In-vivo NIR Diffuse-reflectance Tissue Spectroscopy of Human Subjects

George W. Hopkins, Ganapati R. Mauze
Analytical Medical Laboratory
HP Laboratories Palo Alto
HPL-1999-13
January, 1999

E-mail: [hopkins,mauze]@hpl.hp.com

spectroscopy,
tissue, glucose,
glucometry,
non-invasive,
optical

We made spectral measurements on a diverse group of 19 non-diabetic subjects. Measurements used diffuse reflectance, with a hemispherical fluorite lens and index matching fluid, from the underside of the left forearm. Spectra were collected from 10,000 wave numbers (1.0 microns) to 4,000 wave numbers (2.5 microns) for about three minutes each. Spectra and measurements, with a blood sample, of blood glucose concentration, were taken in fasting, elevated (after a carbohydrate snack) and post-snack conditions. We took three fasting spectra, six elevated spectra, and three post snack spectra. There were monotonic variations in the measured signal as a function of sample number. The multivariate analysis captured about 99 percent of the spectral variance in two principal components (PCs). The first component was a spectrum to spectrum variation in overall level. The second was a variable tilt to the spectra. Those PCs, which correlated best with glucose, captured less than 0.01 percent of the spectral variance. Our calibration model was poor. The error in prediction based on cross validation was on the order of 35 mg/dL. The standard deviation of the reference measurements (which were not normally distributed) was 31.6 mg/dL. One can conclude that our calibration model had no predictive value.

In-vivo NIR diffuse-reflectance tissue spectroscopy of human subjects

George W. Hopkins and Ganapati R. Mauze

Hewlett-Packard Company
3500 Deer Creek Road
Palo Alto, CA 94304-1392 U.S.A.

Telephone: 650-857-5440 (GWH), 650-857-7058 (GRM), Fax: 650-857-2862
E-mail: hopkins@hpl.hp.com, mauze@hpl.hp.com

Summary

We made spectral measurements on a diverse group of 19 non-diabetic subjects. Measurements used diffuse reflectance, with a hemispherical fluorite lens and index matching fluid, from the underside of the left forearm. Spectra were collected from 10,000 wave numbers (1.0 microns) to 4,000 wave numbers (2.5 microns) for about three minutes each. Spectra and measurements, with a blood sample, of blood glucose concentration, were taken in fasting, elevated (after a carbohydrate snack) and post-snack conditions. We took three fasting spectra, six elevated spectra, and three post snack spectra. There were monotonic variations in the measured signal as a function of sample number. The multivariate analysis captured about 99 percent of the spectral variance in two principal components (PCs). The first component was a spectrum to spectrum variation in overall level. The second was a variable tilt to the spectra. Those PCs, which correlated best with glucose, captured less than 0.01 percent of the spectral variance. Our calibration model was poor. The error in prediction based on cross validation was on the order of 35 mg/dL. The standard deviation of the reference measurements (which were not normally distributed) was 31.6 mg/dL. One can conclude that our calibration model had no predictive value.

Introduction

There are few published works (Marbach 1993, 1995) in the field of tissue spectroscopy for glucose self-monitoring, in the wavelength range from 1.0 microns to 2.5 microns, and the error described is too large for clinical use or for home monitoring. Measurements (Arnold 1990, Marbach 1993) of aqueous solutions of glucose, with concentrations in the physiological range, have been promising, including measurements (McNulty 1998) in our laboratory. We chose a subject group that we expected to have a large variation in spectral components, with the knowledge that this small, diverse group would be quite unsuitable for building a calibration model. The goal of our work was to learn the magnitude of variation of components in tissue spectra, other than glucose, and to estimate the relative magnitude of these variations compared to a glucose signal.

Feasibility Study:

A feasibility study was conducted to determine the prospects for experimental work. The study included an estimate of absorption for glucose based on physiological levels of glucose and tissue optical properties, and a calculation of expected instrument signal-to-noise ratio.

After a review of prior work, we concluded that the best prospect for non-invasive measurement of glucose would come from measuring a physical parameter that is directly related to glucose concentration. This parameter is optical absorption in the near infrared (NIR) near 1.57 microns (1570 nanometers or 6350 wave numbers) and near 2.27 microns (2270 nanometers or 4400 wave numbers). At these wavelengths, light penetrates into tissue

sufficiently to pass through capillary blood and interstitial fluid, both of which contain glucose.

Monte-Carlo simulations (Marbach 1993) model the history of light that emerges from tissue after entering and scattering around. Light at 1.57 micron penetrates to an average depth of 0.3 mm. As it scatters, it travels an average distance of 1.1 mm. Light at 2.27 micron penetrates to an average depth of 0.2 mm and travels an average distance of 0.6 mm. Due to these short travel distances, the low molar absorbtivity of glucose, and the modest physiological concentrations, glucose absorption in tissue is small, perhaps 0.1 milliabsorbance units for normal glucose levels. Nevertheless, it is within the capabilities of current instrumentation to measure it. These glucose wavelengths are situated in "water windows", where the absorption of water, although still significant, is lower than at neighboring wavelengths.

