Research in Biomedical Optics

Raman spectroscopy for measurement of blood analytes

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Background

Measurement of the concentrations of blood analytes presently requires withdrawal of one of more blood samples and a measurement process which often involves sample handling, such as serum extraction, addition of various reagents and a delay in the diagnosis process. Withdrawal of blood exposes personnel to biohazards and causes inconvenience and pain to the patient. A non invasive measurement would revolutionize medical diagnosis by providing analytes concentrations quickly, painlessly and without the use of reagents. A non-invasive measurement would be particularly beneficial where the results are needed quickly or where measurements must be taken frequently. An obvious example of this is the measurement of glucose concentration. Millions of people with diabetes must measure their glucose level multiple times per day to maintain their glucose level within prescribed limits so as to reduce the serious long term consequences of this disease. Non-invasive measurement of glucose is a goal of many institutions. Many technologies are being investigated to reach this goal. Among them are absorption spectroscopy, both by diffuse reflectance and transmission, light polarization and light scattering.

Over the past several years, we have been investigating the use of near-infrared (NIR) Raman Spectroscopy for measuring the concentrations of blood analytes. The ultimate goal of this research is to develop a transcutaneous method of measuring clinically important analytes in the blood-tissue matrix by developing a basic understanding of the scientific and engineering issues involved.

The strength of Raman spectroscopy lies in its sharp spectral features, characteristic for each molecule. This strength is ideally suited to blood analyte measurements, where there are many interfering spectra, many of which are much stronger that that of blood analytes. Raman's sharp spectral features enable detection of blood analyte spectra among these strong interfering spectra and in the presence of a large fluorescence background. Figure 1 shows the sharp spectral features of the glucose spectrum and how it is differentiated from the spectra of the many components that exist in skin.

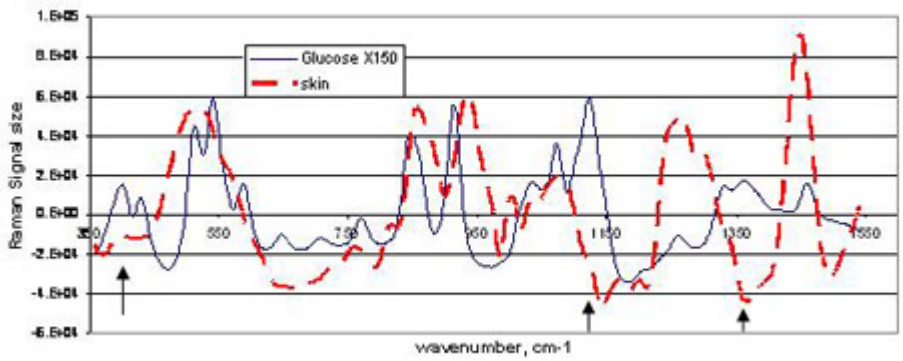

Figure 1. The distinct spectral features, characteristic for a back-ground subtracted Raman spectrum, are here exemplified by a spectrum of glucose dissolved in water. The typical Raman spectrum of skin is shown for comparison with arrows indicating regions that clearly differentiate glucose from the other components in skin. To allow comparison, the glucose spectrum shown is 150 times the estimated size of the spectrum that would be received from a concentration of 5mM glucose in human skin.

However, to measure glucose in tissue is far more complex than indicated by this figure for various reasons: a) the spectra from typical physiological concentrations of glucose in skin are in the order of several hundred times lower than the total spectrum from skin, as shown in an example in Figure 1, b) even with excitation at 830nm, the fluorescence background dominates the signal, as can be seen in figure 2, c) Raman peaks from all other biomolecules that are present overlap and therefore interfere and d) the optical properties of the tissue as well as the probe depth/volume influence the signal.

