Tissue Spectroscopy for Glucose Measurement

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glucose, non-invasive, medical diagnostics, point-of-point testing (POCT), glucometry, home health care

An investigation of the potential for non-invasive optical measurements of glucose concentration in human subjects was conducted from March 1, 1997 to March 31, 1998.

The investigation started with a study of current practice, market potential, activity in the field, and prior work, as evidenced by literature and patents. A feasibility study was conducted, a spectrograph was purchased, a sampling accessory was modified, measurements were made on a small sample of subjects, and the resulting data were analyzed.

We reached conclusions on the business potential and the quality of our measurements, and we recommended a course of action for HP.

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Note: References are indicated by author's name in parentheses, for example, (Marbach 1993).

The DiagNoSTIX investigation:

The DiagNoSTIX investigation was conducted from March 1, 1997 to March 31, 1998. The primary goals of the investigation were a better understanding of tissue spectroscopy and an evaluation of the potential for non-invasive glucose measurement by near infrared (NIR) spectroscopy. DiagNoSTIX is a play on the name of our STIX investigation, which studies invasive blood sampling.

Summary of activities:

We conducted a literature and patent search, which is being continuously updated. Although the relevant literature in refereed journals is small, the number of patents on the subject is large and of highly variable quality.

We then conducted a feasibility study to identify the most promising approach and possible implementations. We chose the 1 to 2.5 micron spectral range (the NIR), because glucose has measurable absorption in this range, and light penetrates into tissue sufficiently to sample glucose in capillary blood and interstitial fluid.

We studied available instrumentation and purchased a Bomem MB 155 FTIR spectrometer with an Indium Arsenide, TE-cooled, detector. We modified a Harrick diffuse reflectance attachment, adding an immersion lens.

We studied regulatory issues regarding exposure of tissue to light. We wrote standard operating procedures and subject consent forms.

We measured tissue spectra in the NIR for a healthy, diverse group of 19 subjects, where fasting and carbohydrate snacks provided variation of blood glucose concentration across the normal range. We made reference measurements of blood glucose concentrations from blood samples obtained with finger sticks.

We analyzed the tissue spectra using multivariate analysis (PCA) and multivariate calibration (PLS). The main components in the spectra, which also accounted for more that 99 percent of the variation in the spectra, were water absorption and a scattering component. Signals, which correlated with glucose absorption, were very small, but they could be identified. The calibration model was very poor, having a RMSECV comparable to the standard deviation of the reference measurements. We found that variations in water absorption appear to correlate with glucose concentration.

The bottom line:

We made good progress toward understanding and defining the problems in non-invasive glucose measurement, and we met the project goals.

We were not successful at measuring blood glucose concentration accurately enough for home monitoring. This was anticipated. Our results were comparable to those

of other researchers in the field, that is, having accuracy about three times worse than needed for home monitoring.

We found evidence that glucose displaces water in tissue, just as it does in aqueous solution. This produces a signal, due to a change in water absorption, that is orders of magnitude larger than glucose absorption. This water signal can be measured with a high signal to (stochastic) noise ratio. Unfortunately, water content in tissue varies due to many causes. For water absorption to be of use in measuring glucose, we would need a means of stabilizing the variation of water or for separating the variation of water absorption due to glucose displacement from that due to other causes. This is a promising and very challenging, but unexplored, line of research.

The main factor limiting the accuracy of our glucose measurements was measurement-to-measurement variation in spectra due to factors other than glucose. Reducing this variation will be a necessary step in continuing research along the present lines and is the logical next step.

The market for home glucose monitoring is about 2.7 billion dollars per year and is growing at a rate of about 14 percent per annum. It is not fragmented; a single product could address a significant portion of the market. This single product would be an instrument.

The potential payback is so large that work will continue until a device can be purchased in your neighborhood drug store. So, someone will succeed in marketing a non-invasive home glucose meter. It might as well be us! Please remember that past work in HP Labs led to pioneering medical products: the ear oximeter, the airway capnometer, and acoustic quantification; and to significant advancements in phased-array ultrasonic imaging, quartz pressure transducers and electrocardiography.

Non-invasive home glucometry is in the research or discovery stage. No one knows how to make the measurement in a way that would lead to a product which could obtain regulatory approval. In the field of invention, the proper analogies are the discovery of photography, the electric lamp or flying machines. The concept was easy to grasp, but the realization came only with insights, a lot of trial and error, and serendipity. The next steps are for researchers, thinking of new ideas and testing them in their laboratories.

Personnel:

George Hopkins conducted this investigation as his primary assignment. Ganapati Mauze, Leslie Leonard, Jerry Zawadski, Paul Lum and Michael Greenstein, Project Manager, assisted. Bo Curry, Dick Lacey, Tirumala (Rangu) Ranganath, Dean Forbes and Peter Webb consulted. Florence Haas of the Research Library monitored activity in literature and patents. Pete Melton, who was Medical Department Manager when the investigation started, has provided support,

encouragement, and guidance throughout the investigation. The investigation began under Rick Pittaro, Project Manager, as the result of a project proposal process. Chris McNulty, an MIT student, conducted a related investigation into spectroscopy of solutions (McNulty).

Situation:

The only non-invasive optical measurement commonly used in clinical settings is pulse oximetry. There has been considerable interest, however, in the use of non-invasive techniques, including optical spectroscopy, for home monitoring of blood glucose concentration. Incentives to develop this measurement are huge market potential (on the order of one billion dollars or more per year) and a belief that diabetics would be willing to measure their blood glucose concentration more often with a non-invasive device than with finger sticks. The resulting increased compliance with their therapy would significantly reduce morbidity and associated health care costs (DCCT, SMBG).

Despite substantial efforts in academia, government laboratories and industry, no satisfactory measurement exists today. The measurement has proven to be very difficult. The current art, based on literature and our results, gives an accuracy that is a factor of three or so worse than needed. Furthermore, the stability of the calibration and the transfer of the calibration from one patient to another have not been demonstrated.

Current practice:

Current devices for home glucose measurement are based on a glucose oxidase or glucose hexokinase reaction. Consumable strips, which are proprietary to each device, are used with a drop of blood from a finger stick to measure blood glucose concentration. Detection uses an electrochemical method or an indicator, which is detected photometrically. The average user spends about $700 per year for these strips (The base devices are provided at essentially no cost through a rebate program).

Competing methods under investigation:

A cure for diabetes would undermine the potential market for a device. Unfortunately, prospects for a cure are non-existent.

The artificial pancreas, a device providing a closed-loop insulin supply, has been sought for years. There are no good prospects, but work continues. There were reports of micro encapsulated islets of Langerhans, which are injected into the peritoneal cavity, reaching animal trials about two years ago (Lanza). There has been no subsequent news.

Four companies are developing devices based on sampling of interstitial fluid. Some of these are in clinical trials, but none has FDA approval. These devices

would eliminate the stick required by present strip testing. Their acceptance by patients has not been tested, and their impact on the prospective market for a non-invasive device is unknown.

Pain-free or minimal pain sampling of blood could also affect the prospective market for a non-invasive device (HP's STIX investigation is studying this field). We suspect that companies already in the market for home glucose monitoring and for lancets and syringes are also investigating or developing. There is a continuing trend in stick and strip monitoring for smaller blood samples (the current art is a 3.5 microliter sample). These smaller samples should be compatible with less-painful sticks.

Market potential:

The worldwide market for home monitoring of glucose is about 2.7 billion dollars per year and is growing at a rate of about 14 percent per annum. Approximately 1 billion dollars is spent in both The United States and Europe. Most income and profit comes from sale of consumable strips. If a device were to exist for non-invasive glucometry, the current business model would not apply. Nevertheless, one could easily imagine that if such a device existed, revenues would be a significant fraction of the current market.

Clearly, this huge market potential is the reason for working toward a non-invasive glucose meter. The market is also relatively monolithic. Suppliers of home glucose meters normally offer a small number of products, which differ mainly in their data management functions. Simple models provide a basic measurement capability in a compact package. More advanced models store and display measurement history and may provide an interface to home computers.

Although we have not studied the market rigorously, we have opinions based on both anecdotal evidence and studies of the effectiveness of insulin therapy.

The population of diabetics includes many sophisticated people, who are motivated to perform accurate monitoring (It's literally a matter of life and death). These folks are knowledgeable consumers. I was once having breakfast at a public restaurant with a diabetic patient. He measured his blood glucose level with a stick and strip meter he had in his pocket and administered an insulin injection. This took between two and three minutes, while he continued to converse with the group, and it was done so discretely that I wouldn't have known what he was doing were I not familiar with the procedure. Any non-invasive measurement must offer the same level of convenience as available with stick and strip measurements (which continue to improve), if it is to gain widespread acceptance. While the dislike of sticking is touted as the reason that non-invasive measurements will be adopted quickly, we must remember that this is an untested hypothesis, and that there are other criteria for patient acceptance besides freedom from lancing.

We must also remember that the customer is the one who pays. Reimbursement criteria affect market acceptance. For governmental health programs, this is a matter of fit to regulations. HMOs, which cover an ever-larger fraction of the population, have recognized the huge impact that effective insulin therapy has in reducing health care costs associated with the horrible consequences of poor control of blood glucose. For this reason alone, a significant market will exist for any proven, cost-effective advance in insulin therapy.

Prior work:

Relevant prior work falls into two categories. One is in vitro reagentless analysis of blood samples by optical spectroscopy. The other is in vivo measurement of analytes using optical tissue spectroscopy.

Reagentless analysis is applied to drawn blood samples or their derivatives. It is more accurate than in vivo measurements due to the relative purity of the sample. Reagentless analysis thus sets a limit on the potential for tissue spectroscopy. It offers the advantage over the current stick and strip methods of not requiring a strip, but the blood sample is still needed.

The second method, tissue spectroscopy, is attractive because it is non-invasive. Tissue spectroscopy is also a significantly different problem from reagentless analysis, and it is much more difficult. The sample is more complex, it has interferences other than the water substrate, glucose information is in other compartments as well as in blood, glucose exists in different forms (bound to proteins and to water), and the sample is poorly controlled. The sample is a portion of a person's tissue. It varies in anatomical structure and is subject to physiological changes due to activity, body position, diet, medications, and environmental factors.

Work in the field is detailed in Appendix 2. Here, we will only note the limits on accuracy today.

The current art for reagentless analysis of glucose is on the order of plus or minus 10 mg/dL. This accuracy is inadequate for the clinical lab, but it is adequate for home monitoring.

