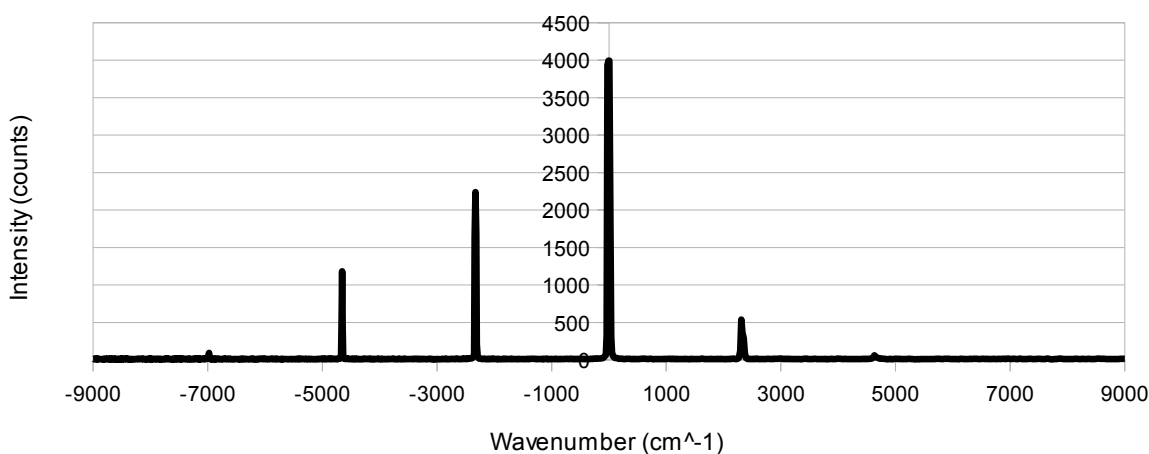


Figure 7. Our data in wavenumbers centered around the pump laser's wavenumber.

In Fig. 7, we can see the Stokes lines are on the left and the anti-Stokes lines are on the right.

3.4 Simulation of Glucose Spectrum

Instead of trying to find a solvent that would allow for a more stable glucose concentration or trying to take the measurements quickly, we created a simulation of the spectrometer. This gives the advantage of repeatability as well as the ability to qualitatively understand error. Simulation allows us to deterministically, repeatably and independently change one variable at a time.

A Raman peak is given by the Lorentzian function,

$$f(\omega) = I \left[\frac{\gamma^2}{(\omega - \omega_0)^2 + \gamma^2} \right] t$$

where I is the intensity per second, γ is the half-width at half-max (HWHM), t is the integration time, and ω is the center frequency. [19]

The Raman signal for the glucose was taken from [4]. Using a photo-editor, the x and y values of the fifteen most intense peaks were copied down and calibrated using the axis of the image. This was then converted into intensity and frequency for the Lorentzian using python code.

