Hollow optical-fiber based infrared spectroscopy for measurement of blood glucose level by using multi-reflection prism

Saiko Kino,1 Suguru Omori,1 Takashi Katagiri,2 and Yuji Matsuura1,*

1Graduate School of Biomedical Engineering, Tohoku University, 6-6-05 Aoba, Sendai 980-8579, Japan
2Graduate School of Engineering, Tohoku University, 6-6-05 Aoba, Sendai 980-8579, Japan
yuji@ecei.tohoku.ac.jp

Abstract: A mid-infrared attenuated total reflection (ATR) spectroscopy system employing hollow optical fibers and a trapezoidal multi-reflection ATR prism has been developed to measure blood glucose levels. Using a multi-reflection prism brought about higher sensitivity, and the flat and wide contact surface of the prism resulted in higher measurement reproducibility. An analysis of in vivo measurements of human inner lip mucosa revealed clear signatures of glucose in the difference spectra between ones taken during the fasting state and ones taken after ingestion of glucose solutions. A calibration plot based on the absorption peak at 1155 cm\(^{-1}\) that originates from the pyranose ring structure of glucose gave measurement errors less than 20%.

©2016 Optical Society of America

OCIS codes: (170.3890) Medical optics instrumentation; (060.2390) Fiber optics, infrared; (300.6300) Spectroscopy, Fourier transforms.

References and links
1. D. D. Cunningham and J. A. Stenken, In Vivo Glucose Sensing (Wiley, 2010).
2. S. K. Vashist, “Non-invasive glucose monitoring technology in diabetes management: a review,” Anal. Chim. Acta 750, 16–27 (2012).
3. N. S. Oliver, C. Tournazou, A. E. G. Cass, and D. G. Johnston, “Glucose sensors: a review of current and emerging technology,” Diabet. Med. 26(3), 197–210 (2009).
4. J. J. Burmeister, M. A. Arnold, and G. W. Small, “Noninvasive blood glucose measurements by near-infrared transmission spectroscopy across human tongues,” Diabetes Technol. Ther. 2(1), 5–16 (2000).
5. J. T. Olesberg, M. A. Arnold, C. Mermelstein, J. Schmitz, and J. Wagner, “Tunable laser diode system for noninvasive blood glucose measurements,” Appl. Spectrosc. 59(12), 1480–1484 (2005).
6. J. J. Burmeister and M. A. Arnold, “Evaluation of measurement sites for noninvasive blood glucose sensing with near-infrared transmission spectroscopy,” Clin. Chem. 45(9), 1621–1627 (1999).
7. J. Yadav, A. Rani, V. Singh, and B. M. Murari, “Prospects and limitations of non-invasive blood glucose monitoring using near-infrared spectroscopy,” Biomed. Signal Process. 18, 214–227 (2015).
8. J. Kottmann, J. M. Rey, J. Luginbühl, E. Reichmann, and M. W. Sigrist, “Glucose sensing in human epidermis using mid-infrared photoacoustic detection,” Biomed. Opt. Express 3(4), 667–680 (2012).
9. H. Lilienfeld-Toala, M. Weidenmüller, A. Xheła, and W. Mantele, “A novel approach to non-invasive glucose measurement by mid-infrared spectroscopy: The combination of quantum cascade lasers (QCL) and photoacoustic detection,” Vib. Spectrosc. 38(1–2), 209–215 (2005).
10. M. A. Pleitez, T. Lieblein, A. Bauer, O. Hertzberg, H. von Lilienfeld-Toal, and W. Mantele, “In vivo noninvasive monitoring of glucose concentration in human epidermis by mid-infrared pulsed photoacoustic spectroscopy,” Anal. Chem. 85(2), 1013–1020 (2013).
11. J. L. Lambert, J. M. Morookian, S. J. Sirk, and M. S. Borchert, “Measurement of aqueous glucose in a model anterior chamber using Raman spectroscopy,” J. Raman Spectrosc. 33(7), 524–529 (2002).
12. A. M. Engejder, T. G. Seccina, J. Oh, M. Hunter, W. C. Shih, S. Sasic, G. L. Horowitz, and M. S. Feld, “Raman spectroscopy for noninvasive glucose measurements,” J. Biomed. Opt. 10(3), 031114 (2005).
13. Y. C. Shen, A. G. Davies, E. H. Linfield, T. S. Elsey, P. F. Taday, and D. D. Arnone, “The use of Fourier-transform infrared spectroscopy for the quantitative determination of glucose concentration in whole blood,” Phys. Med. Biol. 48(13), 2023–2032 (2003).
components. Consequently, multivariate statistics such as principal component analysis are useful but have problems with regard to accuracy and reliability [7]. They detect harmonic overtones of the molecular vibrations of glucose appearing in the near-infrared region that are buried in the overtone peaks of other components (water, blood, protein, etc.) because the concentration and the absorption coefficient of glucose in this region are much lower than those of other components, and the absorption coefficient of glucose is usually necessary for quantitative measurement.