Apparatus:

Measurements were made on a Bomem MB 155 FTIR spectrometer, serial number SZM4908N. The source was an industry standard part, an L9404, 20 watt, tungsten-halogen lamp, supplied in pre-aligned form. It had a filament that is 2.9 mm long by 1.2 mm in diameter. This lamp was operated at 11.0 V (below its 12.0V nominal) to produce 16.8 watts and a color temperature of 2872 K. The spectrum approximated a gray body with an emissivity of 0.425 and an emission peak at 1.01 micrometers. The total collection angle for the MB 155 source was 29.0 degrees. The beam splitter was an extended range NIR/MIR coating on a potassium chloride substrate. The detector was a 1mm diameter, indium arsenide, photodiode, from EG&G Judson, with a sapphire window. It was cooled to –35 degrees Celsius by a three-stage thermoelectric cooler.

We purchased a commercial diffuse reflectance accessory (DRA), the Harrick three-dimensional praying mantis, to avoid the delay and expense of designing and constructing our own DRA. Despite its whimsical name, this accessory was the best off-the-shelf choice. The Harrick DRA illuminated the sample obliquely. It used two 6 to 1 off-axis replicated ellipsoidal mirrors, together with appropriate planar turning mirrors, to illuminate a sample and to collect scattered light. The mirrors subtended 20 percent of a two-pi solid angle. They were oriented so that specular reflection of light onto the sample missed the collecting mirror.

Initially, we made measurements with a subject's forearm at the sample location on the DRA. The opening in the DRA is rectangular, 15.8 mm by 9.5 mm, with fillets in each corner. We used the same portion of the forearm each time, but the subject's skin sagged through the hole. We found that the level of the spectra varied randomly and substantially from one measurement to another. We attributed these variations to the sag of the skin, which is uncontrolled, varying from measurement to measurement.

We then modified the DRA by constructing new covers holding a hemispherical lens with a 20 mm radius of curvature, with its flat side at the sample location. This provides a surface to locate the subject's arm and an advantage of increased signal as well. We had lenses made from fused silica and from fluorite. The fused silica lenses proved to be unsatisfactory due to an absorption band in the silica, and the fluorite lenses were used. Because of the oblique incidence of light on the flat side of the lens, total internal reflection (TIR) of the light occurs. This is avoided by using an index matching fluid between the lens and the subject. A large drop of OxyChem Fluorolube T-80 oil was used to contact the subject's tissue to the lens of the DRA (Messerschmidt 1997). With the lens, we found much less random variation in spectral levels than with no lens, however, we observed systematic changes in spectral characteristics as successive measurements were made. A 25 percent transmission metallic, neutral density filter was used between the source and the sample to avoid saturation of the analog to digital converter at the lowest gain setting on the preamp.

We used a fixture to locate the subject's arm. The distance from a handgrip to the sampled point was fixed for all subjects, but the side-to-side position was determined by an adjustable stop. We instructed each subject to place the center of the forearm over the sample point, adjust the stop, and then leave it fixed for all measurements.

Measurements on subjects:

The data acquisition software used was GRAMS/386 software from Galactic Industries Corporation. The acquisition parameters used were:

Resolution – 4 wave numbers (set on MB 155)

Number of scans – 128

Apodization - Cosine

Spectral range – 10000 to 4000 wave numbers (1.0 to 2.5 microns)

Data type – Single Beam

We initially made spectral measurements on the authors to establish an appropriate operating configuration for the instrument. We then selected a volunteer group of 19 non-diabetic subjects from Hewlett-Packard Company employees and retirees. The group was selected to be diverse with respect to gender, skin pigmentation, age and amount of subcutaneous fat. We sought a diverse group in order to learn as much as possible about variations in tissue spectra, although doing so results in a poor data set for building a calibration model. To have any hope of building a useful calibration model, a much larger number of subjects and a much larger range of glucose concentration (diabetic subjects) would have been required. Finally, we made measurements on the authors to investigate the effects of water, which we thought would confound the glucose measurements.

Measurements were made using diffuse reflectance from the underside of the left forearm. The acquisition time was about three minutes each. Parallel spectra and measurements with a blood sample and analyzer (reference measurements) of blood glucose concentration were taken on the 19 non-diabetic subjects in fasting, elevated (after a carbohydrate snack) and post snack conditions. We took three fasting spectra, six elevated spectra, and three post snack spectra. Reference measurements were made on a YSI model 2300 glucose analyzer, which has an accuracy of plus and minus 2 mg/dL, using a 25 microliter blood sample obtained by a finger stick and milking.

To investigate the effects of water, we used three methods to vary the amount of water in the tissue of the authors. We drank no water for at least three hours, took spectra, then drank a liter of water, and took more spectra. We then used a water chamber to hydrate the skin. Finally, we took spectra with the arm lowered and then elevated with respect to the body. We found that differences in spectra due to drinking lots of water and to lowering and elevating the arm were very small and buried in unrelated spectral variations. Hydrating the skin produced large variations in spectra, as anticipated, but no insights.