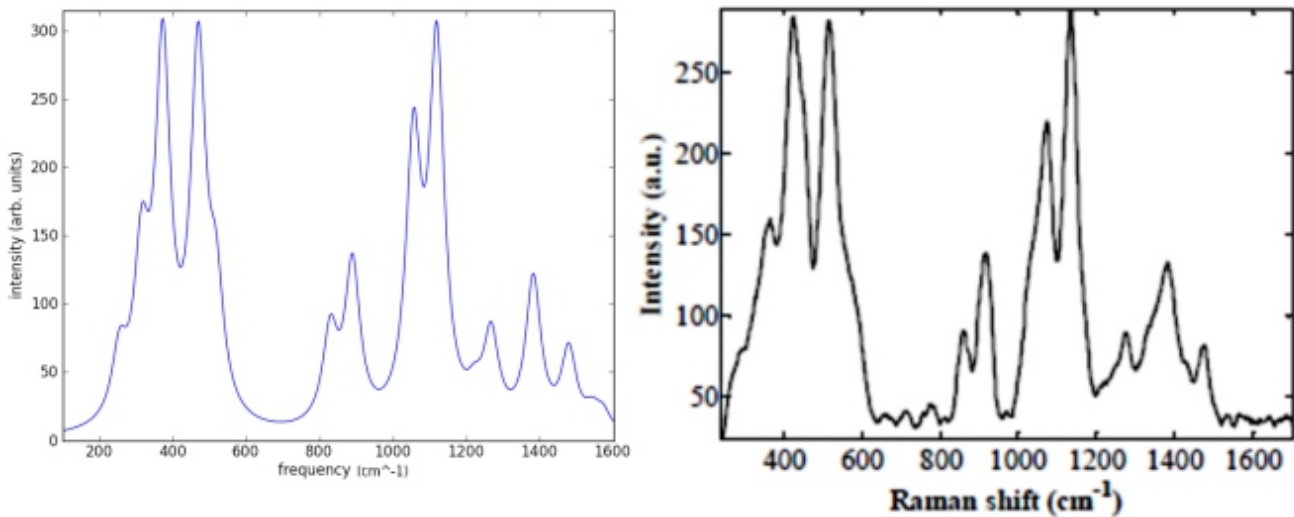


Figure 8: A comparison of the simulated glucose on the left and the actual glucose on the right [4].

We also have several Raman peaks that are not related to glucose levels. These are

Raman peaks that are caused by other molecules in the blood and skin. We can even generate random Raman peaks that are different for each measurement.

The fluorescence is treated as a fourth order polynomial, $I(\omega) = (a_1 + a_2\omega + a_3\omega^2 + a_4\omega^3 + a_5\omega^4)t$. Where a_i are the coefficients of the polynomial, and t is the integration time. The absorption coefficients for each frequency are given by a simple $a(\omega) = a_0 + a_1\omega^2$, where a_0 and a_1 are chosen such that $a(\omega)$ varies from 250 /cm to 275 /cm. This range was chosen based on the absorption window characteristics. The intensity of each frequency is lowered by Beer's law, $I(\omega) = I(\omega) \exp(-a(\omega)z)$, where z is the absorption length.

3.5 Parameters for Noise and Fluorescence Comparison

We can change a number of variables in our simulation. The absorption length, integration time, and the fluorescence. We can also change the initial and final frequencies and the change in frequency. The frequencies usually go from 100 cm^{-1} to 1600 cm^{-1} with a change of 1 cm^{-1} .

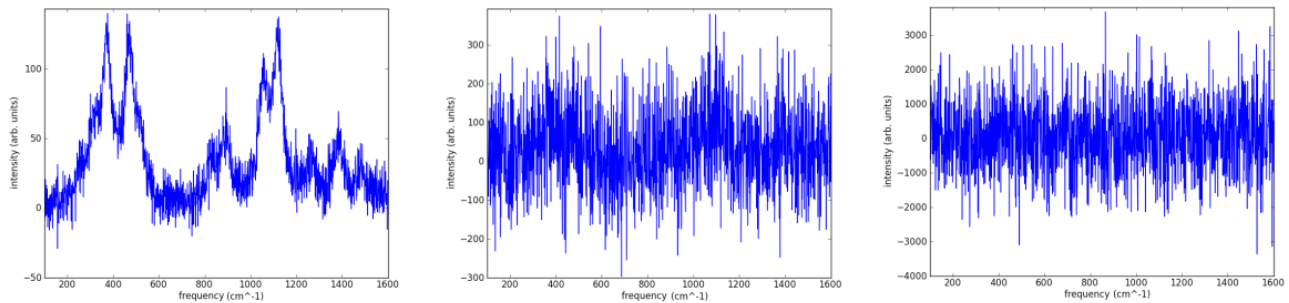


Figure 9: A comparison of the noise. The noise level is 10, 100 and 1000 respectively from left to right.

In fig. 9, we can see the effects of noise on our simulated glucose spectrum. The peaks are visible in the 10 arb. units of noise level. The peaks are washed out to the naked eye when the noise level is increased to 100 arb. units and 1000 arb. units however with careful glucose calibration, it is possible to still make an accurate estimation of glucose levels.