There is no standard for the accuracy required of a non-invasive home glucose meter. For FDA approval, it would be necessary to demonstrate equivalence to the current home glucose monitors. The error for in vivo measurement of glucose by optical tissue spectroscopy is a small integer factor (perhaps 3 times) greater than what would be adequate, even for a single subject and a limited time. More significantly, no one has demonstrated that an accurate measurement can be made on a single subject over an extended time period or on a subject chosen at random from the general population.

Feasibility Study:

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

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 at1.57 micron penetrates to an average depth of 0.3 mm. As it bounces around, 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.

This line of research was initially suggested by Ganapati Mauze in 1990, following initial publications by Arnold and Small and the completion of an HPL task force on hand-held instruments, in which Mauze proposed that HPL develop a home glucose meter.

Calculation of instrument signal-to-noise ratio (for stochastic noise) produced equivocal results. There calculations required too many assumptions, and there is too much uncertainty in the literature and catalog values. The estimates indicate that the signal-to-noise ratio may be a small integer, based on a 10-second measurement, but the spread in the estimates is from less than unity to about a hundred.

We studied the form that a home meter might take, assuming that the measurement could be made. Our vision of a home glucose meter is a diffuse reflectance spectrograph. The source is a tungsten halogen lamp. The spectrograph would be similar to that described in U.S. Patent 5,664,396, *Spectrograph with low focal ratio*, assigned to HP. It uses aspheric glass lenses, a planar diffraction

grating, and an array detector. The array detector will be a hybrid array, composed of discrete strained indium gallium arsenide (InGaAs) photo diodes. The photo diodes would be individually alloyed to have an optimum spectral response for the wavelength being detected. Such a meter could be manufactured at a reasonable cost, perhaps $250 to $500, provided that the production volume is sufficiently large, perhaps 10,000 units per month. Volume is critical; for example, we would have to provide the manufacturer of the hybrid detector array with about two million dollars of business a year, just to make it practical to set up and maintain production.

Procurement of a spectrometer:

We purchased a Fourier transform infrared (FTIR) spectrometer. A dispersive instrument (one with a diffraction grating, as described above in our vision for a home glucose meter) can perform better in particular spectral regions, for example, in the NIR, than an FTIR. Nevertheless, the FTIR is much more flexible; it will outperform a dispersive instrument in the mid infrared. We needed an instrument that would serve current and future projects, as well as our investigation. An FTIR is not at a significant performance disadvantage in the NIR.

We researched suppliers of FTIR spectrometers, and we had each of the three leading suppliers run tests on their instruments. We made a trip to Quebec, PQ, Canada, at the vendor's expense, to visit Bomem, Inc. We purchased a Bomem MB 155 FTIR spectrometer, together with several accessories. We chose the MB 155 because it will be easier to operate the instrument at a clinic or remote site than the others considered. The others offered no significant performance advantage, and, while better automated, would have been much less suited to portable operation. A detailed report of the evaluations is available from the George Hopkins.

Development of a sampling accessory:

We chose to purchase a commercial diffuse reflectance accessory (DRA), the Harrick three-dimensional praying mantis. We chose to use the Harrick 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 illuminates the sample obliquely. It uses 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 subtend 20 percent of a two-pi solid angle. They are oriented so that specular reflection (like off a mirror) of light onto the sample misses 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. This variation was unacceptable.

We then modified the DRA by constructing new covers holding a hemispherical lens with its flat side at the sample location. We had these 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. The combination of lens and index matching fluid is called an immersion lens. It provides a surface to locate the subject's arm and an advantage of increased signal as well. We found less variation in initial measurements, however, we observed systematic changes in spectral characteristics as successive measurements were made. The cause is unknown. We have hypothesized that blood and fluid in the tissue change with time due to pressure or that the index matching fluid migrates into the tissue and changes its scattering characteristics. In any case, this variation in spectra is a problem; it needs to be reduced.

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. This fixture had a depression at the sampling site to allow the subject's tissue to sag into the depression and contact the lens on the DRA. This was done to reduce the pressure on the sampled tissue of the forearm, compared to placing the forearm directly on the DRA.

Instrument characterization:

We tested the MB 155 initially and verified that it was functioning to specifications. The majority of the effort was directed toward determining the characteristics of the DRA and the optimum operating parameters for the measurements.

Measurements on subjects:

We initially made spectral measurements on the author 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. Finally, we made measurements on the author and on Ganapati Mauze to obtain spectra for each individual to look at the effects of water, which we thought would confound the glucose measurements.

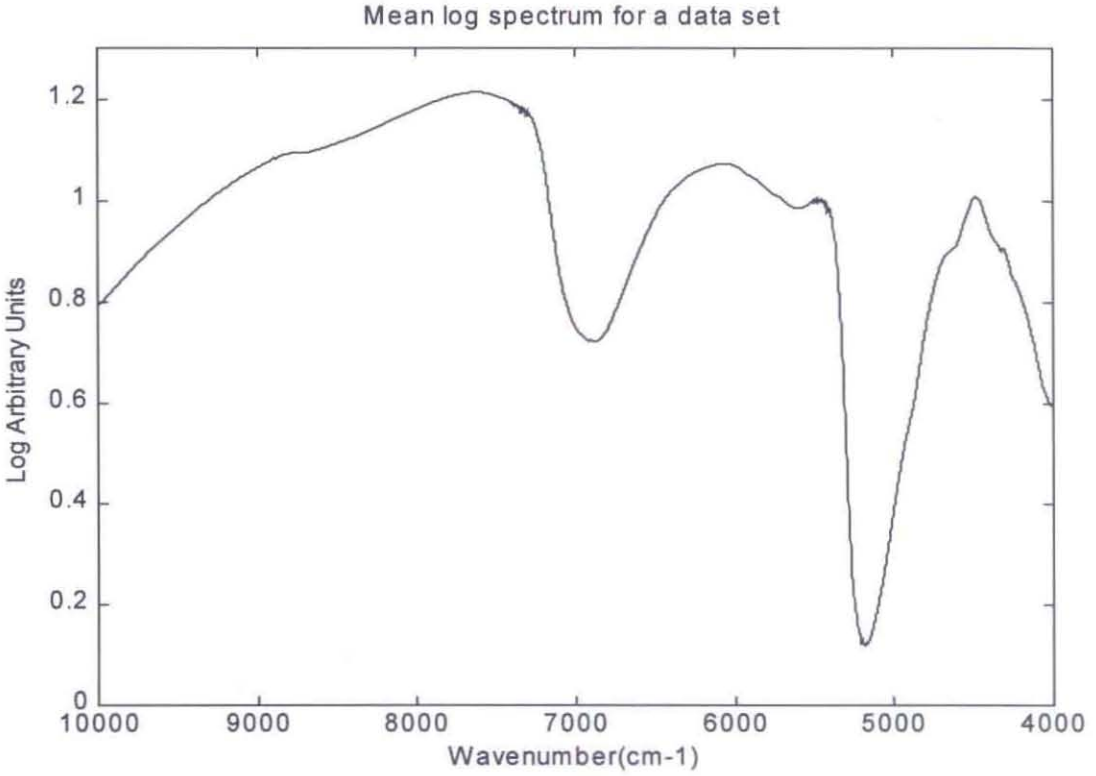
The detailed protocol for the measurements on our 19 subjects is in Appendix 1. Measurements were made using diffuse reflectance from the underside of the left forearm. Spectra were collected from 10,000 wave numbers (1.0 microns) to 4,000 wave numbers (2.5 microns). The acquisition time was about three 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.

We used three methods to vary the amount of water in the tissue of our two subjects. 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.

Data analysis:

We first plotted all spectra (as log spectra) against wave number, both for each subject and as groups. We then plotted the spectra, at selected wave numbers, against sample number. This allowed us to identify anomalous spectra and to identify trends. We eliminated the spectral data for subject number four from the data sets used for further analysis, because it differed significantly from that of the other subjects. A typical tissue spectrum is shown on the next page.

The spectrum below is the mean for a large set of tissue spectra. 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 (perhaps proteins or fatty acids) in the tissue.



Data were then 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 conclude that the gross variations in the spectra between measurement 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. This was done by finding the principal components of pure spectra (water, teflon beads in oil to simulate tissue scattering, pork fat, and so forth). 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. This, however, helped us identify the major causes of variation, which we determined to be related to water absorption and scattering.

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:

An inspection of the spectra indicated that there are anomalous spectra. In particular, the spectra for subject number 4 differ substantially from those of the other subjects. The anomalous spectra are few, and we have no hypotheses for their causes.

There are also monotonic variations in the measured signal as a function of sample number. These variations run over the course of a series of measurements, as long

as the subject's forearm remains in contact, via index matching fluid, with the DRA. We have hypotheses to explain these trends, but we have not verified these hypotheses. The variation may be due to physiological changes resulting from pressure, a change in bodily position before and during the measurement, or migration of the matching fluid into tissue (which should change scattering characteristics).

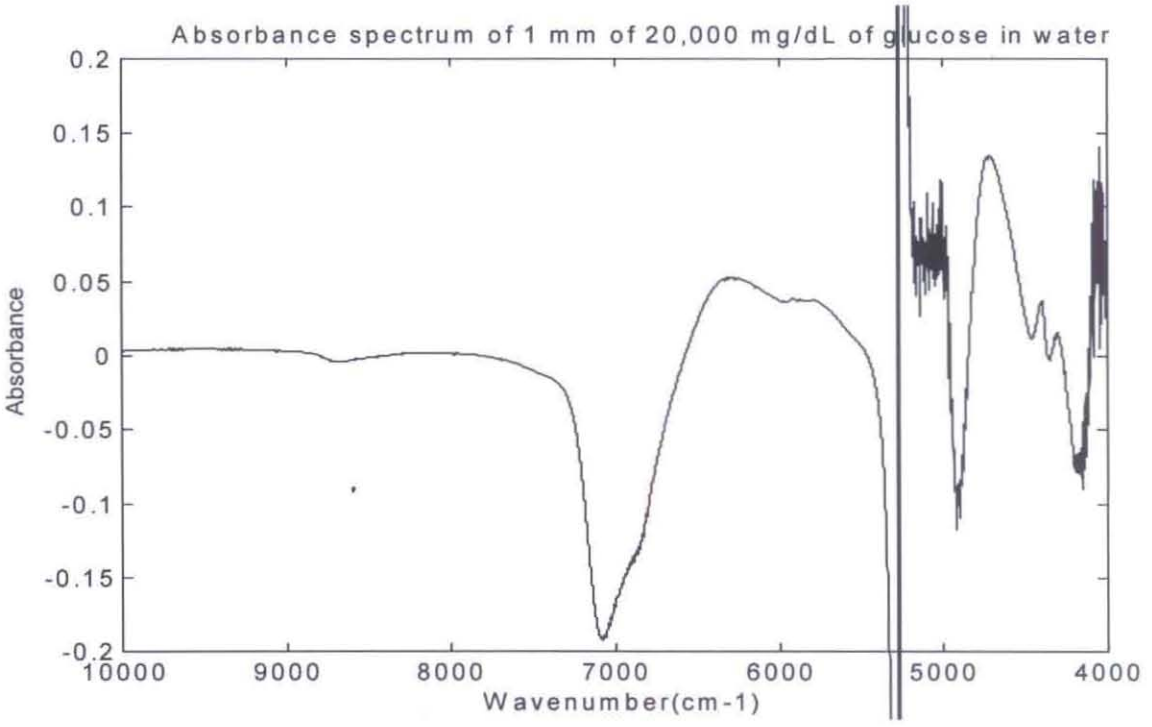
Graphs of spectra demonstrating, anomalies, the range of variations, and trends, as described above, are available from George Hopkins.

