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# Next step of non-invasive glucose monitor by NIR technique from the well controlled measuring condition and results

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**Abstract.** Although non-invasive glucose measurement techniques based on near-infrared (NIR) spectroscopy have been interested for over 20 years, a reliable non-invasive glucose monitoring method has not been established yet. Many hurdles are remained to be solved to extract extremely small glucose information from the complicated NIR spectra. In addition, there are also some ambiguous time-dependent physiological processes, which make the explanation of the model more difficult especially in the universal calibration. In this paper, the optical consideration in instrument to improve the SNR is discussed first, which is a critical way to detect small analyte signal. Then an optical measuring conditions reproducible system is used to reduce the noise from human-spectrometer interface. And an optical probe is designed according to the Monte Carlo simulations to measure the dermis selectively to eliminate the noise from human tissue. Finally, with the well-controlled measuring conditions, the *in vivo* result of single person using the leave one out cross validation within the single day is quite satisfactory. However, the validation results using the validation set from different day are not so good. Further research and extensive model validations are needed to determine if the model is truly based on the glucose information.

**Key words:** glucose, *in vitro, in vivo*, measuring condition, NIR, non-invasive

## 1. Introduction

Diabetes is a serious disease for both the health care system and the individual patient. Appropriate therapy of diabetes such as frequent self-monitoring of blood glucose by patients, however, can reduce the complications of diabetes by up to 75%. Self-monitoring technology has advanced in recent years, for example, the continuous glucose monitoring system (CGMS) developed by MiniMed Inc. is capable of continuous glucose monitoring in a time period spanning over several days, and the GlucoWatch biographer developed by Cygnus Inc. can obtain automatic measurements of glucose concentrations every 20 minutes for up to 12 hours at a time. Non-invasive monitoring of blood glucose offers many advantages, which avoid pain and discomfort from frequent finger-pricking.

Several techniques have been proposed for noninvasive *in vivo* monitoring of blood and tissue glucose in recent years. In polarimetry measurements, the anterior chamber has been suggested as a measurement site for *in vivo* glucose assays (Cameron *et al.* 1999). Compared with near-infrared (NIR) spectroscopy, the fundamental variations from Raman spectroscopy are sharper and less overlap, and water has quite low Raman scattering. Raman spectra of the AH of the eyes from 32 anesthetized rabbits excited at 785 nm showed good correlation with glucose concentration (Borchert *et al.* 1999). Raman spectra of human skin under tissue modulation conditions were also studied in recent years (Chaiken *et al.* 2000, 2001). But the intensity of Raman scattering is so small that high power laser and other signal-enhanced techniques, fluorescence corrections are required, which increase the difficulty of its application on the non-invasive glucose measurement. The OCT technique is promising as the optical measurements targets a restricted area in the skin. The OCT images and signals obtained from skin of Yucatan micropigs and NewZealand rabbits showed that an increase of the ISF glucose concentration in the physiological range may decrease the scattering coefficient by 0.22% $mM^{-1}$ due to cell volume change (Larin *et al.* 2003). No specificity advantage has been established for OCT over other scattering studies (Heinemann *et al.* 2000). The possibility of using diffuse reflectance NIR spectroscopy for determining the blood glucose concentration non-invasively has been demonstrated by many groups (Haaland *et al.* 1992; Marbach *et al.* 1993) and much progress has been made in the past few years. Heise *et al.* (1994, p. 439) presented results through a diffuse reflectance measurement of the oral mucosa in the 1111–1835 nm range with an optimized diffuse reflectance accessory. *In vivo* experiments were conducted on single diabetics using glucose tolerance tests and on a population of 133 different subjects. Malin *et al.* (1999, p.1651), used NIR diffuse reflectance over the 1050–2450 nm wavelength range for non-invasive monitoring of blood glucose in the forearm. The results from the oral glucose tolerance test (OGTT) of three non-diabetic subjects over multiple days yielded a mean standard error of calibration of 1.1 mM. Samann *et al.* (2000, p.406) also used the shorter wavelength region from 800 to 1350 nm to measure diffuse reflection spectra of a finger and investigated the long-term accuracy and stability of calibration models. They obtained a root mean standard error of prediction from 1.03 to 3.00 mM with leave-one-out cross-validation at the beginning of the study, and a root mean standard error from 3.1 to 6.00 mmol/l for the long-term results. Maruo *et al.* (2003, p.1236) designed a new optical fibers to measured *in vivo* the NIR spectra of the human forearm. They obtained the correlation coefficient, R, 0.934 and the standard error of prediction (SEP), 23.7 mg/dL, between the optically predicted blood glucose content and the directly measured content for six subjects, including one Type I dia-

betic. Olesberg *et al*. (2004, p.11) performed *in vivo* measurements of NIR rat skin absorption in the 2000–2500 nm spectral range during a glucose clamp experiment. The result showed that glucose spectral information was present.