3.6 Calibration

To create the regression vector, each frequency was treated as its own linear function,

$$I(\omega) = m(\omega) * g + b(\omega)$$

where I is the intensity measured by the spectrometer at frequency ω , m is the glucose dependence at the current frequency, g is the glucose level at the current frequency, and b is the florescent background. Doing a linear regression on each frequency gives several different values for m and b . This allows for several predictions for the glucose level to be made. Each separate prediction is given by,

$$g = \frac{I(\omega) - b(\omega)}{m(\omega)}$$

We can then take a weighted average of each glucose prediction to obtain a prediction for the glucose level. The weighting, in this case, is done by taking the average r^2 value of each linear regression. If the r^2 value of a particular linear regression is less than the average linear regression then that glucose value is given a weight of zero and so is not considered in the weighted average. If the value is more than the average then it is given a weight of 1. The weighted average is,

$$g = \frac{\sum a_{\omega} g_{\omega}}{\sum a_{\omega}}$$

where a_{ω} is the weight at frequency ω and g_{ω} is the predicted glucose at the frequency. We also considered making the weights above the average the difference between the maximum and the average such that average would be 0, the maximum would be 1 and the halfway point between the average and maximum would be $\frac{1}{2}$ but this was changed in favor of simplicity.

The r^2 value represents the ratio squared of the accuracy between a line whose slope is 0 with a y-intercept of the average, with the linear regression formula for the line.[20] It was chosen as a weight because it captures the fact that some frequencies do not vary as much with glucose levels than other frequencies. This means that those frequencies are more susceptible to variances due to noise. For example, if the noise is 10 units and we have two frequencies to consider. One frequency changes in intensity by 10 units with a glucose change of 10 mg/dl. So this frequency has a signal to noise ratio of 1. The other frequency changes by 40 units with a glucose change of 10 mg/dl. So this frequency has a signal to noise of 4. This means that its linear regression will be more accurate. This means it will have a better r^2 value.

4. Data

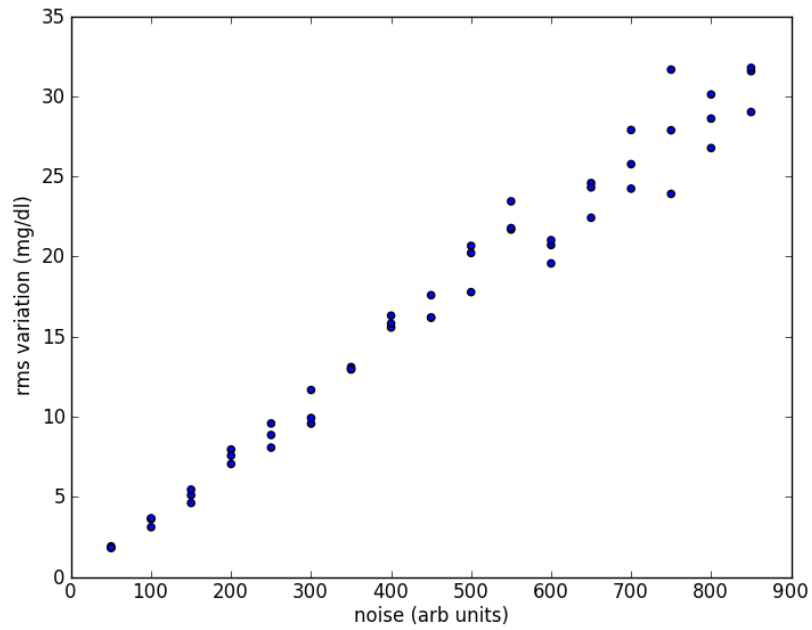


Figure 10: Root mean square deviation vs. noise. The highest glucose peak is about 120 arb. units tall. This was 3 samples per noise level, with noise levels varying from 50 to 850 in steps of

50.