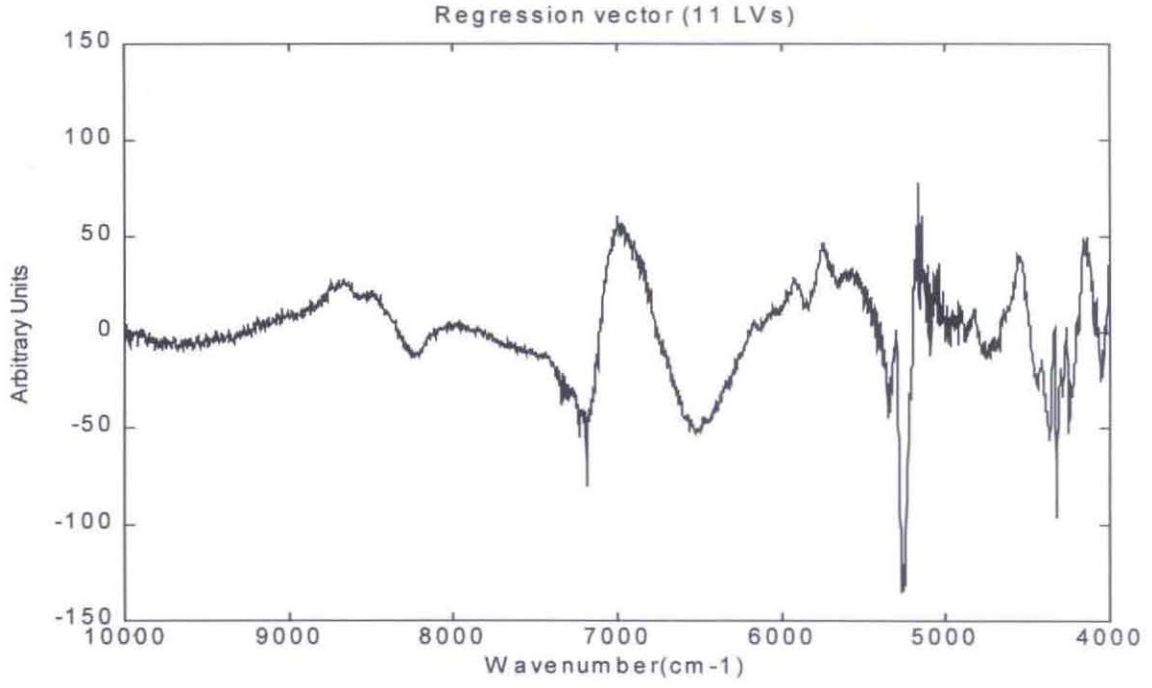
The multivariate analysis is enlightening. About 99 percent of the spectral variance are captured by two principal components (PCs). These PCs are weakly correlated with glucose, capturing about one percent of the variance in the reference measurements. The first component is a spectrum to spectrum variation 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 hadn't expected the glucose information to be so far down in the PC model (as far down as the eighteenth PC).

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. One can safely say that our calibration models have no predictive value! It's impossible to draw any conclusions from the calibration models; however, the error in our predictions is comparable to the error reported in the only published work (Marbach 1993), which describes a calibration model built with data from a single, diabetic subject.

Our regression vectors are similar, feature by feature, to the absorption spectrum of glucose in aqueous solution. We also see large features that match water absorption. 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. This water spectrum, if correlated with glucose concentration, offers a possible way of measuring glucose, provided that other factors are stable or if there is another measurement that can discriminate non-correlated variations in the water spectrum. This similarity is illustrated on the next page.

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.





Recommendations:

Continue the investigation. The market potential for a home glucose meter is both credible and huge. In addition, Pete Melton (Principal Scientist of NCV) says that NCV will inevitably measure glucose in the future, and he is willing to cover at least partial costs of collaborations and clinical trials in follow-on investigations.

Brainstorm. Take a fresh look at the problem, using a brain storming and idea evaluation approach. The problems associated with continuing the current line of research are formidable; we need breakthrough ideas to test.

Solve the two main problems. The first is dealing with short-term noise. We need a large enough signal-to-noise ratio for the glucose signal that we can predict glucose concentration to the plus or minus 10 mg/dL level. The second is keeping the calibration stable for a single subject over a period of days or longer. A third problem, which becomes important after the first two problems have been solved, is providing a calibration that is valid across a large population of subjects. We have ideas for attacking these problems. Instrumentation improvements, enhancement of the sample, or measurements on glucose-related substances may increase the short-term signal-to-noise ratio. Differential measurements on tissues that differ due to application of heat, pressure, chemicals or other stimuli may provide stability in calibration. We may be able to detect a pulsatile component of glucose in arterial blood.

Build a small team. This type of research is inherently multidisciplinary. It can obviously be done with one person, but the scope of the work will be severely limited. The team members do not have to be full time; the needed skills can be obtained from team members who are working on other projects as well. The project will progress faster, however, with more full-time members. Based on experiences during the investigation, consultation is of limited value; the author still had to acquire at least minimal competence in several disciplines. This takes time, and work is done by the minimally competent (I often see work done by the minimally competent in my own field of optics, and I can only imagine what I might be doing!).

Collaborate. At the appropriate time, and, at a minimum, we will need to work with a clinician in order to study diabetic patients. We have potential HP volunteers, who are diabetic. However, we will not ask anyone to deviate from her doctor's instructions, and we will want to measure highly-elevated glucose levels.

Stabilize the spectra. Continuing the present line of work, we would concentrate first on removing measurement-to-measurement variation in spectra due to factors other than glucose.

Mine the data. Due to time constraints, we were unable to study the data as thoroughly as planned. We did not study the variation in spectra across subjects,

we did not refine spectral subtraction, we did not look at windowing (analyzing selected portions of the spectra), and we did not investigate non-linear calibration methods. We don't yet understand whether spectral variations result from subject differences or are "outliers" resulting from measurement error. So, some additional analysis of the data we already have is desirable. This is unlikely to produce a breakthrough, but it may lead to better understanding.

Study a single subject. Our group of subjects was well chosen for studying variables in tissue spectroscopy, but the data from them are not suited to building a calibration model. In addition to improvements in repeatability, a much larger number of samples and greater range of variation of blood glucose concentration (that is, use of diabetic subjects) will be needed to build a better calibration model. Initially, these data should come from a single subject or from a small group of subjects.

Final comments:

HP is in a good position to market the first non-invasive glucose meter. We have no existing business to protect. The product is an instrument, and we know how to develop instruments (The hand-held calculator is an example). Surely, the field is well plowed, the challenges are daunting, and there are large and competent competitors working on the problem. Our situation is not unlike those, which faced researchers of the past, who were looking for ways to make photographs, light bulbs, flying machines or photocopies. The problem is finding the key or keys. Initially, HP only has to decide to take a serious look for the keys.

Please understand some important points. This effort is research, rather than development. Furthermore, ideas are a commodity today; there are several linear feet of patents on every aspect of non-invasive glucose measurement. Value, and eventual success, will come from discovery. Discovery will come from lots of hard work. Understanding the relevant aspects of tissue optics, spectroscopy, anatomy, physiology, biology and medicine will lead to insights, which will point to new directions. At the same time, we must remember that the best measure of our research productivity is the number of new ideas, ***which we have tested in the laboratory***. We have ideas, insights, and some good data. The potential reward is huge. We need to get going.

Appendix 1 – Experimental protocol

Measurement of glucose in living tissue using diffuse reflectance spectroscopy. George W. Hopkins, Second Revision, 8/27/97

Purpose

This protocol outlines steps to be followed in experimental setup, data acquisition and data analysis for tissue measurements on human subjects. The goals of the experiment are (a) characterization of the measurement problem of determining analytes (particularly glucose concentration) in tissue, and (b) assessment of the potential for this non-invasive glucose measurement. Additional measurements, which are not described here, will also be made as needed.

Summary

Suitable non-diabetic subjects are recruited. These subjects are (a) tested in a fasting state, (b) fed a carbohydrate snack with a high glycemic index and tested, and (c) fed a meal and tested. Blood glucose concentration is measured from a finger stick, for each test, and diffuse reflectance measurement are promptly made in the near infrared on the lower (ventral) surface of the left forearm. The data are analyzed as described below. The experimental apparatus and protocol are refined, if needed, and additional tests on the healthy subjects are performed. After data on healthy subjects have been evaluated, and if the results are promising, diabetic patients are tested.

Equipment

A Bomem MB 155 Fourier transform infrared (FTIR) spectrometer and a modified Harrick upward-looking, three-dimensional "Praying Mantis" diffuse-reflectance attachment (DRA) are used. The specific modifications include an immersion lens, an index-matching fluid and a support for the subject's forearm.

The Bomem MB 155 is equipped with a 20 W tungsten halogen lamp (operated on low current to shift the radiation toward the near infrared) as a source and a potassium chloride beam splitter with an extended range coating. The detector is a 1 mm diameter, thermoelectrically cooled indium arsenide unit. Neutral density filters with metallic coatings on a glass (BK7) substrate are used to avoid saturation of the analog to digital converter (a 25 % ND filter is normally used during tissue measurements) and to reduce tissue exposure.

Alignment

The Harrick DRA is supplied with a tilted-mirror alignment device. Using the lid for the DRA that was supplied with it (no lens), a suitable filter (typically 1 % transmission) and the alignment device, the screws at the back of the DRA are

adjusted alternately for maximum signal. The lid with the calcium fluoride immersion lens is installed, and the subject's forearm is contacted to the lens with matching fluid. The screws at the back of the DRA on the right or detector side only are adjusted alternately for maximum signal.

The subject's forearm must be aligned carefully and repeatably. This is done with the handgrip and an adjustable stop that centers the subject's forearm over the lens. The subject is asked to grip the handgrip in the same manner for each measurement.