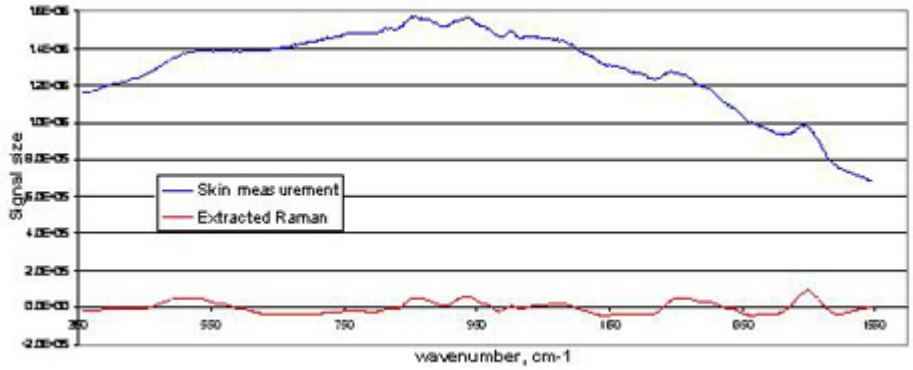

Figure 2. A signal collected from a transcutaneous measurement, is shown in blue. It consists of a dominating fluorescence background on top of which the Raman peaks from all present biomolecules is superimposed. The extracted Raman signal is shown in red.

Previous Research

Previous work determined that the measurements of blood analytes in a serum matrix were feasible. From that foundation, the Raman system was improved and then utilized to demonstrate the feasibility of the measurement of Glucose, Urea, Triglyceride, Total Protein, Albumin, Hemoglobin and Hematocrit in whole blood [8]. Based upon these successes, our focus moved to transcutaneous measurement of analytes. To support this objective, the Raman system was further improved to significantly increase its light collection and detection efficiency. The system developed for this application is shown in figure 3. The Raman light generated in the tissue is collected by a paraboloidal mirror, designed for both wide-angle and large-area light collection optimal for light being emitted from highly scattering skin. A circular-to-linear shaped fiber bundle efficiently guides the collected Raman light to the spectrograph and a large area CCD, enabling recordings of Raman spectra with high sensitivity.

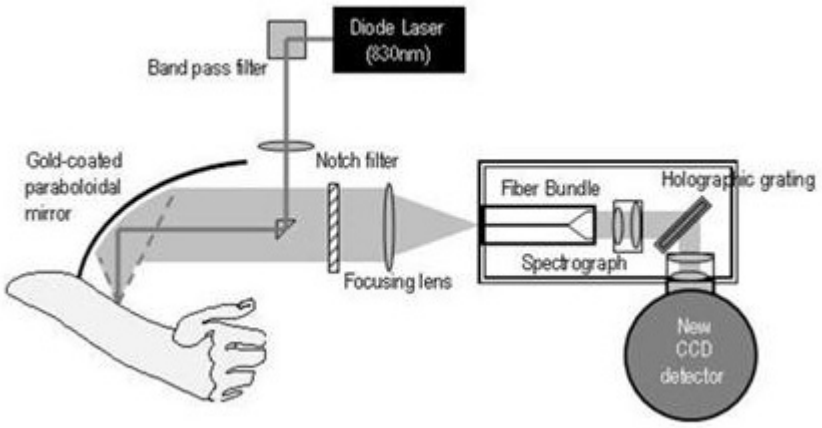

Figure 3. Diagram of the high sensitivity Raman spectroscopy system used for transcutaneous measurements

Transcutaneous Measurements
As an initial evaluation of the ability of Raman spectroscopy to measure glucose transcutaneously, a series of spectra were collected on human volunteers in conjunction with a glucose tolerance test. This involves the intake of a high-glucose containing fluid (SUN-DEX), after which the glucose levels were elevated to more than twice that found under fasting conditions. Raman spectra, each measured for 3 minutes, and reference glucose concentrations from blood samples were measured periodically during the 2-2 ½ hour duration of the procedure for each volunteer. A Hemocue glucose analyzer provided the reference measurement for the blood analysis. The manufacturer's specification for precision is SD < 6 mg/dl.

The Raman spectra were extracted from the large fluorescence background using a 5th order polynomial to fit the background. A calibration algorithm was generated individually from the data from each volunteer using the Partial Least Squares (PLS) regression method. Each calibration algorithm was validated using hold-out-one cross validation.

A comparison of the predicted glucose concentrations to the corresponding reference data from one of the volunteers is shown in figure 4. The average error in the validated data (Standard Error of Validation, SEV) is 9.8 mg/dl with an R^2 of 0.91.