Although, satisfactory prediction results have been obtained by most groups in these published papers, problems remain to be solved in order to achieve reliable and precise results. There are several critical obstacles preventing from the success of measuring glucose non-invasively. First, glucose specific information presented within the spectral region probed is so small. Second, the noise from the instrumentation and from human body is unavoidable and should be reduced as low as enough. Finally, the complication of the spectral makes it difficult to validate the calibration model.

In this paper, the optical consideration in instrument to improve the SNR is discussed first, which are critical for successful non-invasive clinical measurements. Then an optical measurement reproducible system is proposed to reduce the noise from human-spectrometer interface. In order to eliminate the noise from tissue, the optical probe is designed according to the Monte Carlo simulations to measure the dermis selectively and some *in vitro* experiments are conducted to evaluate it. Finally, with the well-controlled measuring conditions system, the *in vivo* result of single person using the leave-one-out cross validation within the single day is quite satisfactory. However, the validation results using the validation set from different day are not so good. So further research are required to eliminate the chance correlation, and extensive model validations are needed to determine if the model is truly based on the glucose information.

## 2. High performance instrumentation to detect small analyte signal

A custom-built NIR spectrometer with a fiber optic accessory was developed for the tissue sampling on the left palm. This system was equipped with a tungsten-halogen lamp (PG64623, OSRAM, German), a non-collinear $TeO_2$ acousto-optic tunable filter (TEAF10-1.0-1.8-S), RF driver (VFI-80-50-DDS-B1-C2-E, Brimrose, U.S.A) and InGaAs PIN photodiode (G5851-21, Hamamatsu Photonics K.K, Japan). The light returned from the tissue was received by the fiber optic and collected by the photodiode. Then the signal was digitized by a 16-bit data acquisition card (PCI-MIO-16XE-50, National Instrument, U.S.A). For the detail information, please refer to the reference (Xu *et al*. 2003).

For NIR spectra of aqueous based clinical samples, water is always a critical matrix component. Glucose absorption exists at the first overtone band (1530–1850 nm) and at the combination band (2080–2340 nm). The combination band exhibits higher absorbance signal but only very shallow

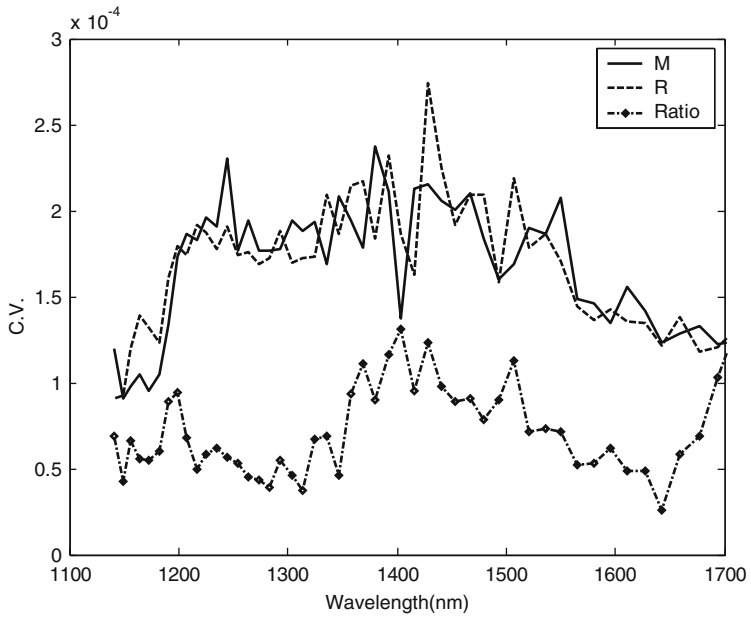


Fig. 1. Double beam design can improve the SNR of spectrometer.

penetration depths, because the water-absorption coefficient at this band is about three times than that at the overtone band of glucose (Yoon *et al.* 2002). The spectral range 1100–1700 nm is investigated in our group because of its deeper penetration into tissue, and the maximum radiation path length of this region is expected to be a few millimeters.