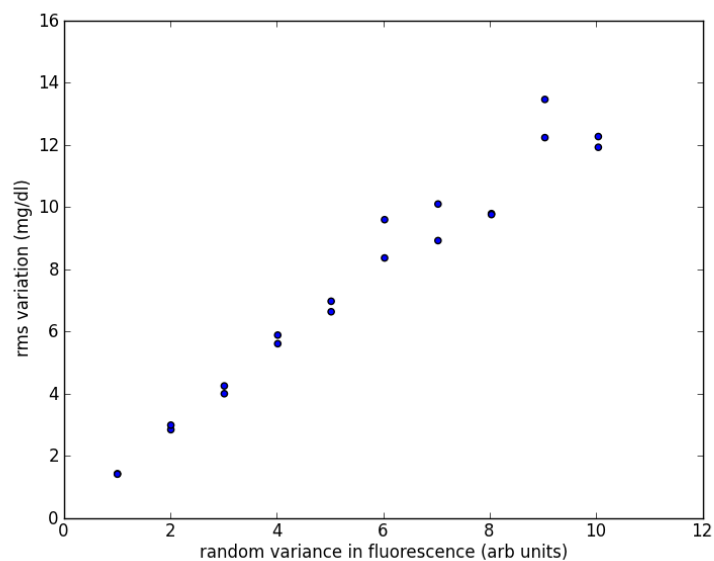


Figure 11: Root mean square variation vs. Random variance in fluorescence. The slope is 1.2414 mg/dl per arb unit of random variation.

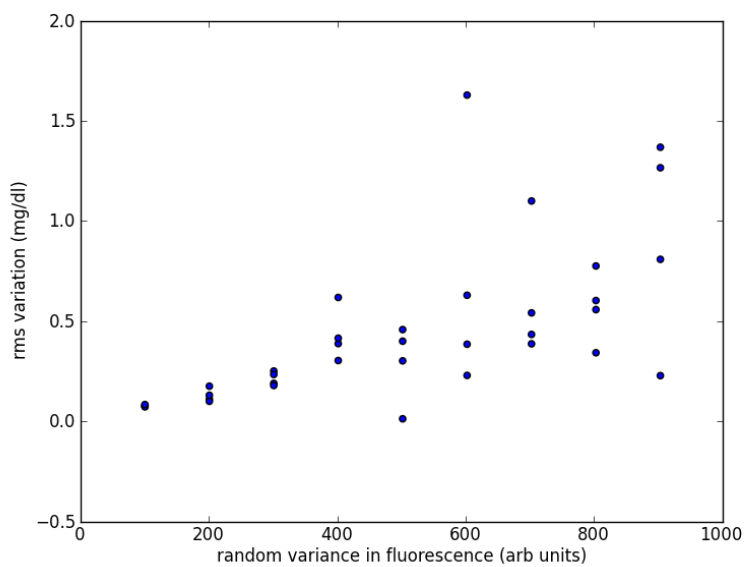


Figure 12: Root mean square variation vs. Random variance in fluorescence. The slope is about 9.5825×10^{-4} mg/dl per arb unit of random variation, 1306 times better than Fig. 11.

5. Discussion

We can see that fluorescence differences between people can be filtered using a high pass filter in the Fourier transform of the spectrum. The result is 1306 times better. In Fig. 13, we can see why this happens. The high-pass filter filters out the differences in fluorescence so that we are left with almost overlapping spectra. In Fig. 13 d) we can see that they spectra overlap almost perfectly whereas in Fig. 13 c) the variance is enough to drown out the glucose signal.