In vivo measurement of stratum corneum thickness from water concentration profiles obtained with Raman spectroscopy,” Acta Derm. Venereol. 87(1), 4–8 (2007).

P. W. Wertz, D. C. Swartzendruber, and C. A. Squier, “Regional variation in the structure and permeability of oral mucosa and skin,” Adv. Drug Deliv. Rev. 12(1–2), 1–12 (1993).

T. Kimura, H. Yamano, A. Tanaka, T. Matsuura, M. Ueda, K. Ogawara, and K. Higaki, “Transport of D-glucose across cultured stratified cell layer of human oral mucosal cells,” J. Pharm. Pharmacol. 54(2), 213–219 (2002).

P. Garidel, “Mid-FTIR-Microspectroscopy of stratum corneum single cells and stratum corneum tissue,” Phys. Chem. Chem. Phys. 4(22), 5671–5677 (2002).

1. Introduction

A non-invasive device for monitoring blood glucose level is highly desired [1–3] because it would eliminate the pain and infection risk associated with the commonly used glucose monitoring devices that measure electrochemical properties of blood taken from a fingertip. Various blood glucose measurement methods based on near-infrared absorption spectroscopy have been proposed [4–6] and some products using them have been marketed, although all have problems with regard to accuracy and reliability [7]. They detect harmonic overtones of molecular vibrations of glucose appearing in the near-infrared region that are buried in the overtone peaks of other components (water, blood, protein, etc.) because the concentration and the absorption coefficient of glucose in this region are much lower than those of other components. Consequently, multivariate statistics such as principal component analysis are usually necessary for quantitative measurement.

Among other optically based non-invasive techniques, including photoacoustic spectroscopy [8–10] and Raman spectroscopy [11,12], ones based on mid-infrared absorption spectroscopy have advantages of a lower scattering effect and higher absorption than near infrared spectroscopy because they detect the fundamental vibrations of glucose that are much stronger, sharper, and more isolated than the overtone peaks in the near infrared [13–15]. Because the penetration depth of mid-infrared light is limited to a few microns, mid-infrared spectroscopy methods detect glucose in interstitial fluid that reflects the blood glucose level [16]. Glucose measurement systems based on infrared spectroscopy usually use an attenuated total reflection (ATR) prism to deal with the small penetration depth of mid-infrared light. These ATR prisms are made of high-index materials such as Si, Ge, and diamond, and when
light is totally reflected at the surface of the prism in contact the samples surface, the evanescent field is absorbed by the sample.

In most ATR measurement systems, the prism is in a rather bulky housing, so the area that can be measured is usually limited to skin surfaces such as the fingertip, which has a thick stratum corneum. This layer makes it difficult for infrared light to penetrate deeper tissues. In contrast, oral mucosa such as that of the inner lips has no keratinized layer and has interested many researchers as a target tissue for mid-infrared ATR spectroscopy. Here, the advent of measurement systems using fiber optic probes has made it easier to make ATR measurements on oral mucosa. In particular, some researchers have used ATR fiber probes consisting of a bare polycrystalline fiber [17], while others have developed optical fiber probes incorporating a prism made of zinc selenide (ZnSe) at the distal end [18].