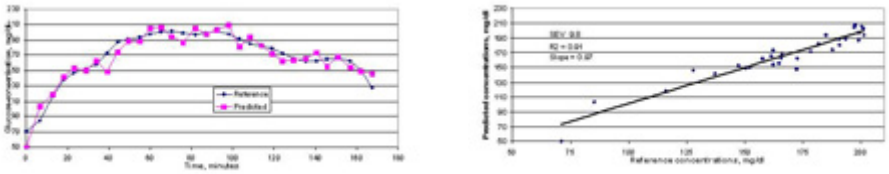

Figure 4. The left chart shows the predicted glucose tracking the reference values for one volunteer. The correlation of the same data is shown on the right, with an average error of 9.8mg/dl and an R^2 of 0.91.

The procedure was applied individually to data from each of 16 volunteers and the validated prediction results combined into one chart, shown in figure 5. For the data from all 16 volunteers, the average prediction error is 13.2 mg/dl and the R^2 is 0.79.

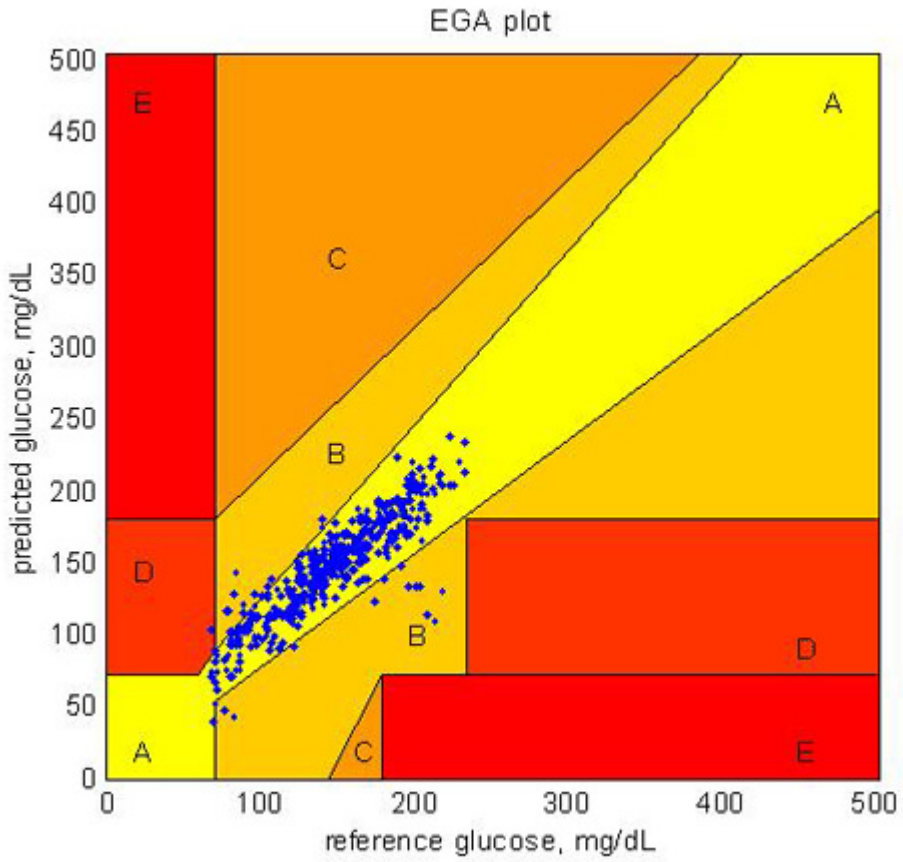

Figure 5. Cross validated results for 16 volunteers calibrated individually. The average prediction error for this set is 13.2 mg/dl and the R2 is 0.79.

A question that occurs with this kind of procedure is whether the calibration is based upon glucose. Raman Spectroscopy offers a way to obtain an indication of whether glucose is an important factor in this calibration; by comparing the calibration regression vector to the spectrum of glucose. Figure 6 compares the regression vector for the calibration shown in figure 4 to the spectrum of glucose in water, scaled to fit on the same chart. Due to the sharp features of Raman Spectroscopy, there are numerous peaks in the glucose spectrum that appear in the regression vector.

Unlike many methods of measuring glucose, where there are valid questions of whether glucose is being measured, the existence of numerous glucose peaks in the regression vector developed from Raman measurements provides direct evidence that glucose is being measured.