As we know, the absorbance change at the concentration of 10 mg/dl with the pathlength of 1 mm at 1600 nm is about $2.2 \times 10^{-5}$ A.U., which is quite small. So the SNR of the spectrometer must be high enough to detect the small analyte signal variations. Double beam design is an effective method to improve the SNR of spectrometer, which is used in our system. The pure water is as the sample solution and its spectral intensity is measured eight times in the range of 1100–1700 nm to evaluate the performance of instrumentation. The coefficient of variation (CV, the reciprocal of SNR) before and after the ratio of light intensity in reference channel is shown in Fig. 1. The coefficient of variation for both measurement channel and reference channel is about $5000:1 \sim 10000:1$ in each wavelength. However, after the ratio to the reference light, the SNR is about 10000:1~20000:1. So the double beam design is an effect way to improve the performance of instrument.

Based on above optical designs, a series of *in vitro* experiments were conducted to examine the validity of glucose analysis. The transmission mea-

Table 1. The result of *in vitro* experiments.

| solvent | Water | Albumin | Plasma | 2% Intralipid | Whole blood |
|---|---|---|---|---|---|
| Concentration range(mg/dl) | 10–300 | 10–300 | 20–800 | 10–300 | 20–600 |
| Number of samples | 30 | 30 | 40 | 30 | 30 |
| Correlation coefficient | 0.994 | 0.974 | 0.988 | 0.971 | 0.987 |
| RMSEP(mg/dl) | 6.96 | 16.65 | 34.28 | 21.60 | 26.51 |

surements (glucose in water, albumin, and plasma) and diffuse reflectance measurements (glucose in 2% intralipid solution, and whole blood) were investigated in the *in vitro* experiments. The optical pathlength for the transmission measurement is, 1 mm. For the detail information about the experimental design, please refers to the reference (Xu *et al.* 2004).

## 3. Optical measuring reproducible system to reduce the effect of interface

For the diffuse reflectance spectroscopy, contact measurement is firstly adopted to reduce the effect of specular reflection. However, there remain many other problems, such as difference in sampling positions and contact pressure, which have great influences on the spectral repeatability.

Based on the custom-built spectrometer, influence of variations in sampling position during spectroscopic measurement of palm was analyzed in the wavelength region from 1100 and 1700 nm. A young male volunteer took the experiment. Keeping the contact pressure constant, the diffuse reflectance spectrum of left palm was collected. And then the palm was put up and down again to change the sampling position slightly. The spectra collected with same contact pressure in different sampling position are shown in Fig. 2. Furthermore, at the same sampling position, the palm kept fixedly and the probe was controlled to change the contact status. The diffuse reflectance spectra collected with the changing contact pressure are shown in Fig. 3.

As shown in Fig. 2, there's a considerable difference between spectra with same contact pressure at different sampling positions. And the contact pressure also has great influence on the spectra. So the proper measurement of blood glucose concentrations using NIR diffuse reflectance spectroscopy is complicated by these problems of spectrometer-human interface. The main reason is that improper interface leads to the changes in the tissue volume being sampled and the distribution of analytes is not as uniform as that in simple aqueous solution. Glucose, water and other analytes have different local concentrations within blood vessels, interstitial fluid, and skin layers. Thus, how to maintain the measuring conditions is quite important for *in vivo* sampling.