Instrument/Software Parameters

The data acquisition software used will be GRAMS/386 software from Galactic Industries Corporation. The parameters will be:

Resolution – 4 wave numbers (set on MB 155)

Number of scans – 128

Apodization - Cosine

Spectral range – 10000 to 4000 wave numbers

Data type – Single Beam

Data Storage

The data are stored on the hard disc of the computer that is used for data acquisition. The file used will be C:\BGRAMS\DATA\GEORGE\SUBJxxx\ where xxx is the patient's number. The tests on a given patient will be numbered alphabetically, beginning with sdddddda (dddddd is the date in the form YYMMDD, for example 980822). A separate log, correlated with the data files, of patient information and test conditions will be kept in George Hopkins' current research notebook.

Recruitment of Subjects

Subjects for the initial tests will be volunteer Hewlett-Packard Employees and Retirees who have not been diagnosed with either Type I or Type II diabetes. Subjects for later tests, which will be conducted under medical supervision, will have been diagnosed with diabetes. Subjects in each group will be chosen for diversity in gender, age, pigmentation and amount of subcutaneous fat in the forearms. Subjects must sign a consent form prior to participation in this study.

Subject Protocol

Subjects will be asked to fast after the evening meal prior to measurements (A fasting period of 10 to 16 hours is desirable). Subjects may drink water as needed to quench thirst but will be asked to refrain from other beverages. Measurements will begin immediately upon arrival at work in the morning. Three series of measurements will be taken, each series consisting of a finger stick and a measurement of blood glucose concentration and the acquisition of spectra. The first series will be taken upon arrival. Then the subject will eat a carbohydrate snack, composed of foods with a high glycemic index and no fat, that is equivalent in calories to 70 to

98 grams of glucose. At 30 minutes after the snack has been finished, a second series of measurements will be taken. The subject will then be allowed to eat breakfast. At two hours after the initial snack was finished, or at one to two hours after breakfast, the third series of measurements will be taken.

Data Acquisition

Subjects will be asked to wash their hands and to lance a finger tip, to accumulate about 50 microliters of blood in a micro centrifuge tube, and to present this sample to the sipper on the analyzer. The investigators will assist the subjects and will operate the controls of the Yellow Springs Instruments Model 2300 Glucose Analyzer to measure the glucose concentration in the subject's blood. Subjects will asked to position their left arm on an armrest, which is designed for a high degree of repeatability in repositioning. The rest has a handgrip and an adjustable stop, which will be set for each subject. This locates the same portion of the subject's forearm over the sampling point for any given measurement. A large drop of OxyChem Fluorolube T-80 oil will be used contact the subject's tissue to the lens of the DRA. Three spectra will acquired using the Instrument/Software Parameters above for the fasting and post meal states. Six spectra will acquired using the Instrument/Software Parameters above after the carbohydrate snack. The subjects will be asked to wash their left forearms with soap and water before and after the measurements. The DRA and arm support will be cleaned with lens tissue and isopropyl alcohol between subjects. After measurements, subjects fingers will be cleaned of blood with alchohol wipes and washing with soap and water.

Data Analysis and Interpretation

Data will be analyzed by two methods. Principal Component Analysis (PCA) will be used to identify the portions of spectra that vary during our measurements. These components will be associated, to the extent possible, with physiological variables and with sources of non-repeatability in measurements. Partial Least Squares (PLS) Analysis will be used, together with blood glucose concentration determined from a finger stick, to construct a calibration model.

Together, these analyses will characterize the measurement problem. Armed with this information, we can determine if the measurement is practical and estimate whether causes of uncertainty can be reduced and whether or not it might be possible to enhance the desired components by increasing their variation using external means.

Appendix 2 – Workers in the field

Workers in the non-invasive glucose measurement field fall into two general categories, academia/government and industry. These categories are not orthogonal. Most academicians seem to have some industrial affiliation, and industries often support academic research. Some employees of government laboratories consult for industry, and the government often supports companies through CRADAs (cooperative research and development agreements) or through contracts. The primary difference is the degree of openness. Industry rarely publishes or presents its work, other than through patent documents, or if the work has been abandoned. Workers in academia and government publish and present their work often.

This list is not all-inclusive. We have not listed workers, whom we believe to be inactive, but have tried to include major, active workers.

We list workers alphabetically, using company/institution name or researcher name:

Abbot Laboratories of Abbott Park, IL sells strip home glucose meters through their subsidiary MediSense, Inc. of Bedford, MA. Their meters, sold under the Exactech and Precision trade names, account for a modest share of the U. S. market, about 4 percent in 1995. They likely have a program in NIGM, but little is known. They hold USP 5,725,480 (Non-invasive calibration and categorization of individuals for subsequent non-invasive detection of biological compounds, 3/10/98), and WO 97/34521 (Non-invasive measurement of optically active compounds). Abbott is partnering with SpectRx, Inc. to distribute a HGM that samples interstitial fluid with a laser.

Alam – M. Kathleen (Kathy) Alam of Sandia National Laboratory presented "Near-infrared spectroscopy of lysed blood: pH effects" at BiOS '96, SPIE 2680-15, where pH was predicted from NIR spectra.

ArithMed – Formerly MedScience

Bayer Diagnostics acquired the Ames Company and markets meters under the trade names of Elite and Encore. They have registered "Glucometer" as a trade name. In 1995, they had about a 12 percent share of the U. S. market. We have no evidence of a non-invasive measurements program at Bayer.

Biocontrol Technology, Inc. (BICO) of Pittsburgh, PA, and their subsidiary, Diasense, Inc. of Indiana, PA, have been developing a non-invasive glucose meter since about 1989. We received a video from BICO in the September 1996, which describes their company and their product. The company claims to have invested

$60M over 8 years, growing from 7 employees to 150 employees. They offer their device, the "Diasensor 1000", overseas, for the U.S. equivalent of about $8k. They have manufactured 30 to 50 of the devices. The company failed to obtain approval from the U.S. Food and Drug Administration (FDA) to market their product in the U.S. There was subsequent public activity, involving both an FDA review panel and hearings before the Committee on Commerce of the U.S. House of Representatives. BICO still does not have FDA approval. It is defending a class action suit, filed in the U.S. District Court for the Western District of Pennsylvania, alleging that they engaged in a scheme to deceive the investing public in promoting their "Diasensor 1000" analyzer. BICO canceled supplier contracts in June 1997, but it continued testing. According to Michael Heise (Ralf Marbach's thesis advisor), Ralf Marbach, who published some of the best work on non-invasive glucose measurement by tissue spectroscopy, began working for BICO in 1993 and is currently employed there. Their instrument is a table-top instrument and contains a spectrograph that is about 8 by 8 by 2 inches (shown in their video). We do not know the wavelength range. A salesperson for Sensors Unlimited told me that BICO had bought a 2.2 micron cutoff InGaAs detector array from them in July 1995. I doubt that this range is being used in the "Diasensor 1000", but I frankly don't know. The unit measures on the underside of the patient's forearm. They hold USP 5,070,874 (Non-invasive determination of glucose concentration in body of patients), USP 5,460,177 (Method for non-invasive measurement of concentration of analytes in blood using continuous radiation spectrum), and WO 97/30629 (Method and apparatus for non-invasive blood glucose sensing).

Biotronics Technologies, Inc. of Waukesha, WI, developed an NIR technique for noninvasive measurement of human blood chemistry (Schlager). The principal in the company is Kenneth J. Schlager. Called TAMM, for Transcutaneous Analyte Measuring Method, it used an InGaAs array. Experimental work, involving over 1000 patients, was funded by the U.S. Navy under an SBIR program and a CRADA with the Naval Health Research center. The results are describe in SPIE Vol. 2386, pp. 174-184 (1995). Nine analytes, including glucose, with a 14% error of predicition, were measured, but details on experimental conditions are lacking. Biotronics conducted a road show in 1995 in search of funding to develop a medical product. The current status of their effort is unknown. Biotronics, together with W. R. Grace and Co., hold USP 5,242,602 (Spectrophotometric monitoring of multiple water treatment performance indicators using chemometrics, 9/7/93).

Block – Myron J. Block is an inventor. He holds several patents on tissue spectroscopy, most of which have very broad claims, including USP 5,424,545 (Non-invasive, non-spectrometric infrared measurement of blood analyte concentrations, 6/13/95). Recent patents are assigned to Optix LP of Jensen Beach, FL.

Böcker – Dirk Böcker of Boehringer Mannheim has published and patented extensively in NIOM.

Boehringer Mannheim GmbH. (BM) dominates the European market for stick and strip home glucose testing and had a 30 percent share of the U. S. market in 1995. They market meters under the trade name Accu-Chek. BM has a substantial program to develop non-invasive glucometry. BM had been a privately-owned business, but it was acquired by Roche Pharmaceuticals in 1998. BM holds U. S. Patents 5,710,630 (Method and apparatus for determining glucose concentration in a biological sample),and 5,692,504 (Method and apparatus for the analysis of glucose in a biological matrix).

CME Telemetrix (CMET) of Waterloo, ON, Canada, announced on 8/28/97, the completion of the first phase of limited clinical tests of its non-invasive glucose monitor at the McMaster Medical Center in Hamilton, ON. They judged their measurements clinically acceptable based on a Clark Error Grid analysis. No details are known.

Cohen – Glenn M. Cohen, professor of biological sciences at Florida State University, Tallahassee, FL, is collaborating on development of a device for non-invasive monitoring of blood glucose.

Cote – Gerard L. Cote is a professor in the Bioengineering program at Texa A&M University at College Station, TX. He and his students are regular presenters at the conferences on biomedical optics; papers are often on in-vitro spectroscopic methods. He is an inventor of a polarimetry method described in USP 5,209,231 (Optical glucose sensor apparatus and method, 5/11/93, assigned to U. of Connecticut).

Cygnus, Inc. of Redwood City, CA is developing the GlucoWatch, a meter, worn like a wrist watch. It measures the glucose concentration in interstitial fluid, which is obtained by reverse iontophoresis. Cygnus is publicly traded. It had a $20M cooperative agreement with Becton Dickinson, Inc. from about March 1996 until April, 1998. The agreement was terminated because Cygnus did not submit a 510(k) application to the FDA on schedule. Cygnus has conducted clinical trials, and says that it will apply for 510k approval for its product in the third quarter of 1998. It cited delays in development due to technical problems with the device's components.

Douglas – Joel Douglas is CTO of Mercury Diagnostics, Inc.