The above fiber-based systems are not on the market, partly because of the photochemical instability of polycrystalline fiber and the possible toxicity of ZnSe. As a way of resolving these problems, we developed a mid-infrared ATR spectroscopy system using a flexible hollow optical fiber with a rooftop-shaped diamond ATR prism at the distal end [19]. The fiber is made from a safe flexible material that is an excellent delivery medium of mid-infrared light. In a previous study, we used this system to measure the absorption spectra of oral mucosa and found that the intensity of the absorption peak at 1035 cm$^{-1}$ was related to blood glucose levels [20]. However, due to the very small absorption of glucose in the interstitial fluid that was detected by mid-infrared ATR spectroscopy, the signal-to-noise ratio in the measurements was low. In addition, reproducibility was not high because the contact pressure changed between measurements.

In this paper, we describe a new hollow optical fiber probe with a trapezoidal multi-reflection prism for ATR spectroscopy. The increase in the number of reflections brings about higher sensitivity, and the flat and wide contact surface of the prism results in higher measurement reproducibility.

2. Experiment

Figure 1 shows the experimental setup schematically. Infrared light from an FT-IR spectrometer (Bruker Tensor27) was focused on the input end of a hollow optical fiber by using a gold-coated off-axis mirror with a focal length of 100 mm. We used hollow optical fibers with an inner silver coating for transmission of mid-infrared light [21]. The fibers are based on a flexible polycarbonate tube with an inner diameter of 2 mm, and their minimum bending radius is about 5 cm. A hollow optical fiber with a relatively large diameter has the advantage of high total transmitted power, especially for incoherent light from a spectrometer that cannot be focused onto a small spot. A trapezoidal prism is fixed at the distal end of the hollow optical fiber, and the incident light is totally reflected multiple times at the surfaces of the prism. An evanescent field is produced when the incident light is reflected, and the absorption of the sample touching the prism surface is detected as an attenuation of the transmitted light. The detected light enters another hollow optical fiber and is transmitted to a mercury cadmium telluride (MCT) detector. In the spectral measurement, a single measurement with a frequency resolution of 4 cm$^{-1}$ and 128 integration times takes 60 seconds.

We used a trapezoidal prism providing nine reflections. Its dimensions are shown in the inset of Fig. 1. We chose zinc “sulfide” (ZnS) as the prism material because of its chemical stability, nontoxicity, and lower cost than diamond. Unlike zinc “selenide” (ZnSe), ZnS is not considered carcinogenic and is even utilized as a pigment (known as “lithopone”) in cosmetics and dental materials [22]. It has a low refractive index of 2.2, and hence, has a larger penetration depth than high index materials such as germanium and silicon.
We measured the absorption spectra of inner lip mucosa in order to determine the change in blood glucose levels during an oral glucose tolerance test. In this test, volunteers took 75 g of glucose sugar dissolved in 150 ml of water, and reference blood sugar levels were measured by using a blood glucose measurement system in which blood samples are taken from the fingertips (Terumo Medisafe Mini). At the same time as the glucose tolerance test, the absorption spectra at the inner lip mucosa were measured by holding the prism between the upper and lower lips. Here, the transmitted power spectra measured when the prism was held in air were used as a reference, and saliva was carefully wiped from the lips before the measurement in order to suppress the effect of saliva components. When measuring spectra of human lips, the total transmitted power of the FT-IR signal that was the integral power of whole wavelengths was monitored and we kept it constant for all measurements. By doing this, we could suppress effects of fluctuation of pressure applied on the prism and absorption of saliva, because amplitude of water absorption that changes with both of applied pressure and water in saliva is much stronger than those of other components such as glucose. Our protocol was approved by the ethical committee of Tohoku University on the Use of Humans as Experimental Subjects, and informed consent was obtained from the examinees.