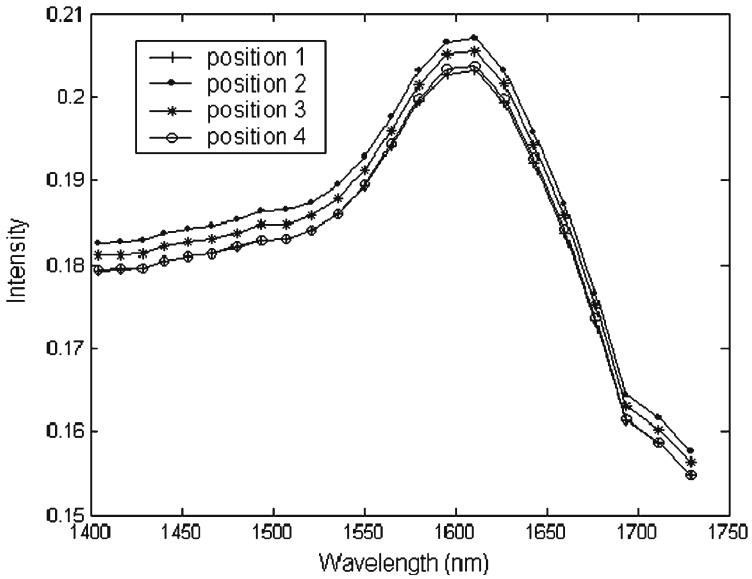


Fig. 2. Variation of spectral energy with different sampling position.

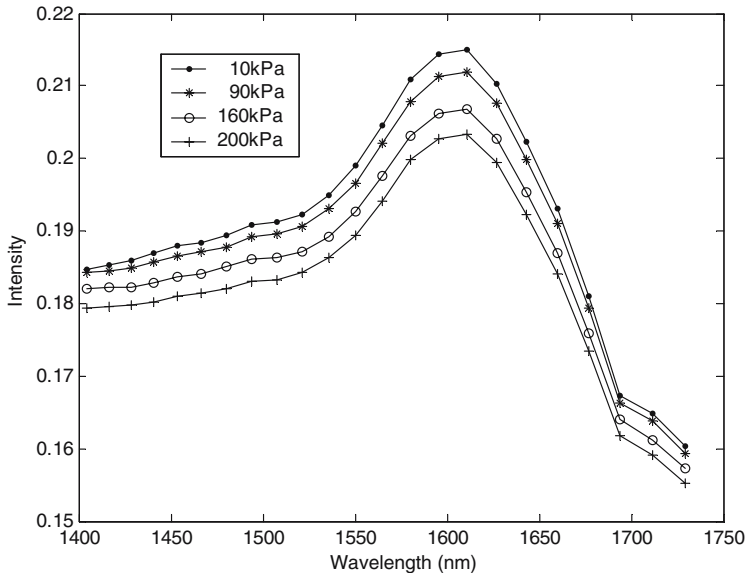


Fig. 3. Variation of spectral energy with changing contact pressure.

In order to decrease the effect of human-spectrometer on the diffuse reflectance spectra, an optical measuring conditions reproducible (OMCR) system is developed, shown in Fig. 4. That means, a fixed spectrometer-human interface is determined for each subject and it can be reproduced with same contact pressure and same sampling position, whenever for calibration or prediction. In the OMCR system, an image orientation system is introduced to achieve the reproduction of sampling positions, and a pressure analysis system is used to keep the contact pressures constant. For each subject, a unique ID is assigned and a template image is captured by the CCD camera and saved in the special databases as the reference for further experiments. Before each measurement, a new image of palm print is collected and matched with the reference template. The OMCR system automatically computes their difference in $X$ and $Y$ axis, which is used to control the movement of optical fiber. Thus the precise sampling position is obtained. Pressure sensor is another key element of OMCR system. For each subject, variation curve of diffuse reflectance spectra with contact pressure is first analyzed to determine the optimal contact status point, and the corresponding coordinate in $Z$ axis of the probe is recorded in the specific database for this individual. For next experiment, the three-dimensional traveling platform is driven to reach the coordinate in $Z$ axis saved in the database. Thus, the contact pressure is also reproduced.

A young volunteer took part in the evaluation experiment. After a long time fast, the physiological state of the volunteer was quite steady. Without the OMCR system, the palm was put on the probe randomly and the diffuse reflectance spectrum was collected by the custom-built spectrometer. Repeated this process eight times and computed the coefficient of variation (CV) of these spectra. Then the same procedures were followed with the OMCR system. The CVs without and with OMCR system

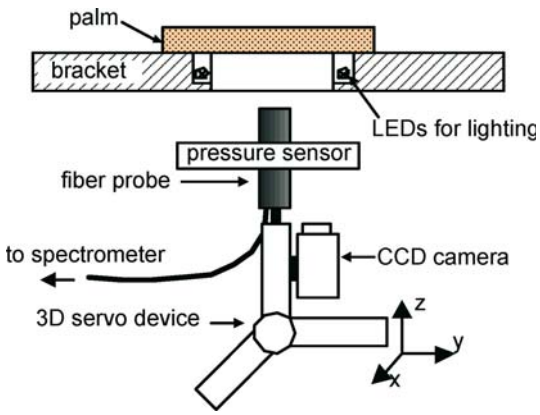


Fig. 4. The diagram of OMCR system.

in the wavelength region 1100–1700 nm is indicated in Fig. 5. Obviously, spectral repeatability is greatly enhanced in each wavelength after applying the OMCR system.