EMBA-GmbH (EMBA), Handel Vogel GmbH , has announced a non-invasive HGM. EMBA stand for Einstichfrei messende Blutzucker Anzeigesysteme. Nothing more is known about this product. Source was www.diabetiker-mailbox.com.

Essenpreis – Matthias Essenpreis of Boehringer Mannheim has published and patented extensively in NIOM. He was a Principal in the Technology Assessment Office in Fremont, CA on 4/15/98.

Futrex Medical Instrumentation, Inc. of Gaithersburg, MD is developing a non-invasive measuring device called the "Dream Beam". On September 23, 1996, the U.S. Securities and Exchange Commission filed a fraud action in U.S. District Court for the District of Columbia against Futrex and its CEO, Robert D. Rosenthal, for falsifying study results for its "Dream Beam" analyzer, and work has been discontinued. Futrex began researching the potential of using near infrared (probably in the "silicon region, 0.7 to 1.1 microns) technology to perform non-invasive glucose measurement in 1988. Futrex established a CRADA with the U.S. National Institutes of Health in 1990. This research was unable to demonstrate a calibration suitable for multiple patients, but accurate measurement for a single individual was claimed (I have not seen published data). In 1991, Futrex completed development of a research instrument called the SORTRONIC and conducted a field study of over 700 volunteers at the Mt. Sanai Medical Center in NYC. They claimed success at the June 1991 meeting of the American Diabetes Association (ADA). At his time, Futrex began development of a small, battery-powered, non-invasive meter. The instruments developed from 1991 to 1994 did not provide needed accuracy. In 1994, Futrex abandoned the idea of a universal calibration and began trials of a meter which required individual patient calibration. In order to finance trials, Futrex scheduled an initial public offering (IPO) of its stock for December 1995. This was abandoned, and the SEC action is connected with this IPO. The SEC action alleges that data from as early as the 1991 Mt. Sanai study failed to demonstrate any correlation between the infrared scans and blood glucose levels. Robert D. Rosenthal, inventor, and Futrex, assignee, have a large number of U.S. Patents on non-invasive blood glucose measurment, including 5,703,364 (Method and apparatus for near infrared quantitative analysis, 12/30/97), 5,438,201 (Method and apparatus for restraining fingr motion in blood analyte optical measurement, 8/1/95), 5,086,229 (Non-invasive measurement of blood glucose, 2/4/92); and world patents 95/31133 (Non-invasive near-infrared quantitative measurement instrument), and 95/20906 (Procedure for verifying the accuracy of non-invasive blood glucose measurement instruments).

Haaland - Haaland, David M. of Sandia National Laboratories is a widely-recognized expert in chemometrics. He was a pioneer in the work that is now continuing at UMN and RGMT.

Heriot-Watt University, Edinburgh, Scotland – Hugh A. McKenzie, with his student, Helen Ashton, has a program on photoacoustic spectroscopy. Earlier work was done by Arlene Campbell (Campbell), who left the group in 1996.

Integ, Inc. of St. Paul, MN, is developing the LifeGuide, a HGM, which takes a sub microliter sample of interstitial fluid and uses IR to measure glucose concentration. In June, 1998, Integ presented a summary of studies conducted at Mayo Clinic and the University of Minnesota. Integ has not stated when it intends to apply for FDA approval.

Kaiser – Nils Kaiser was one of the earliest workers in spectroscopic analysis of blood. He described a method based on ATR in *Laser Absorption Spectroscopy with an ATR Prism*, IEEE Transactions BME-26, No. 10, pp. 597-600, 1979 and in USP 4,169,676 (Method for determining rh contents of metabolic products in the blood, 10/2/79).

Kurabo Industries, Ltd. of Osaka, Japan, presumably has an IR device under development.

Kupershmidt - Vladimir Kupershmidt is associated with Sunshine Medical Instruments, Inc. of Sausilito, CA.

LifeScan, of Milipitas, CA, a Johnson and Johnson Company, dominates the U.S. market for stick and strip home glucose testing. Lifescan was bought by Johnson and Johnson for $930M in 1995. LifeScan is thought to have conducted internal investigations in the past and is believed to now have a large program to develop non-invasive glucometry through contracts with Rio Grande Medical Technology (see note below), though to be about 3 million dollars per year.

MedScience – Presumably their current name is ArithMed, and they have a partnership with Samsung Fine Chemicals, Ltd.

Mercury Diagnostics, Inc. of Palo Alto, CA is developing a "painless" system for HGM which they claim will be available to the public in October 1998. It does a stick in tissue of a body part other than the finger tip. It has a "milking" device and uses conventional enzyme measurement with a photometer for detection. They have filed numerous world patent applications, which are not listed in this report. Joel Douglas, CTO, provided this information at a public talk on 4/15/98.

MiniMed of Sylmar, CA has a business in insulin delivery through infusion pumps. They are involved in a closed-loop delivery system using a removable in-vivo sensor. They are reported to be developing a system for continuous monitoring of blood glucose level, but no details have been disclosed. They hold U.S. patent 5,569,186.

Mitsui Mining & Smelting Co., Ltd., of Tokyo, Japan, developed a blood glucose level meter that is painless and simply requires having the patient insert a finger into the device. It is as accurate as conventional devices. The company will supply trial equipment to four major hospitals n Japan in April 1998. Mitsui is a multinational company, primarily a supplier of materials and a manufacturer, with about 3000 employees and annual revenues of approximately $2B. Ref: Nikkan Kogyo Shimbum, 01/21/98, p.23 and Mitsui web site.

Novo Nordisk A/S of Bagsvaerd, Denmark is assignee to USP 5,452,716 (Method and device for in vivo measuring the concentration of a substance in the blood, 9/26/95), which claims the use of one or more additional wavelengths to remove inteferences, where at least one wavelength is in the range 3 to 10 microns. Novo

Nordisk is the world leader in insulin and diabetes care and also manufactures and markets a variety of other pharmaceutical products.

Robinson – Mark Ries Robinson, an EE from Stanford and an MD, is a principal in Rio Grande Medical Technologies (He goes by "Ries"). He was an early researcher in non-invasive glucose measurement at the University of New Mexico. He visited HPL in 1992 in an attempt to get HP to support his work. Robinson was first author of "Nonnvasive Glucose Monitoring in Diabetic Patients: A Preliminary Evaluation", Clinical Chemistry 38/9, 1618-1622 (1992).

Rio Grande Medical Technologies, Inc. (RGMT) of Albuquerque, NM is a major player in non-invasive glucose monitoring R&D. It was founded by Ries Robinson, who did early studies in non-invasive glucose measurement. It is closely allied with The University of New Mexico (UNM) and it's medical center (UNMMC) and has facilities on the campus. It is also closely allied with Sandia Laboratories, where David Haaland, an expert in chemometrics and consultant to RGMT, is employed. RGMT also employees Bob Messerschmidt, an optical engineer who, as a SpectraTech employee, designed many accessories for optical spectrometers. Altogether, RGMT is thought to have about 30 employees. RGMT began with a CRADA with Sandia National Laboratories and has a U.S. Department of Defense contract to build a non-invasive medical analyzer for electrolytes. It is thought to be in the third year of a 3M$ per year contract with LifeScan to develop a non-invasive glucose meter and is reported to be in clinical trials with about 600 patient at the UNMMC. Supposedly, they are making good progress. None of this information, however, has been verified. Ken Ward, an HP employee at the InkJet Business Unit (IJBU) in Corvallis, OR, was a member of the first team at UMN. Ries Robinson, an EE from Stanford and an MD, was taking an endocrinology class from Phil Eaton, an endocrinologist an UNM. Robinson, Eaton, Haaland and Ward started a project in the mid infrared (MIR) using attenuated total reflection (ATR) with blood samples. They worked with diabetic patients, using a meal test. The predictive power of the MIR measurements was 10 mg/dL at one sigma. Then they moved to the NIR. Ken left shortly after this. They had found that, when the model for one patient was used with another patient, there was a bias or intercept offset, but the slopes were consistent. Even so, about 10 to 20 percent of patients had fatty acid metabolism problems, and their slopes were different. Offsets occurred even on the same patient over a period of months. Ken guessed that RGMT had up to 37 employees in mid 1996, with 25 of these actively researching glucose. He suspected that they were using 1.6 microns and had abandoned use of fiber optics. At the end of 1997, they were still working and were making good progress, with a clinical trial in process involving more than 600 patients at UNMMC. Ken, of course, has no internal information, so his suppositions about RGMT, based on hearsay, should be treated accordingly. RGMT has a number of U.S. Patents (USP) and world patents (WO), including WO 97/06425 (Improved diffuse reflectance monitoring apparatus,

2/20/97); WO 97/05819 (Method for non-invasive blood analyte measurement with improved optical interface); USP 5,435,309 (Systematic wavelength selection for improved multivariate spectral analysis, 7/25/95, assigned to UNM); and USP 4,975,581 (Method of and apparatus for determining the similarity of a biological analyte from a model constructed from known biological fluids, 12/4/90, assigned to UNM).

Sandia National Laboratories – M. Kathleen (Kathy) Alam and David M. Haaland are researchers at Sandia, who have done recent work in NIGM and related fields.

Schlager - Kenneth J. Schlager, is a principal in Biotronics Technologies, Inc.

Solid State Farms of Reno, NV has a non-invasive measurement technology for analytes and electrolytes, including glucose, based on RF measurements. They approached HP in 1995 and provided details of their technology under a CDA. HP chose not to work with them. They hold USP 5,363,052 (Permittivity spectroscopy apparatus and method, 11/8/94), USP 4,765,179 (Radio frequency spectroscopy and method using multiple frequency waveforms, 8/23/88), USP 4,679,426 (Wave shape chemical analysis apparatus and method, 7/14/87), and several world patents.

SpectRx of Norcross, GA is developing a personal glucose monitoring system based on a transdermal technique for drawing interstitial fluid, called micropore, that is claimed to be rapid and painless. SpectRx made a $14M IPO in July 1997 and is now traded on the NASDAQ exchange under SPRX. SpectRx, Inc. expected to launch its first non-invasive medical diagnostic product in 1997 and build on its product development agreements with Abbott Laboratories, BM, and Healthdyne Technologies, according to its underwriter, Hambrecht and Quist. To date, there has been no news of any product introductions. SpectRx has announced a bilirubin meter called the BiliCheck, which it is developing with Healthdyne. SpectRx has an agreement with Abbott Laboratories, which made a $500k progress payment to SpectRx on 2/4/98, to market its home glucose meter. SpectRx is also developing a diabetes screening system, which will be marketed by BM.