Spectral data:

To demonstrate the features of the spectra, we present the mean for a large set of tissue spectra. In this case, and in all cases, the $\log_{10}$ of the single-beam spectrum is plotted. The overall spectral profile is the instrument profile as modified by the scattering and absorption characteristics of tissue and the collection of the DRA. The large dips are due to water absorption. The "noisy" regions, near the water dips, are due to residual water vapor. There are additional small structures in the spectrum due to absorption by other components, such as proteins or fatty acids, in the tissue. Glucose absorption is not visible at this scale.

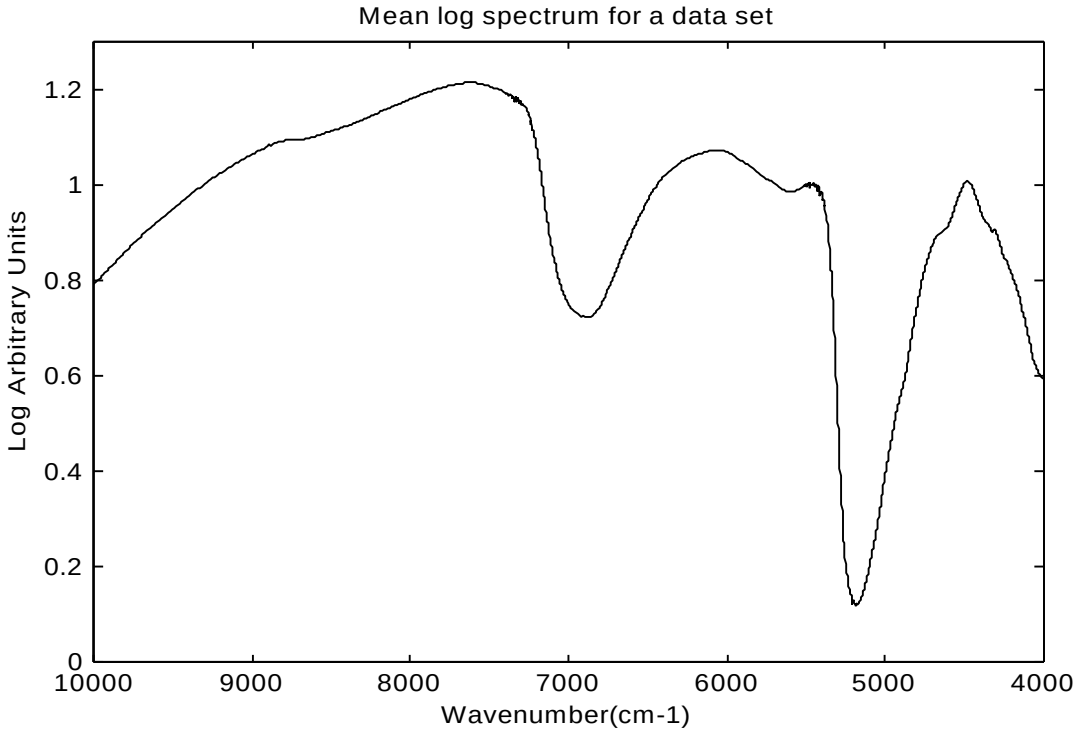

Figure 1: Typical diffuse reflectance tissue spectrum from the forearm of human subjects.

We first plotted all spectra, as $\log_{10}$ spectra, against wave number, for each subject. We also plotted the spectral values at seven wavenumbers, at the extremes of the spectral range and at major maxima and minima, as a function of sample number, to identify trends. Shown on the next page is a set of spectra and a time sequence for a typical subject (Subject #1).

The spectra show a large variation from sample to sample. The time sequence is by sample number. The first three samples (1,2,3, fasting) are taken with the forearm in continuous contact with the lens. The next six (4 through 9, elevated) are taken sequentially, again with the forearm in continuous contact with the lens, and the final three (10,11,12, post-snack) are also taken with the forearm in continuous contact with the lens. There are obvious systematic changes in the spectra. Measured values tend to decrease. The trends were consistent, but the extent of the systematic changes and the occurrence of occassional inconsistencies varied from subject to subject. We formulated hypotheses for these trends, including penetration of the matching fluid into the tissue and physiological changes due to pressure or remaining in one position for several minutes, but we did not test these hypotheses.