## 4. Optical probe to measure dermis tissue selectively

The skin tissue is regarded as the most suitable measurement media because of its simple anatomical structure (i.e., epidermis, dermis, and subcutaneous tissue) and shallow depth. Glucose in the epidermis, which contains stratum corneum, and the subcutaneous tissue, which composed of fatty tissue, does not correspond to changes of glucose in blood. However, glucose in the dermis is assumed to correlate with the blood glucose in the same way as that in the interstitial fluid (Thennadil *et al.* 2001). In order to insure higher probability photon to penetrate the epidermis, an optical probe was designed properly to selectively measure the dermis tissue using Monte Carlo simulation, which is a very flexible algorithm that simulates light transport through the medium according to its absorption and scattering properties. It is typically used to generate an approximate relationship between the measured light intensity and the absorption occurring in the tissue. Measurement sites explored in our system is the palm, and the depth of epidermis and dermis are 0.5 and 1.0 mm, respectively. The opti-

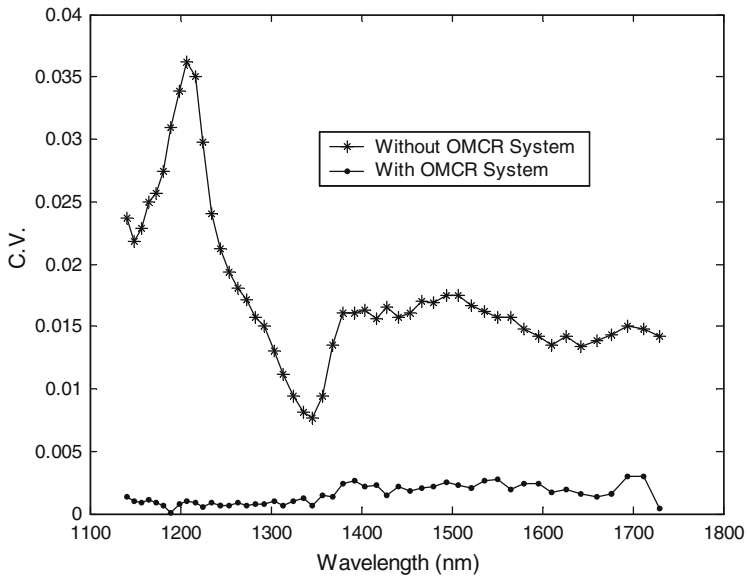


Fig. 5. CVs without and with OMCR system.

cal fiber probe consists of the central source fiber bundle and the detector fiber bundle arranged in a circle. Such a design for optical fiber probe means more useful light signals are detected through enlargement of receiving area.

Optical parameters at 1600 nm [$\mu_a = 0.511$, $\mu_s = 10.43$, $g = 0.4$ (Troy and Thennadil 2001)] are applied for simulation. To collect the NIR light that has traveled through the dermis layer of tissue, the minimum and average source-detector separation distance is about 0.3 and 2.25 mm, respectively. The total number of photons used for simulation is $5 \times 10^6$. Photons distribution at each penetration depth is shown in Fig. 6. There are 87 percentages of all received photons with a penetration depth larger than 0.5 mm. The average photon penetration depth at measuring potion of optical fiber probe is 0.975 mm. Therefore, requirements for glucose sensing within dermis layer of tissue can be satisfied.

The custom-built NIR spectrometer and optical probe described above was used to collect the NIR diffuse reflectance of the dermis layer of palm tissue selectively in the *in vivo* experiment. And the OMCR system was used to ensure that the fiber probe reaches to the same measuring position during every measurement with the same contact pressure.