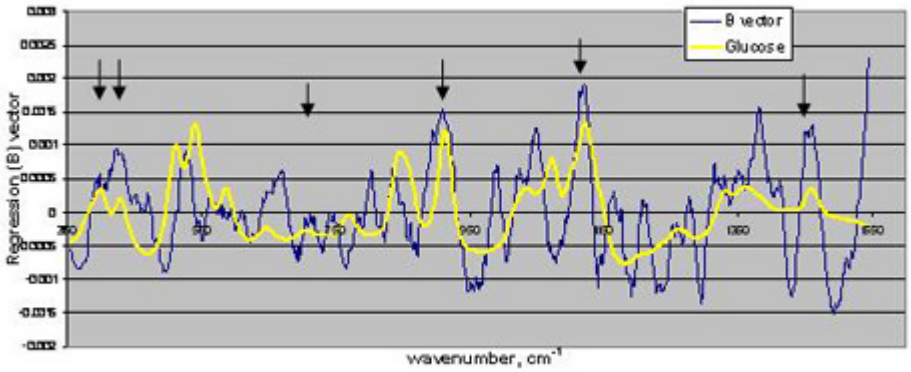

Figure 6. The regression (B) vector for the calibration shown in figure 4 and the spectrum of glucose, scaled to fit on the same chart. Numerous peaks in

the glucose spectrum match peaks in the regression vector, indicating that glucose in an important part of the calibration.

Current Work

The data from this series of tests in volunteers has provided a wealth of knowledge for us. We are continuing to analyze the data to identify opportunities to improve results. From the analysis and further testing of system characteristics, we have generated a number of instrument improvements to be made and identified a number of causes for error. Addressing those causes and making identified instrument improvements are expected to decrease measurement error.

We have further analyzed the optical and stability characteristics of our system. Based upon that, we have improved light collection efficiency by over 30% by changing to a higher NA fiber in the bundle that couples light to the spectrometer. We are also taking steps to make our system more stable and developing techniques to accurately and precisely measure and correct for the drifts that remain.

We are also developing new data processing techniques to extract more information from our measurements. Our goal is to utilize all the techniques we are learning to obtain reduced error levels in an expended human volunteer study.

Recent Publications

1. "Measurement of blood analytes in turbid biological tissue using near-infrared Raman spectroscopy", Tae-Woong Koo, Doctoral thesis, Massachusetts Institute of Technology, 2001
2. "Prospects for In Vivo Raman Spectroscopy", Eugene B. Hanlon, Ramasamy Manoharan, Tae-Woong Koo, Karen E. Shafer, Jason T. Motz, Maryann Fitzmaurice, John R. Kramer, Irving Itzkan, Ramachandra R. Dasari, and Michael S. Feld, *Physics in Medicine and Biology* **45**(2), R1-R59 (2000)
3. "Reagentless Blood Analysis by Near-Infrared Raman Spectroscopy", Tae-Woong Koo, Andrew J. Berger, Irving Itzkan, Gary Horowitz, and Michael S. Feld, *Diabetes Technology & Therapeutics* **1**(2), 153-157 (1999).
4. "Multicomponent Blood Analysis by Near-Infrared Raman Spectroscopy", Andrew J. Berger, Tae-Woong Koo, Irving Itzkan, Gary Horowitz, and Michael S. Feld, *Applied Optics* **38**(13), 2916-2926 (1999).
5. "Measurement of Glucose in Human Blood Serum using Raman Spectroscopy", Tae-Woong Koo, Andrew J. Berger, Irving Itzkan, Gary Horowitz, and Michael S. Feld, *IEEE-LEOS Newslette* **12**(2) 18 (1998).
6. "An Enhanced Algorithm for Linear Multivariate Calibration", Andrew J. Berger, Tae-Woong Koo, Irving Itzkan, and Michael S. Feld, *Analytical Chemistry* **70**(3), 623-627 (1998).
7. "Measurements of analytes in whole blood by means of Raman spectroscopy" Annika M. K. Enejder, Tae-Woong Koo, Jeankun Oh, Gary L. Horowitz, Ramachandra R. Dasari, and Michael S. Feld, *SPIE's BIOS 2002*, 19-25 January 2002, San Jose, California, USA
8. "Blood Analysis by Raman Spectroscopy" Annika M. K. Enejder, Tae-Woong Koo, Jeankun Oh, Martin Hunter, Slobodan Sasic, Gary L. Horowitz, Michael S. Feld. *Optics Letters* **27**, 2004-2006 (2002).

Support

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