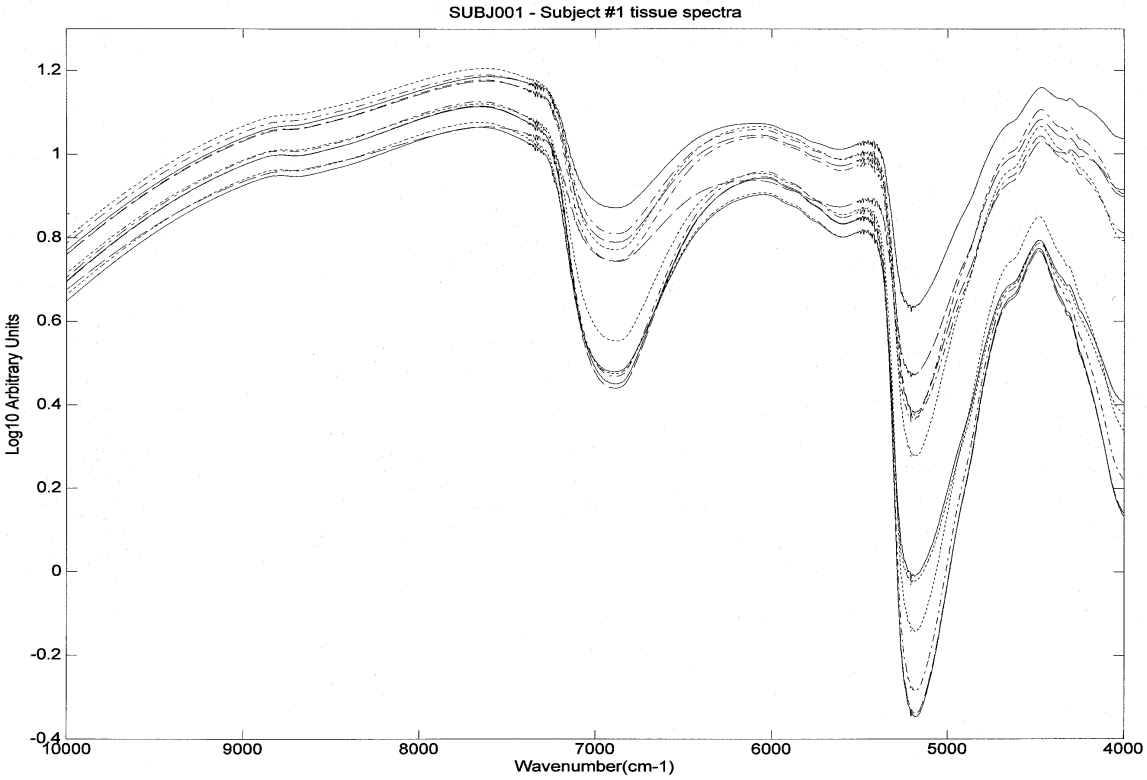

Figure 2: Tissue spectra for a single subject, taken in 3 sets of 3, 6 and 3 measurements.

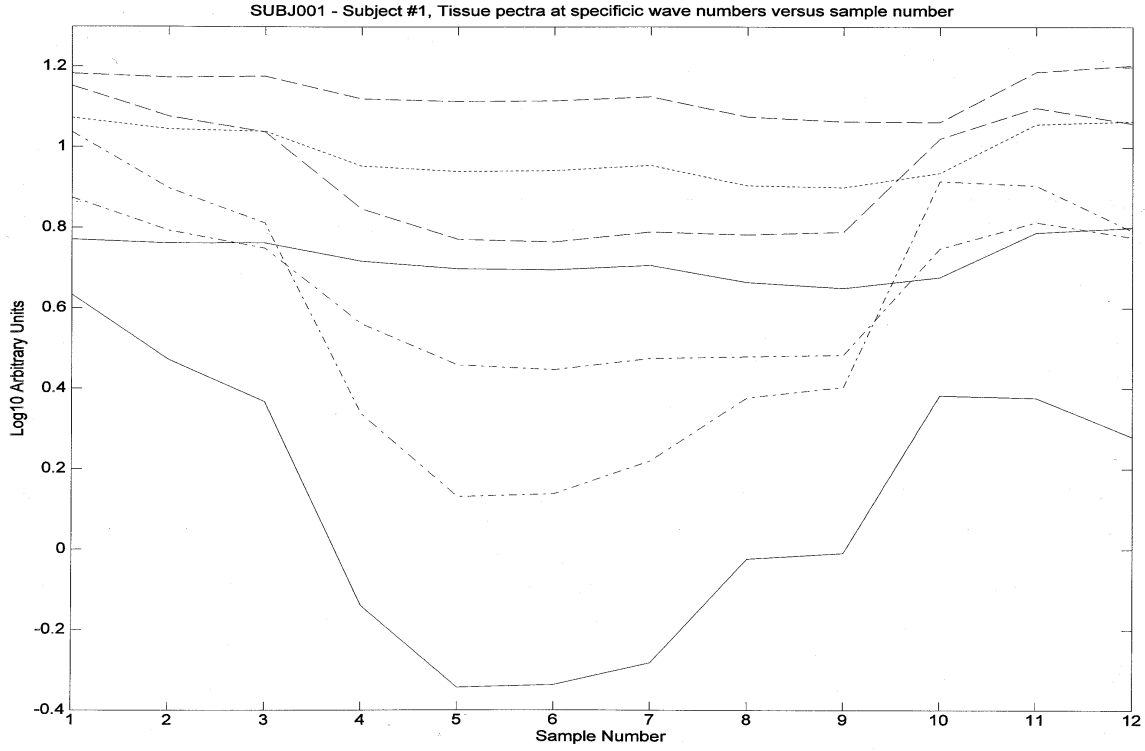

Figure 3: Tissue spectral values for a single subject at seven wavenumbers versus sequential sample number.

The spectra for subject four were anomalous. This was noticed during data acquisition, and we rechecked our instrumentation and technique and then repeated the measurements. The occurrence of anomalous spectra below was repeatable. Subject four had very low subcutaneous fat levels, but subject seventeen also had very low subcutaneous fat levels and had typical spectra. Although there were variations in the mean spectra for other subjects, these variations from subject to subject were much smaller than the variations within a measurement set for a given subject. The cause of the spectral anomalies for subject four was not identified, and spectra from subject four were not used in analyses.