More than 30 volunteers suffered from type II diabetes and healthy volunteers took part in the OGTT (oral glucose tolerance test) experiments. Since the normal proportion of glucose in blood and tissue is only about

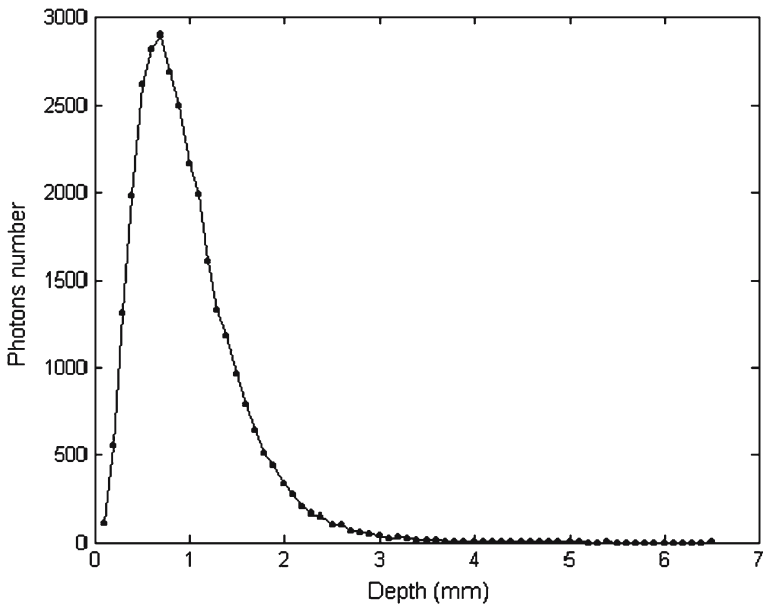


Fig. 6. Depth distribution of photons at 1600 nm.

0.1% of the water, the spectral variations due to the change of glucose concentration are extremely small. And the spectra are further complicated by some other main components of living tissue. In addition time dependent physiological activities and psychological variations are response to the NIR diffuse reflectance spectra. So multivariate spectroscopic strategies, e.g. partial least squares (PLS) regression, must be used for quantitative analysis from non-invasive near infrared spectra due to the complexity of *in vivo* signal. Typically, PLS is designed to involve all sources of spectral variation (Arnold *et al*. 1998, 2004) and calibration models are treated in a "black box" manner with no regard for the chemical information. So it is difficult to distinguish analyte-specific spectral variations.

For i*n vivo* experiments, OGTT is the simplest method to achieve a wide range of glucose concentration. So the multivariate regression model for glucose is usually built and validated through the OGTT process. If the individual model is validated by leave-one-out cross validation or prediction set within single day, most of the prediction results are between 0.5 and 1.0 mmol/l and the correlation coefficient between predicted concentration and reference concentration is higher than 0.8, which are seemed to be quite satisfactory. But until now, no one can give the proof that the model is based on the blood glucose information.

For a patient suffered from type II diabetic, different prediction sets are used to validate the *in vivo* multivariate models. As shown in Fig. 7, the multivariate model of blood glucose concentration built using the even sam-

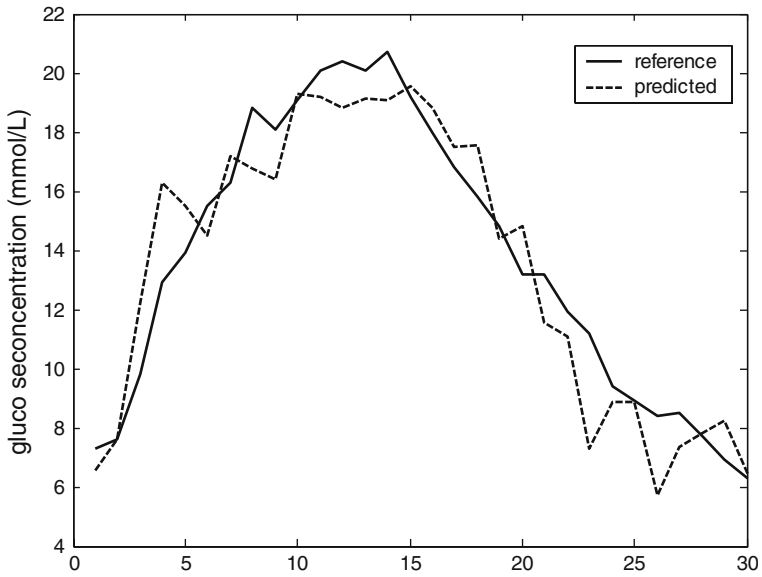


Fig. 7. The prediction result using the prediction set within one day.