Sunshine Medical Instruments, Inc. of Sausilito, CA holds USP 5,448,992 (Method and apparatus for non-invasive phase sensitive measurement of blood glucose concentration, 9/12/95), which claims a measurement of glucose in tissue using circular dichroism, and USP 5,398,681 (Pocket-type instument for non-invasive measurement of blood glucose concentration, 3/21/95). The inventor for these patents is Vladimir Kupershmidt.

Technical Chemicals and Products, Inc. (TCPI), of Pompano Beach, FL, is developing the TD Glucose Monitoring System. It is a transdermal device for the home market. Development has been completed, and clinical trial were supposedly beginning in the fall of 1997.

Technomedica of Mosow, Russia, markets a non-invasive bilirubin meter. Technomedica seems to have sponsored work by Aristarchof and Balashowsky of the Russian academy of Science on the influence of glucose on water infrared spectra, and it may be working on a non-invasive glucose meter.

University College London – Mark Cope and Matthias Kohl have collaborated with BM on studies of scattering in tissue and glucose measurement (Kohl).

VivaScan Corp. of Worcester, MA is a small startup company developing a non-invasive glucose monitor. They claim to use an Optical Bridge ™ technology. Source: Resume of Rebecca A. Kupcinskas, 5/97.

VTT Electronics of Oulu, Finland, posted an abstract, *Non-invasive glucose measurement by near infrared spectroscopy*, on the web on 5/16/97, but the link was dead and nothing more is known. The key author was Jussi Tenhunen.

Ward – Kenneth J. Ward works for HP's Ink Jet Business Unit in Corvallis, OR. He was part of the pioneering team with Rees Robinson at UNM. Ken visited with us on June 7, 1996 to tell us about his experiences and to advise us on our investigation.

Appendix 3 – Instrumentation

We used a Bomem MB 155 FTIR spectrometer, serial number SZM4908N, for this study. It was ordered in January 1996 at a system cost of over $50,000 and was received in April 1996. We purchased both NIR and MIR sources and detectors and diffuse reflectance and ATR accessories, because the instrument had to be suitable for this investigation and for Chris McNulty's work with glucose solutions, as well as being a resource for future projects within the Analytical/Medical Lab.

The source for NIR is a 20 watt, tungsten-halogen lamp. Although Bomem supplies it in pre-aligned form, the lamp is an industry standard part, an L9404. It has a filament that is 2.9 mm long by 1.2 mm in diameter. This lamp is operated at 11.0 V (below its 12.0V nominal) to produce 16.8 watts and a color temperature of 2872 K. The spectrum approximates 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 is 29.0 degrees.

The beam splitter has an extended range NIR/MIR coating on a potassium chloride substrate.

The detector is a 1mm diameter, InAs, unit from EG&G Judson with a sapphire window. It is cooled to –35 degrees Celsius by a three-stage thermoelectric cooler. We would gain by going to a larger detector or by using an InSb detector with a cold stop and a cold filter, but this type of detector must be cooled by liquid nitrogen.

We considered four FTIR instruments, ones from Bomem, Perkin-Elmer, BioRad and Nicolet. We either ran, or the vendor ran, a performance test which measured noise in the "water windows" in the NIR. The Bomem proved to be as good as the other instruments, and it will be much more convenient if we use it for field trials. All instruments were essentially limited by their A/D converters, rather than by shot noise, in this test. We wrote an extensive report, available from George Hopkins, on these tests.

An FTIR distributes noise across the entire spectrum, which can be important when the source spectrum, modified by absorbance, is not uniform. If detection is shot noise limited, rather than detector or background limited, the multiplex advantage of the FTIR is not realized. A dispersive (grating) spectrometer is limited by shot noise that is proportional to the square root of the signal. This can be an advantage, and a final product might use dispersive optics. Using an FTIR, however, was not a serious compromise.

Our measurements on tissue were made with a Harrick, 3-D, upward-looking, Praying Mantis, DRA. Marbach used a custom DRA, with optics similar to a Cassegrain telescope, with nearly normal illumination. Messerschmidt (WO 97/06425) reported that the Harrick DRA is not suited to tissue measurements

because tissue scatters light strongly forward. Our tissue optics model indicates that light is quite diffusely reflected from a subject due to multiple scattering events. In use, our diffusely reflected signal needs to be attenuated, indicating that the Harrick DRA collects adequate scattered light.

Appendix 4 – Regulatory issues

Regulatory issues fall into three categories. The first is regulation of devices, the second is radiation safety, and the third is environmental health and safety.

The Division of Clinical Laboratory Devices of the CDRH of the FDA regulates medical devices and medical diagnostic tests, such as a non-invasive glucose meter for HGM. Approval requires that these devices be demonstrated to be equivalent to existing HGMs (510(k) approval), which requires proving that a device is safe and effective on a suitable patient population. A discussion about obtaining such approval is beyond the scope of this study, but it has proven to be elusive for two developers, Futrex and Biocontrol. Suffice it to say that his procedure requires extensive testing, which would be quite expensive. Such approval would be needed for any new HGM.

Radiation safety is also an issue, because we irradiate tissue with light in the course of our measurement. The underlying physics are heating of tissue, which our radiation causes, and the breaking of chemical bonds, which our radiation does not do. Tissue may die if heated to 41 degrees Celsius or hotter, which is 4 degrees above the normal body temperature of 37 degrees Celsius. Our measurement does not cause this degree of heating. The situation in complex, because the tissue may be hotter than normal initially, due to fever, and the natural cooling function of the body, which is very efficient, does depend on an individual's health. There is also concern that radiation may cause health problems, independent of heating and the breaking of chemical bonds. This can only be determined by epidemiological studies and by politics. Presently, there is no regulatory standard for incandescent light exposure, but a regulatory standard may be introduced. Joe Tajnai of HP is a committee member is the group studying change to IEC 825, which may introduce regulation of exposure to incandescent light.

The following two standard operating procedures (SOP) cover methodology for our subject investigation and are required by OSHA for environmental health and safety. The Subject Consent Form is required due to liability concerns.

DS&D SOP DiagNoSTIX-1 Original Version 7/17/97

Sterilization of Spectroscopic Apparatus and Subject Hygiene

1.0 Purpose

This Standard Operating Procedure (SOP) documents the procedure for subject hygiene and sterilization of the diffuse reflectance attachment and arm support used for tissue spectroscopy.

2.0 Scope

The procedures detailed here are to be followed by all individuals, who will be testing subjects, working specifically on the "DiagNoSTIX" project (tissue spectroscopy).

3.0 Responsibility

The primary responsibility for compliance with this SOP resides with the individuals carrying out the outlined tasks. Ultimate responsibility for this SOP resides with the Project Manager, Michael Greenstein.

4.0 Operation

1. Subjects are requested to wash their left forearms and hands with soap and water in the restroom prior to measurements.
2. The immersion lens, diffuse reflectance attachment (DRA) lid, and arm support will be wiped with a pad saturated with isopropyl alcohol prior to a measurement or series of measurements on any one subject.
3. Subjects are requested to wash their left forearms and hands with soap and water in the restroom after measurements.

DS&D SOP DiagNoSTIX-2 Original Version 7/17/97
Measurement of Blood Glucose Concentration

1.0 Purpose

This Standard Operating Procedure (SOP) documents the procedure for measurement of blood glucose concentration using a finger stick and the Yellow Springs Instruments Model 2300 STAT Plus Glucose and Lactate Analyzer (YSI 2300).

2.0 Scope

The procedures detailed here are to be followed by all individuals, who will be testing subjects, working specifically on the "DiagNoSTIX" project (tissue spectroscopy).

3.0 Responsibility

The primary responsibility for compliance with this SOP resides with the individuals carrying out the outlined tasks. Ultimate responsibility for this SOP resides with the Project Manager, Michael Greenstein.

4.0 Operation

4. Subjects will perform finger sticks on themselves.

5. A lancet, in its original, sanitary packaging is used. Any lancets found that are not in original, sanitary packaging will not be used and will be placed in biohazard sharps container immediately and disposed of according to STIX SOP-3.
6. Prior to lancing, subjects will clean the end of the lancing device that will contact the skin with a pad saturated with isopropyl alcohol.
7. Prior to lancing, subjects will clean the skin on the fingertips that they will lance by washing with soap and water and air dry.
8. Subjects will squeeze a large drop or two of blood into a micro-centrifuge tube (A 25 microliter sample, minimum, is required).
9. Subjects will take the sample to the YSI 2300 and enter the sample for analysis.
10. Subjects will place the used lancet and micro-centrifuge tube in biohazard containers immediately. These will be disposed of according to STIX SOP-3.
11. Individuals involved in testing subjects and who will handle any used lancets or lancing devices that have not been sterilized with alcohol, and individuals performing maintenance on the YSI 2300, will wear latex gloves to prevent possible contact with any subject's blood.

DiagNoSTIX SUBJECT CONSENT FORM

The following discussion outlines the risks involved in participating in this protocol and the waiver of confidentiality should accidental exposure to blood by either of the parties occur. Please understand that we regard this as a serious issue and have instituted safety procedures with this in mind.

Statement of Risks:

Working with blood and/or blood products always carries some risk to those handling these reagents. In these experiments, both the test subject and the investigator have the potential to be exposed to blood.

1. You, the subject, will only be exposed to your own blood; at no time will you be exposed to someone else's blood.
2. We will ask you, the subject, to lance your fingertips and provide blood samples for analysis of blood glucose concentration. To avoid infection, we will ask you to wash the site of the lancing with soap and water immediately prior to lancing. We will ask you to squeeze a large drop of blood into a micro centrifuge tube and to load this tube into a blood glucose analyzer. We will ask you to dispose of the lancet and the micro centrifuge tube in a bio hazard container, and to bandage your wounds.

The source for the spectroscopic measurements is an incandescent lamp that is imaged onto your tissue by the optics of the spectroscope. This has been tested by the investigators for extended periods and has been observed to produce no sensation of heat or after effects; however, there are no standards for exposure, and there have been no studies of long-term effects of incandescent light on tissue.

The index matching fluid, which will be applied to your fore arm, is an inert fluorocarbon oil, OxyChem Fluorolube T-80. The Material Safety Data Sheet (MSDS) is available for your inspection. We recommend that you avoid contact of the oil with your eyes; in the event of eye contact, flush your eyes with water immediately and notify the investigators. We recommend that you remove the oil from your skin after the experiment by washing with soap and water. Please notify the investigators immediately if skin irritation occurs.

The experiment requires overnight fasting and eating of meals with high carbohydrate and/or fat content. This poses no short-term health risk to a healthy person. Please notify the investigators if you have any dietary restrictions.

The measurements of blood glucose concentration could lead to a diagnosis of disease in you, the subject. Measurements on each subject will be available to the subject and to the investigators. The investigators will notify any subject if their fasting blood glucose concentration is considered to be abnormally high.