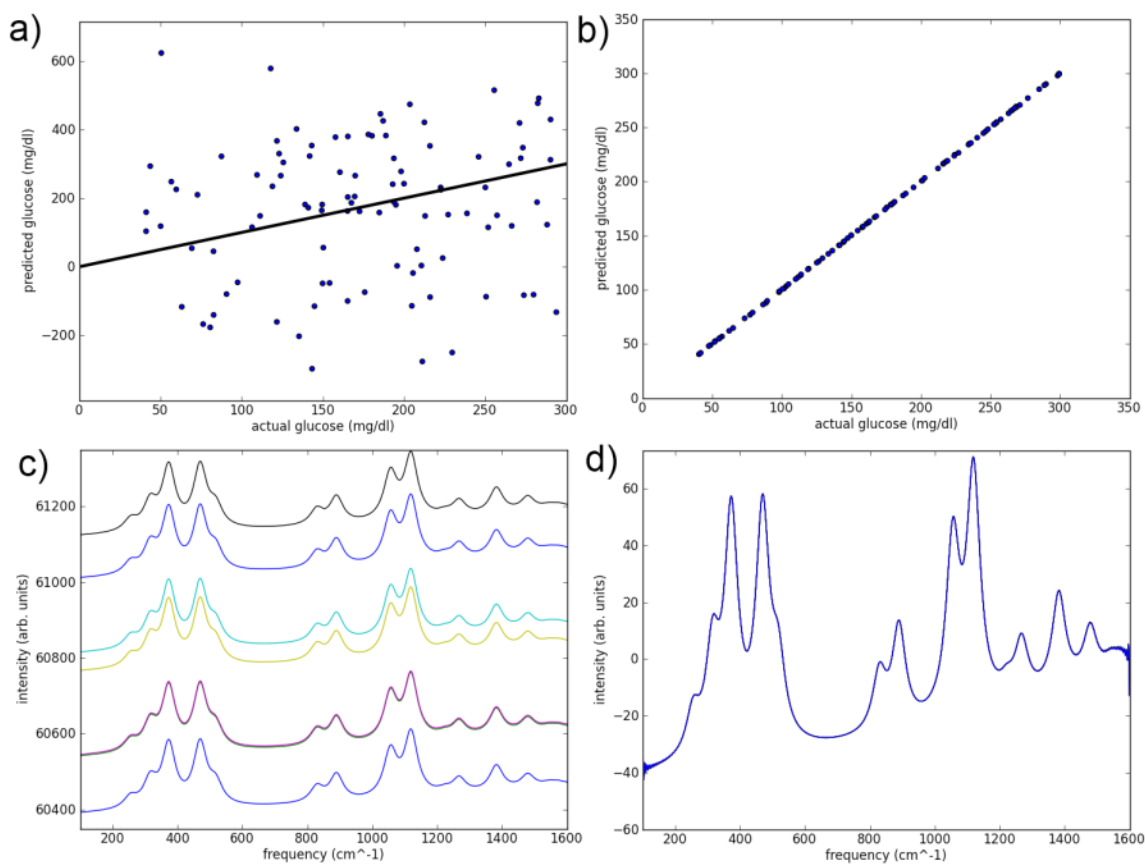


Figure 13: a) predicted vs. actual glucose without Fourier high-pass filter. The black line represents a perfect match b) predicted vs. actual glucose with Fourier high-pass filter applied to the spectrum before processing. c) eight different glucose spectra with an average variance in

fluorescence of 500 arb. units. This corresponds to the data of a). d) eight glucose spectra after filtering with a high-pass filter. The spectra are almost perfectly overlapped and the data corresponds to the graph of b).

We can see the Fourier high pass filters work well to reduce the effects of fluorescence. To demonstrate on real spectra, we took the following absorption spectrum of molecular iodine measured with a white Tungsten lamp.