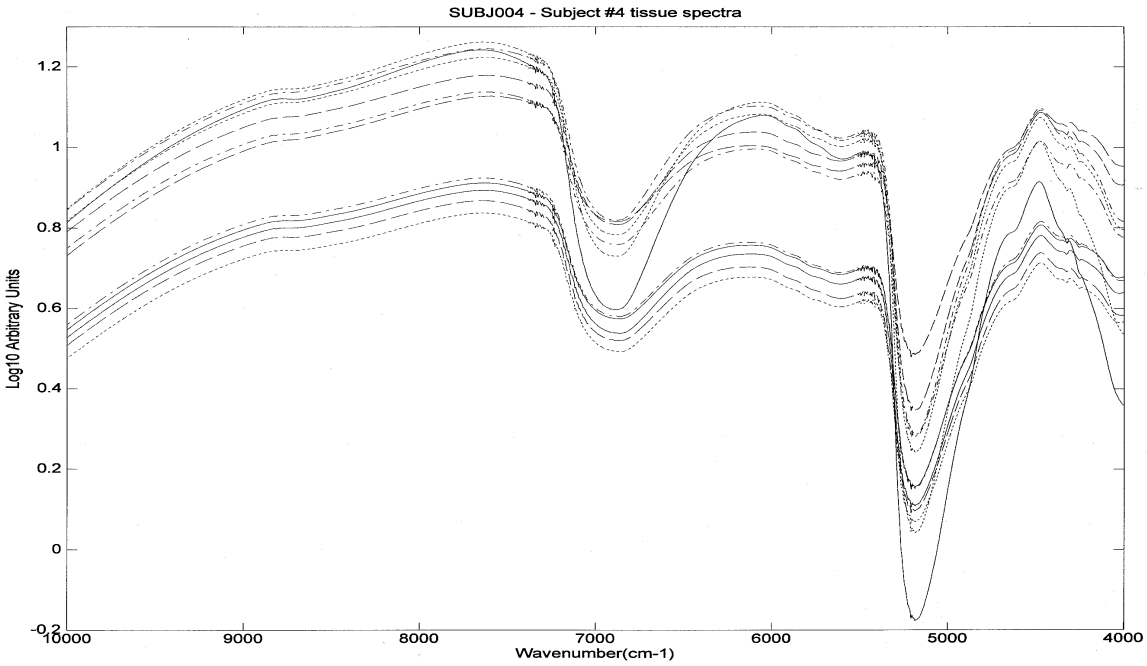

Figure 4: Tissue spectra for subject four, showing anomalous spectra. Compare to Fig. 2.

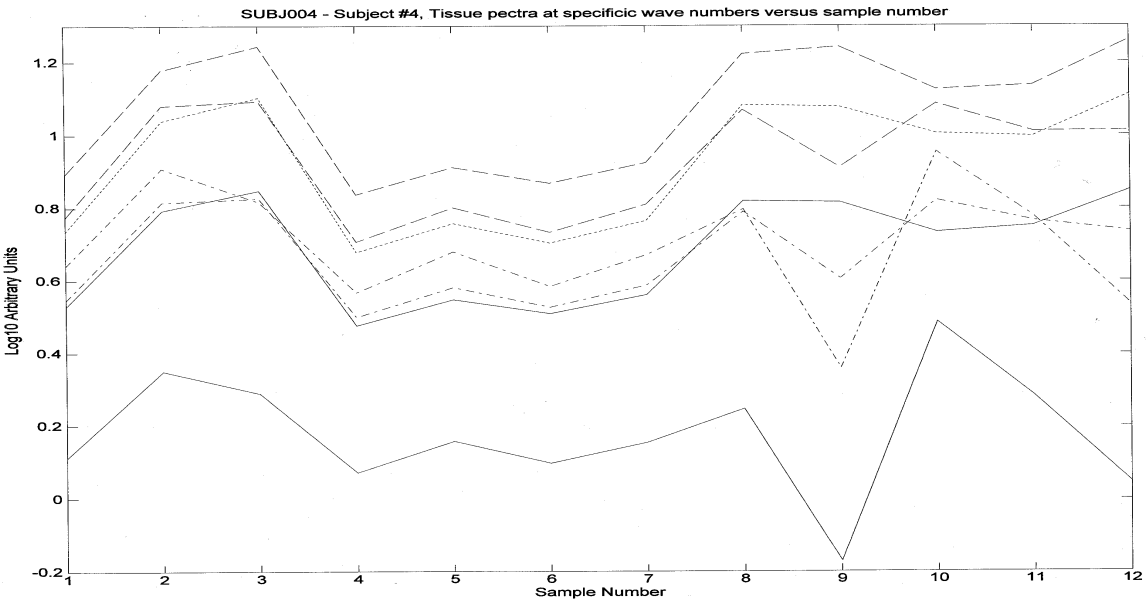

Figure 5: Tissue spectral values for a subject four at seven wavenumbers versus sequential sample number. The sample numbers of the anomalous spectra can be identified. Compare to Fig. 3.