Results of measurements may be made known to individuals other than the investigators, but anonymity will be preserved unless specific written permission to identify data is given by the subject.

Implied Consent:

You have the right to privacy with respect to having your blood tested for the presence of the infectious agents, Hepatitis B and HIV. It is important for you to know that by taking part in these experiments, you *waive* your right to that confidentiality. If the investigator accidentally sticks himself/herself with lancets contaminated with your blood, OSHA policy dictates that your blood will be tested for the infectious viruses, Hepatitis B and HIV. It is important for you to understand that, in this situation, you waive your right to confidentiality, even if you do not want to have your blood tested. This is a limited loss of confidentiality: in the event of such a situation, both parties will be referred to an outside health care professional who will handle the testing and reporting between the two parties. With the exception of the two people involved and the Occupational Health Nurse, no one at HP will know the results.

Your signature and date indicates that you have read and understand the risk and implied consent as outlined above. A copy of this form will be on file with the HP Occupational Health Nurse.

Printed Name Signature
Date

DiagNoSTIX Project, HPL Medical Department, 8/21/97

Appendix 5 – Anatomy

The anatomy of the skin is important in non-invasive optical measurements. We will not discuss it in detail here, because it is well covered by Marbach (Marbach 1995 and 1993). The non-homogeneity of the skin is a factor in degrading repeatability of measurements, but this has not been well studied. Such a study will be required if work is continued.

Skin contains light absorbing pigments, principally melanin. Light absorption by melanin is important in the visible and for the infrared out to about 1 micrometer wavelength, but it is not thought to be an issue for the wavelengths used in this work.

Many aspects of the skin, including anatomy, are discussed in detail in these references: (Spearman), (Jarrett), (Elsner), (Berardesca 1994), (Wilhelm), and (Berardesca 1995).

Appendix 6 – Physiology

The mechanisms of glucose metabolism are complex. In addition, there are physiological changes, which can interfere with glucose measurement. Some of these, for example, the effect of hematocrit on determination of glucose concentration, are well known. For non-invasive measurements, the situation is more complex. Water content of the blood and tissue is a major confounding factor in non-invasive optical measurements, and it can vary up to 10 percent due to body position. Young (Young) treats these issues thoroughly.

Many aspects of the skin, including physiology, are discussed in detail in these references: (Spearman), (Jarrett), (Elsner), (Berardesca 1994), (Wilhelm), and (Berardesca 1995).

Appendix 7 – Tissue optics

Tissue optics is discussed in the short course notes of Steve Jacques and in several papers in Tuchin's collection (Tuchin). Although there is an extensive literature on theory for propagation of light in turbid media (tissue is an example), the analytical work is of little practical use. Computer modeling, using Monte Carlo simulations, is the best approach. Even this is hampered by a lack of good measured parameters for wavelengths other than those used for laser therapy. A further complication in modeling is the complex structure of real skin, which must be represented by a simple structure in the computer models. We did no work in computer modeling due to a lack of resources. Fortunately, Marbach has done simple computer models of tissue at wavelengths in the NIR where glucose absorbs light. These models were useful in helping us understand the measurement problem and giving us confidence that we were making valid measurements.

Using the log of spectra is common practice in diffuse reflectance spectroscopy. It is clearly advantageous in tissue spectroscopy as well, but tissue is not a Beer's Law absorber (meaning that the log of the signal or absorbance is not proportional to analyte concentration). Scattering complicates the relationship of signal from tissue to analyte concentration. In the limit where tissue can be represented by a diffusion model, analyical expressions can be written for the relationship between transmission and path length (Schmitt, equations 4 through 10). Schmitt also measured phantoms and demonstrated, by scaling, the bottom line: this relationship is non-linear. This is a source of error in measurement, because the multivariate calibration methods we used assume that the relationship between the measured quantity and analyte concentration is linear. In fact, it is complicated by turbid medium variations. Schmitt describes techniques for processing spectra under turbid conditions, but we learned about his work too late and did not have sufficient resources to test these in our investigation.

Appendix 8 – Multivariate analysis and calibration (chemometrics)

The classic reference on the mathematical techniques that we used for data analysis is Martens. This is an excellent, but rather sophisticated, book. A better starting point for anyone who is not familiar with chemometrics is Esbensen.

Our data analysis was performed using the PLS_Toolox Version 1.5 from Eigenvector Research, Inc. of Manson, WA. This software package, developed by Barry M. Wise and Neal B. Gallagher, runs under MatLab, a mathematical computing environment, which is well suited for matrix manipulation. For PCA, we used the functions mncn (mean centering) and pca. For PLS, we used the functions mncn and plscvblk. A dual processor HP Vectra XU 6/200, 200 MHz Pentium Pro, computer, was used. This machine was adequate for our work.

Appendix 9 – Data organization and location

The feasibility study for DiagNoSTIX is recorded in pages 54 through 94 of HP Laboratories research notebook number HPL 1846. This study includes the description of a proposed device, which is described pages 80 and 81 of HP Laboratories research notebook number HPL 1846 and in invention disclosure 10980956.

Modifications to the Harrick DRA are described in pages 98 through 102 and on page 106 of HP Laboratories research notebook number HPL 1846.

The fixture for tissue spectroscopy is described in pages 126 and 127 of HP Laboratories research notebook number HPL 1846 and in invention disclosure 10980955.

Safety issues related to exposure of subjects to light are treated in pages 129 through 133 of HP Laboratories research notebook number HPL 1846.

Measurements on the 19 subjects are described pages in 134 through 144 of HP Laboratories research notebook number HPL 1846.

Data for all measurements made on the FTIR are stored in C:\bgrams\data\george, in appropriately-named sub directories, on gorbash, the computer which controls the FTIR. The data for the 19 subjects are in directories named subj001, etc. The data for the 19 subjects and additional test are also on floppy disks in the possession of George Hopkins. This data is also in C:\george\data1, in appropriately named sub directories, on hplens, George Hopkins' PC.

There are notes in the scratch notebooks of George Hopkins, by date, which apply to the project. These include discussions with Diane Fiore of Sensors Unlimited (7/13/95), Rangu Ranganath of HP (7/31/95), Angelo Guimento of Grasby Infrared (11/3/95), Don Green of Judson (11/6/95), Henry Buijs of Bomem on the MB155 (12/22/95 and 1/24/96), and Ken Ward (6/7/96).

There are documents in directories under /users/george/niom/ on hplrat1, George Hopkins' unix workstation, including patent and literature searches and reports and memos.

The spectra for the instrument characterization and development of the DRA modifications are in loose-leaf notebooks in the possession of George Hopkins. George also has the spectra from the subject tests in folders, organized by test set. These spectra are quite voluminous; hence, they are not included in this report.

Appendix 10 – Intellectual property

The intellectual property related to non-invasive glucose measurement is extensive, complex, and of highly variable quality. There is no clear patent holder, who would control the field, or to whom one might go for licensing. We did a thorough search of the field, but we have not studied all of the following listed patents in detail.

After a list of the IP resulting from our work, we list U. S. patents, world patents, European patents and German patents. The world patents are of particular interest, because information is disclosed in these before U. S. patents might issue. An important example of this are the patents assigned to RGMT.

The lists are in order of patent number, with most recent first.

The following invention disclosures were filed in the course of our investigation:

Docket #	Title
10950912	Quantitation of blood anlytes by imaging blood vessels of the sclera.
10980955	Method and apparatus for tissue spectroscopy.
10980956	Improved detector array for optical spectrographs.
10980955	Method for measurement of analytes in vivo by tissue spectroscopy.

The following U. S. Patents are relevant to NIGM:

USP #	Inventor(s)	Assignee	Title
5,729,333	Osten et al.	Minnesota Mining &...	Characterizing biological matter in a dynamic condition using near infrared spectroscopy spectrum.
5,725,480	Oosta et al.	Abbot Laboratories	Non-invasive calibration and characterization of individuals and subsequent non-invasive detection of biological compounds.
5,710,630	Essenpreis et al.	Boehringer Mannheim	Method and apparatus for determining glucose in a biological sample.
5,703,364	Rosenthal	Futrex, Inc.	Method and apparatus for near-infrared quantitative analysis.
5,694,930	Pries et al.	Yook-Ok Kim	Device for qualitative and/or quantitative analysis of a sample.

5,692,504 Essenpreis et al. Boehringer Mannheim Method and apparatus for the analysis of glucose in a biological matrix.

5,685,300 Kuenstner not assigned Noninvasive and in-vitro measurement of glucose and cholesterol by nuclear magnetic resonance spectroscopy.

5,672,875 Block et al. Optix LP Methods of minimizing scattering and improving tissue sampling in non-invasive testing and imaging.

5,671,301 Kupershmidt Sunshine Medical Inst. Optical phase modulator for high resolution phase measurements.

5,666,956 Buchert not assigned Instrument and method for non-invasive monitoring of human tissue analyte by measuring the body's infrared radiation.

5,644,396 Hopkins Hewlett-Packard Co. Spectrograph with low focal ratio.

5,638,816 Kiani-Azarbayj... Masimo Corporation Active pulse blood constituent monitoring.

5,617,852 MacGregor not assigned Method and apparatus for non-invasively determining blood analytes.

5,615,672 Braig et al. Optiscan, Inc. Self-emission noninvasive infrared spectrophotometer with body temperature compensation.

5,601,079 Wong et al. not assigned Non-invasive quantitation of glucose control, aging, and advanced maillard products by stimulated fluorescence.

5,598,842 Ishihara et al. Toa Medical Electronics Non-invasive blood analyzer and method using the same.

5,553,616 Ham et al. Floride Institute of Tech. Determination of concentration of biological substances using Raman spectroscopy and artificial neural network discriminator.

5,553,613 Parker Pfizer Inc. Non invasive blood analyte sensor.

5,535,744 DiNino not assigned Method and apparatus for blood chemistry analysis.

5,533,509 Koashi et al. Kurashiki Boeseki Kab... Method and apparatus for non-invasive measurement of blood sugar level.

5,529,755 Higashio et al. Minolta Co., Ltd. Apparatus for measuring a glucose concentration.

5,487,384 Lee Blue Marble Research Kinematic assay of plasma glucose without blood sampling.

5,460,177 Purdy et al. Diasense, Inc. Method for non-invasive measurement of concentration of analytes in blood using continuous radiation spectrum.

5,452,716 Clift Novo Nordisk A/S Method and device for in vivo measuring the concentration of a substance in the blood.

5,448,992 Kupershmidt Sunshine Medical Inst. Method and apparatus for non-invasive phase sensitive measurement of blood glucose concentration.