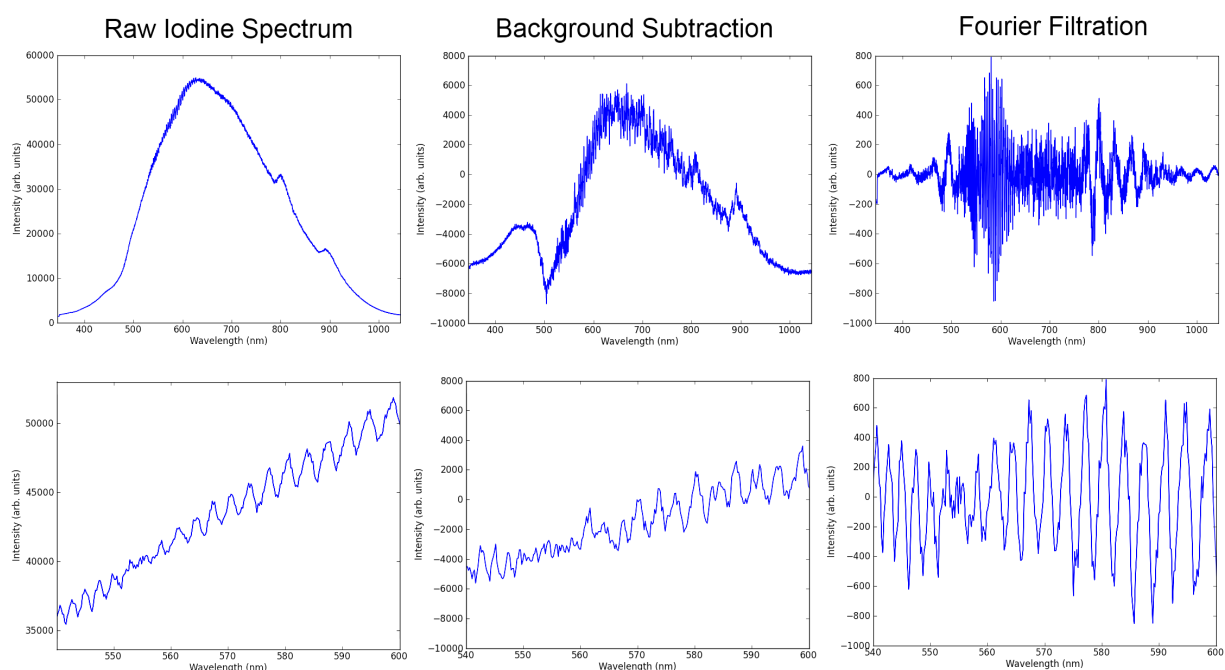


Figure 14. The absorption spectra of molecular iodine using different techniques. The bottom row represents the same spectra but zoomed in from 540 nm to 600 nm.

Note that the background is filtered out much better when using Fourier filtration compared to when we take the spectrum without the iodine present and then subtract that background. With the subtraction we are left with a linear signal in the 540 nm to 600 nm range that could change the calibration. Also note that the height differences of the absorption peaks is

preserved in the Fourier filtered data in Fig. 14.

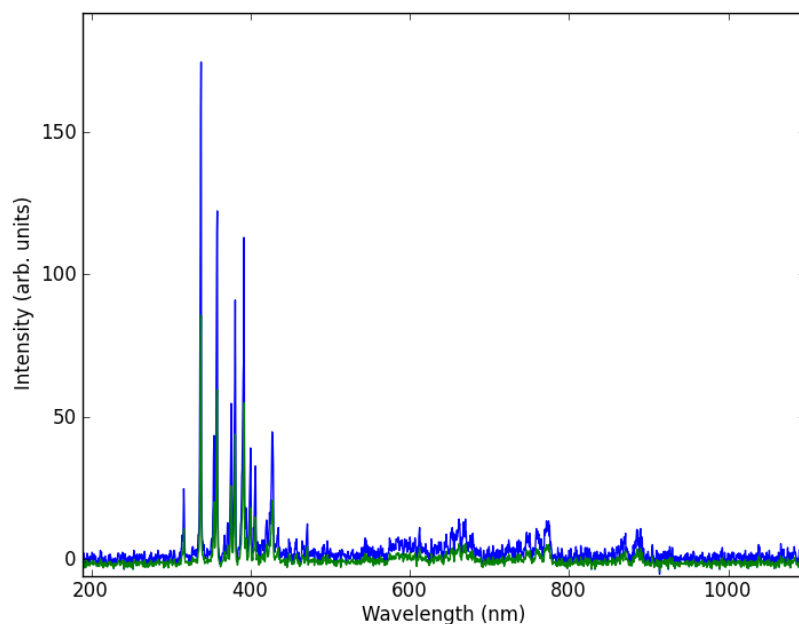


Figure 15. N_2 spectrum before (blue) and after filtration.

Using data from nitrogen ions, we find that the Fourier filtration does not substantially effect the Raman peaks. Instead, the filter gets rid of the offset.

6. Conclusion and future direction

More work needs to be done into understanding where to filter the Fourier transform. Too much filtering can remove important data. When combined with noise the quality of the filtration goes down as if some of the data is being lost. Picking the filtration cutoff frequency more carefully in the future could perhaps give better results. More work also needs to be done to understand the cause of the outliers in Fig. 12.

Creating a more accurate simulation of skin is an important step for the future. It would be interesting to see how the simulation of different layers of the skin might effect the results. The simulation in its current form relies on a net fluorescence and a net absorption as a result of skin. Instead, different layers would allow for better understanding of where the ideal test location is. It would answer questions such as, what variance in skin thickness is acceptable. A more accurate simulation might also take into account changes in glucose levels during the time that the spectrometer is taking data.

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