There was substantial variation among spectra for each and every subject. The following plot shows mean centered data for 18 subjects, with subject four's data removed from the set.

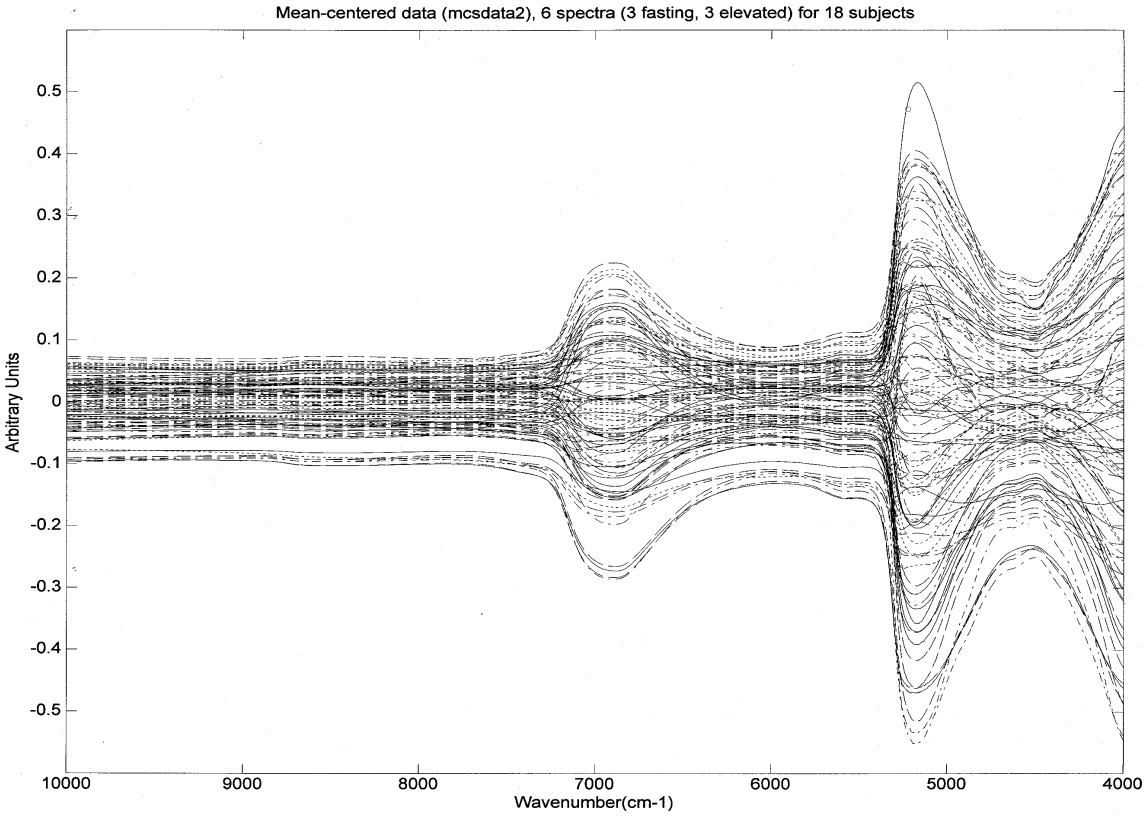

Figure 6: Six mean centered spectra from each of 18 subjects shows the range of spectral variation.

Data analysis

Data were analyzed using multivariate analysis and calibration methods.

We split the measurements on the remaining eighteen subjects into three data sets. We had observed that there was monotonic variation in spectra as successive spectra were taken during a series of measurements, when the arm was in continuous contact with the DRA. We had taken three spectra in the fasting and post snack conditions and six in the elevated conditions. Because of the variations, we made up two data sets using only the first three elevated spectra, which were matched with the fasting and then with both the fasting and post snack spectra. The third data set consisted of all spectra.

First, we used principal component analysis (PCA), an analysis technique which simplifies the data model. It determines orthogonal spectra, which successively capture the greatest variance in data. We found that components capture the variance faster when we take the logarithm of the spectra data, indicating that the data is more linear in its logarithmic form. We subsequently used log spectra. We then found that the first PC captured over 96 percent, the second PC over 3 percent, and the remaining PCs captured a tiny fraction of one percent of the variance. The loading vector for the first PC strongly resembles a water vector; the only non-water feature is a dip-peak-dip/peak-dip-peak between 4200 and 4800 wave numbers. The second PC again resembles water, but inverted, and the non-water features are more pronounced. We did not identify the non-water features, which are likely due to proteins or fatty acids. We concluded that the gross variations in the spectra between

measurements are related to water, and that these variations are non-linear (otherwise, the first PC would have captured them).

We next experimented with spectral subtraction. We did this by finding the principal components of pure spectra. Water, teflon beads in oil to simulate tissue scattering, and pork fat were used. These could then be subtracted from subject spectra. In theory, this is unnecessary, because the multivariate techniques should separate these effects, but spectral subtraction has proven useful in other applications, such as MRI. For us, spectral subtraction had shortcomings. Our water spectrum, taken on liquid water, was a poor match for the water spectrum in tissue, and subtraction failed to remove the water component. We did verify the major causes of variation, which matched the spectra of water absorption and scattering by the teflon beads in oil.