ples is validated by the odd samples within the same day. This means, there is same systematic drift and physiological variations for both the calibration set and prediction set. But for a prediction set from another day, both the instrumentation status and human status have changed. As shown in Fig. 8, the model is validated by the data of different day. Obviously, the predicted concentration is quite different from the reference value. It's a bad situation for the NIR spectroscopic analysis. Some time-dependent factors and unmeasured physiological activities (Small and Arnold 1998), which are potential factors to chance correlations, make it quite difficult to explain the model and extract the glucose information from the spectra directly. Now, with the same measuring conditions and different validation method, both satisfactory and unsatisfactory results are obtained for the same subject. Further research and extensive model validations are needed to determine if the model is truly based on the glucose information.

## 5. Conclusion

With the custom built spectrometer and optical accessory, high SNR has been achieved by the double beam design and some *in intro* measurements, including transmission measurements in the simple aqueous solution and diffuse reflectance measurements in the complicated whole blood samples,

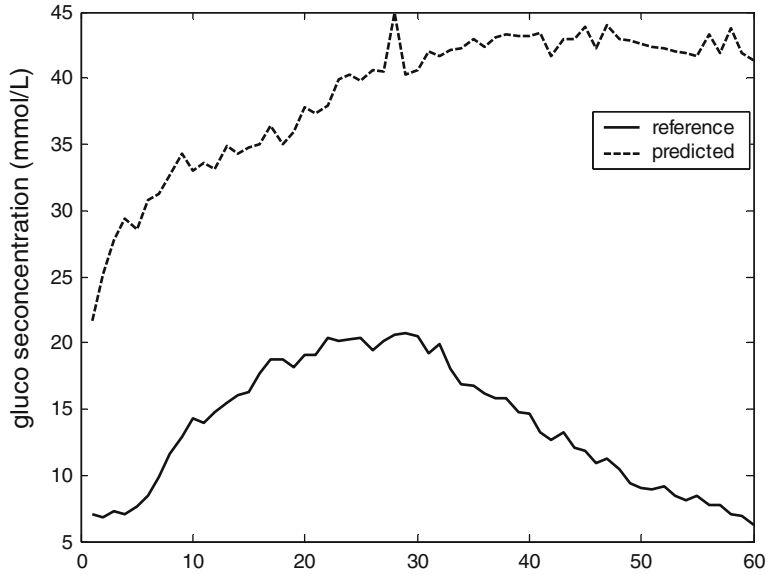


Fig. 8. The prediction result using the prediction set from different day.

have been conducted. Results show that, under the SNR of 10000:1∼20000:1, the glucose information can be extracted from NIR spectra.

For *in vivo* experiments, OGTT is usually an effective method to achieve a wide range of glucose concentration and the prediction results within single day are also remarkable. But human body is so complicated that many other interferences, including time-dependent factors and physiological factors, are all response to the NIR spectra. In addition random samplings are difficult to be performed in *in vivo* experiments. The calibration based on many days is a good choice to eliminate the effect of some time-dependent factors, but some other sources, e.g. temperature, humidity and the status of instrument, may result in the spectra more complicated. So OGTT process with well controlled conditions is a good choice to obtain the glucose information from *in vivo* spectra, although some unmeasured physiological factors may make contributions to the calibration. By admitting the correlation of glucose with other physiological components, and as long as the glucose-interference concentration relationship maintains for the specific individual, the accurate glucose measurement is possible by use of the specific model.

With our system, both satisfactory and unsatisfactory results are obtained in *in vivo* experiments and more carefully designed protocols should be adapted in order to avoid the chance correlation in the *in vivo* measurement of glucose. Some data processing methods, such as net analyte signal, selectivity analysis, orthogonal signal analysis, are required to extract the glucose-dependent information and remove other irrelevant interferences. Calibration of the instruments and the models under different environmental conditions and physiological conditions must be further performed. Nowadays, the application may have to be calibrated to individual users. The ultimate objective is to develop the universal model based on well-developed and effective extraction of glucose-dependent information.

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