5,438,201 Rosenthal et al. Futrex, Inc. Method and apparatus for restraining finger motion in blood analyte optical measurement.

5,435,309 Thomas et al. not assigned Systematic wavelength selection for improved multivariate spectral analysis.

5,424,545 Block et al. Myron J. Block Non-invasive non-spectrophotometric infrared measurement of blood analyte concentrations.

5,396,681 Kupershmidt Sunshine Medical Inst. Pocket-type instrument for non-invasive measurement of blood glucose concentration.

5,363,052 McKee Solid State Farms, Inc. Permittivity spectroscopy apparatus and method.

5,243,983 Tarr et al. Georgia Tech Research Non-invasive blood glucose measuring system and method using stimulated Raman spectroscopy.

5,209,231 Cote et al. U. of Connecticut Optical glucose sensor apparatus and method.

5,203,328 Samuels et al. Georgia Tech Research Apparatus and method for quantitatively measuring molecular changes in the ocular lens.

5,086,229 Rosenthal et al. Futrex, Inc. Non-invasive measurement of blood glucose.

5,070,874 Barnes et al. Biocontrol Technology Non-invasive determination of glucose concentration in body of patients.

5,054,487 Clarke Boston Advanced Tech. Laser systems for material analysis based on reflectance ratio detection.

5,025,785 Weiss not assigned Diabetes detection method.

4,975,581 Robinson et al. U. of New Mexico Method of and apparatus for determining the similarity of a biological analyte from a model constructed from known biological fluids.

4,765,179 Fuller et al. Solid State Farms, Inc. Radio frequency spectroscopy apparatus and method using multiple frequency wave forms.

4,750,830 Lee Arnold St. J. Lee Method and apparatus for monitoring blood-glucose concentration by measuring focal properties of the eye.

4,427,889 Müller Carl Zeiss Stiftung Method and apparatus for molecular spectroscopy, particularly for the determination of products of metabolism.

4,169,676 Kaiser not assigned Method for determining the contents of metabolic products in the blood.

4,014,321 March not assigned Non-invasive glucose sensor.

3,958,560 March not assigned Non-invasive automatic glucose sensor.

3,963,019 Quandt not assigned Ocular testing method and apparatus.

The following World Patents (PCT) are relevant to NIGM:

97/39686 Castano not assigned Optical method and device for determining blood glucose levels.

97/39341 Fuller et al. Solid State Farms, Inc. Improving radio frequency spectral analysis for in vitro or in vivo environments.

97/34521 Pezzaniti Abbott Laboratories Non-invasive measurement of optically active compounds.

97/32521 Oosta et al. Abbot Laboratories Calibration for subsequent monitoring of biological compounds (See USP 5,725,480).

97/30629 Griffith et.al. Diasense, Inc. Method and apparatus for non-invasive blood glucose sensing.

97/30341 Rosenthal Futrex, Inc. Method and apparatus for near-infrared quantitative analysis.

97/28437 Malin et al. Instrumentation Metrics Method and apparatus for multi-spectral analysis in noninvasive infrared spectroscopy.

97/27800 Raber et al. Diasense, Inc. Methods and apparatus for non-invasive glucose sensing: non-invasive probe.

97/24066 Ishihara Toa Medical Electronics Noninvasive blood examination apparatus (in Japanese).

97/13448 Müller Laser und Medizin-Tech. Device for determining blood glucose level (in German).

97/06425 Messerschmidt et Rio Grande Medical Tch. Improved diffuse reflectance monitoring apparatus.

97/05819 Messerschmidt Rio Grande Medical Tch. Method for non-invasive blood analyte measurement with improved optical interface.

97/02781 Rosencwaig not assigned Apparatus for non-invasive analysis of biological compounds.

96/41151 Lepper et al. Masimo Corp. Blood glucose monitoring system.

96/39922 Block et al. Optix LP Methods of minimizing scattering and improving tissue sampling in non-invasive testing and imaging (see USP 5,672,875).

96/37259 Domjan not assigned Method and apparatus for rapid non-invasive determination of blood composition parameters.

96/35370 Gosani Mass. Inst. of Tech. Apparatus and method for non-invasive blood analyte measurement.

96/17546 Braig et al. Optiscan, Inc. Self-emission noninvaisve infrared spectrophotometer with body temperature compensation (see USP 5,615,672).

96/14567 Block et al. Myron J. Block Rapid non-invasive optical analysis using broad bandpass spectral processing.

96/04840 Parker not assigned Non invasive blood analyte sensor (see USP 5,553,613).

95/31930 Braig et al. Optiscan, Inc. Self-emission noninvaisve infrared spectrophotometer.

95/31928 Künst not assigned Transcutaneous, non-blood determination of the concentration of substances in the blood (in German).

95/31133 Quintana Futrex, Inc. Non-invasive near-infrared quantitative measurement instrument.

95/22046 Small et al. U. of Iowa Res. Foundtn. Method and apparatus for non-invasive detection of physiological chemicals, particularly glucose.

95/20906 Rosenthal Futrex,Inc. Procedure for verifying the accuracy of non-invasive blood glucose measurement instruments.

95/15711 Cho not assigned Process and device for non-invasive determination of glucose concentration in parts of the human body (in German).

95/13739 Fischbacher et al. Jenoptik GmbH Method and apparatus for the non-invasive transcutanean detrmination of the concentrations of substances in human body fluids or tissues (in German).

95/12348 Essenpreis et al. Boehringer Mannheim Process and device for the determining the glucose concentration in a biological matrix (in German, see USP 5,692,504).

95/04496 Fuller et al. Solid State Farms, Inc. Apparatus and method for radio frequency spectroscopy using spectral analysis.

94/19701 Mckee Solid State Farms, Inc. Permittivity spectroscopy apparatus and method (see USP 5,363,052).

92/10131 Tarr et al. Georgia Tech Res. Corp. Non-invasive blood glucose measurement system (see USP 5,243,983).

92/07511 Cote et al. U. of Connecticut Optical glucose sensor apparatus and method (see USP 5,209,231).

The following European Patents (EPO) are relevant to NIGM:

0 822 349 Matsuoka et al. Kyoto Dai-ichi Kagaku Optical measuring device with wavelength selective light source.

0 807 812 Tolda et al. Fuji Photo Film Co. Glucose concentration measuring method and apparatus.

0 714 628 Ishihara et al. Toa Medical Electronics Non-invasive blood analyzer (see USP 5,598,842 and WO 97/24066).

0 680 727 Böcker Boehringer Mannheim Analysis system for monitoring the concentration of an analyte in the blood of a patient (in German).

0 673 622 Gilkfeld U. of California A method of substantially continuously monitoring the level of a bioactive material.

0 603 658 Backhaus et al. Boehringer Mannheim Apparatus for in-vivo determination of optical properties of the interocular fluids of the eye.

0 589 191 Stark Edward W. Stark Non-invasive measurement method and apparatus.

0 426 358 Yang Won Suck Yang A non-invasive method and apparatus for measuring blood chemical concentration.

0 160 768 Dähne et al. Batelle Memorial Inst. Spectrophotometric method and apparatus for the non-invasive determination of glucose in body tissues.

The following German Patents (DE) are relevant to NIGM:

196 29 342 Fischbacher et al. EPSa Elektronik Method and apparatus for the non-invasive transcutaneous determination of the concentrations of substances in human body fluids or tissues (in German, see WO 95/13739).

4 23 663 A1 Cho Med Science GmbH Method and apparatus for determination of heat exchange effects in parts of the human body and the appropriate measuring apparatus and the correlation with glucose concentration in human blood (in German, see WO 95/15711).

44 17 639 Pfeifer Boehringer Mannheim Method for determination of an analyte in a biological sample (in German).

42 00 332 Kuhlmann Fritz Kuhlmann Method and apparatus for non-invasive quantitative determination of the concentration of substances in human bodily fluids (in German).

25 38 985 March Wayne F. March Non-invasive glucose sensor (see USP 4,014,321).

Appendix 11 – DiagNoSTIX Proposal

The DiagNoSTIX investigation proposal is available from George Hopkins. Basically, all of the goals were accomplished. We had planned to carry out two series of measurements on subjects, which would have allowed us to improve our experimental methodology. This proved to be impossible, and only one series of measurements was made. We had delays in obtaining the lenses for modifications to the DRA. It took longer to deal with regulatory issues of safety and radiation exposure than we had anticipated. Finally, we chose to rent a glucose analyzer for our reference measurements. This cost us about $2500; purchase would have cost about $6500. The analyzer was not available for a sufficiently long time to make a second series of measurements.

Appendix 12 – A glossary of abbreviations

A/D – Analog to digital, as in A/D converter, an electronic component

ATR – Attenuated total reflection, a spectroscopic sampling technique

BICO - Biocontrol Technology, Inc.

BM – Boehringer Mannheim GmbH., also Boehringer Mannheim Diagnostics

CDA – Confidential Disclosure Agreement

CDRH – Center for Devices and Radiological Health (U. S. Govt./FDA)

CRADA - Cooperative Research and Development Agreement (U. S. Govt.)

CTO – Chief Technology Officer (of a company)

FDA – Food and Drug Administration (U. S. Govt.)

FTIR – Fourier transform infrared (spectrometer or spectroscopy)

HGM – Home glucose monitoring

HP – Hewlett-Packard Company

HPL – Hewlett-Packard Laboratories

InAs – Indium Arsenide, a semiconductor used in photodetectors

InGaAs – Indium Gallium Arsenide, a semiconductor used in photodetectors

InSb – Indium Antimonide, a semiconductor used in photodetectors

IP – Intellectual Property (Usually patents, but also trade secrets)

IPO – Initial Public Offering (of shares in a formerly private company)

ISBN – International Standard Book Number (a unique specifier for a book)

LED – Light emitting diode, a semiconductor device which emits light

MIR – Mid infrared (2.5 microns to 25 microns)

NIGM – non-invasive glucose measurement

NIR – Near infrared (700 nm to 2500 nm, sometimes 700 nm to 1100 nm)

OSHA – Occupational Health and Safety Administration (U. S. Govt.)

PCA – Principal component analysis, a data analysis technique

PLS – Partial least squares, a calibration technique

RF – Radio frequency, usually referring to radiation from 3 kHz to 300 GHz

RGMT - Rio Grande Medical Technologies, Inc.

SPIE – The International Society for Optical Engineering

SBIR - Small Business Innovation Research Program (U. S. Govt.)

TE – Thermoelectric (as in "thermoelectric effect")

UNM – University of New Mexico, also UNM Medical School/Center

USP – United State Patent

WO – World "Patent" (Actually an application filed under the World IP Org.)

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