Finally, we used PLS (partial least squares or projection to latent structures). This is a calibration method, which finds latent variables, similar to principal components, but which correlate more strongly with the reference (glucose) measurements. The validity of the calibration model can be determined by eliminating measurements from one subject, building a calibration model from the remaining subjects' measurements, using this model to predict the eliminated subject's glucose value, and comparing it to the reference measurement. The resulting error, called the root mean square error of cross validation (RMSECV) would be a standard deviation if the data were normally distributed. We performed this validity check, called cross validation, on the data for all eighteen subjects. We then split the data into two arbitrary sets of nine subjects each, built PLS calibration models, and used one set to predict the other and then vice versa. This allowed for a graphical presentation of predicted versus reference values for each data set.

Results:

The multivariate analysis is enlightening. About 99 percent of the spectral variance are captured by two principal components (PCs). These PCs are weakly correlated with glucose, capturing only about one percent of the variance in the reference measurements. The first component is a spectrum to spectrum variation in overall level. The second is a variable tilt to the spectra, which may be due to a scattering component. Those PCs, which correlate best with glucose, capture less than 0.01 percent of the spectral variance. We had expected, since glucose absorption is small, that the PCs correlated with glucose would be "buried" in the larger variations. We were surprised to find the glucose information so far down in the PC model. Those components with strongest correlation with glucose were as far down as the eighteenth PC, and in all cases were in PCs where noise in the components was very large.

Our calibration models were poor. The errors in prediction based on cross validation were on the order of 35 mg/dL. The standard deviation of the reference measurements (which are not normally distributed) was 31.6 mg/dL. The use of either half of the data to predict the other half produced random scatter plots (Linear regression coefficients were $r = -0.1682$ and $r = 0.2844$). We can safely conclude that our calibration models had no predictive value.

Our regression vectors have features in common with the absorption spectrum of glucose in aqueous solution, including large water absorption features. We cannot say whether these water features in our regression vectors are due to water displaced by glucose or are due to randomly correlated variations. We have observed, in solutions, that the presence of glucose in water changes the water spectrum, presumably by displacing water. This effect is much larger than glucose absorption and is distinct from band shifts due to temperature changes. It can be observed in the spectrum, on the next page, of an aqueous solution of glucose. The spectrum immediately below is a typical regression vector. Note that, in the regression vector, there are poorly defined features, which align with glucose absorption peaks, and well-defined features, which align with water absorption peaks.

The spectra below show glucose in water (top) and the regression vector (bottom) for one data set. The features of the water spectrum between about 5300 and 4900 wave numbers are not real, but are artifacts due to the very large absorption of water in this region. The small peaks are due to glucose absorption.

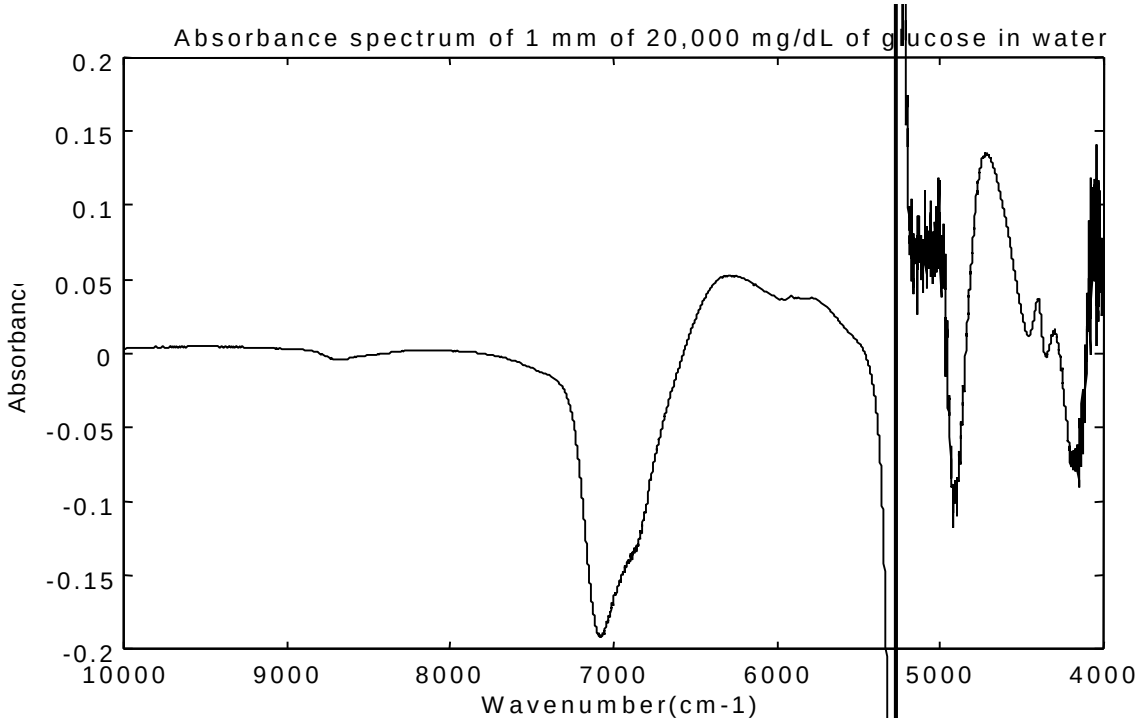

Figure 7: Absorbance spectrum, in transmission, of a concentrated aqueous glucose solution.

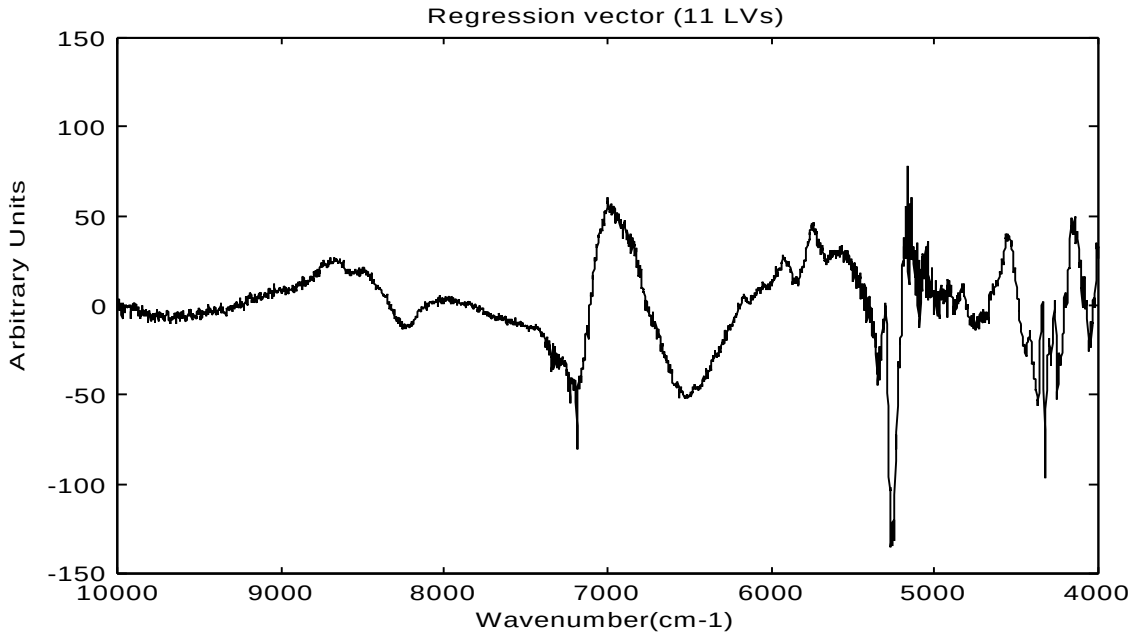

Figure 8: A regression vector for predicting glucose concentration. Compare the features of this vector to the features of the aqueous glucose spectrum above.

Conclusions

The variations in spectra for any single subject, with characteristics of water absorption and scattering, were larger than subject to subject variations, for our diverse sample of 18. The single exception is the unexplained variation in spectra for subject four. Although one cannot conclude that glucose measurement cannot be done using the method of this paper, the results from our small sample with glucose variation in the normal physiological range show no promise. There appears to be correlation between spectra of aqueous solutions of glucose and regression vectors, but the principal components, which correlate best with glucose, are buried in noise.

Acknowledgements

We gratefully acknowledge the advice and assistance of Leslie Leonard, Jerry Zawadski, Paul Lum, Michael Greenstein, Bo Curry, Dick Lacey, Tirumala (Rangu) Ranganath, Dean Forbes, Peter Webb, Florence Haas, Pete Melton, and Rick Pittaro. Chris McNulty and Delphine Le Cocq, visiting MIT students, conducted related investigation into spectroscopy of solutions (McNulty).

References

Arnold 1990 - Mark A. Arnold and Gary W. Small, "Determination of Physiological Levels of Glucose in an Aqueous Matrix with Digitally Filtered Fourier Transform Near-Infrared Spectra", *Anal. Chem.* 1990, 62, 1457-1464.

Marbach 1995 – R. Marbach and H. M. Heise, "Optical diffuse reflectance accessory for measurements of skin tissue by near-infrared spectroscopy", *Applied Optics*, Vol. 4, No. 4, 1 February 1995, pp. 610 – 621.

Marbach 1993 – Ralf Marbach, *A measurement method for determination of blood glucose by IR spectroscopy* (in German). VDI Verlag, Series 8, #346, 1993, ISBN 3-18-144608-4.

McNulty 1998 – Christopher S. McNulty and Ganapati Mauze, *Applications of Wavelet Analysis for Determining Glucose Concentration of Aqueous Solutions Using NIR Spectroscopy*, HP Laboratories Technical Report HPL-98-53, March 1998.

Messerschmidt 1997 – World Patent 97/05819, *Method for non-invasive blood analyte measurement with improved optical interface,* by Messerschmidt, assigned to Rio Grande Medical Technology.