A measured absorption spectrum obtained in the fasting state is shown in Fig. 2. The large peaks around 3300 cm$^{-1}$ and 1670 cm$^{-1}$ were assigned to the OH stretching and bending bands of water molecules, respectively. The small peak around 2400 cm$^{-1}$ was caused by CO$_2$ gas of breath that penetrated the fiber’s bore during the measurement. Absorption peaks of amide I and II can be seen at 1670 cm$^{-1}$ and 1550 cm$^{-1}$, respectively, and the former overlapped the peak of the OH bending band. The inset of Fig. 2 shows the small absorption peaks of glucose around 1155 and 1080 cm$^{-1}$.
To see these small absorption peaks more clearly, we calculated the second derivative spectra. Some of these spectra are shown in Fig. 3. Figure 4 shows calibration plots of the peak heights of the second derivatives at 1155 cm⁻¹ and 1080 cm⁻¹. The coefficients of determination $R^2$ are 0.60 for 1155 cm⁻¹ and 0.28 for 1080 cm⁻¹. The larger errors in the 1080 cm⁻¹ plot may be due to components other than glucose. It is known that phospholipids and nucleic acids in saliva produce an absorption peak at 1084 cm⁻¹ [23]. The peak at 1155 cm⁻¹, which arises from the combination of C-C and C-O bonds [24] in the pyranose ring of glucose, may better reflect the quantity of glucose molecules.

Fig. 3. Second derivative spectra of measured absorption of lip mucosa.

![Second derivative spectra of measured absorption of lip mucosa.](image)

Fig. 4. Calibration plots of the peak height of second derivatives at (a) 1155 cm⁻¹ and (b) 1080 cm⁻¹.

![Calibration plots of the peak height of second derivatives.](image)
To improve the measurement accuracy, we calculated difference spectra by subtracting from each measured spectrum taken at the higher blood glucose levels the spectrum measured in the fasting state. These difference spectra should correspond to absorption of only glucose and therefore can reduce errors caused by other components in human tissues such as water, carbohydrate, protein, and sugars that do not change with the blood glucose level. In addition, this calculation of the difference spectra can suppress errors due to individual differences in the spectral shape. Figure 5 shows difference spectra obtained at blood glucose levels of 84 and 157 mg/dl. A spectrum measured in the fasting state with a glucose level of 72 mg/dl was used as the subtrahend. In calculating these spectra, spectrum baselines drawn from 1173 to 982 cm\(^{-1}\) that correspond to the absorption band of glucose were subtracted from the originally measured spectra. Figure 5 also shows glucose solution spectra that were fitted to the measured spectra. A glucose solution with a concentration of 1% was measured with a conventional ATR spectroscope and then a least-squares spectrum fitting was done using the solution spectrum as the base spectrum. For the fitted spectra shown in Fig. 5, the weights applied to the base spectrum were 0.17 for 84 mg/dl and 0.48 for 157 mg/dl. The difference spectra clearly shows the absorption peaks that fit those of the glucose solution, and in addition to the peaks at 1155 cm\(^{-1}\) and 1080 cm\(^{-1}\) described above, there are peaks at 1110, 1035, and 990 cm\(^{-1}\). It is reported that the peaks at 1080 cm\(^{-1}\) and 1035 cm\(^{-1}\) arise from C-O bonds and the ones at 1155, 1110 and 990 cm\(^{-1}\) are from a combination of C-C and C-O bonds [24]. Some researchers reported that it was not possible to detect any glucose signatures correlated with blood glucose concentrations because of the limited penetration depth of mid-infrared ATR spectroscopy [15]. In our setup, the calculated penetration depth is around 1.9 μm at the wavenumber of 1000 cm\(^{-1}\) assuming refractive index of ZnS as 2.2 and that of oral mucosa as 1.3. Although the calculated penetration depth is smaller than thicknesses of stratum corneum layer (~10 μm) that have been shown [25, 26], it is also reported that the thickness of stratum corneum of oral mucosa is much smaller than that of skin and the thickness varies considerably by location in oral cavity. Also it is reported that the permeability of oral mucosa for water is much higher than that of skin [27] and sugar transporters in human oral mucosal cells transport D-glucose across the stratified epithelial layer [28]. From these facts, we suppose that a small amount of interstitial fluid that reflects the blood glucose level penetrates to the thin and permeable stratum corneum of oral mucosa and that it can be detected by multi-reflection prisms with much higher sensitivity than other common ATR prisms.

Fig. 5. Difference spectra between the fasting state and state with higher blood glucose levels. Also shown (the thinner lines) are solution spectra created from least-squares fittings.

We first tried to obtain a calibration plot for blood glucose level by using the weights derived by the least-squares fitting, but the coefficient of determination R\(^2\) of the plot was smaller than 0.5. This was mainly because the shapes of the absorption spectra of lip mucosa
were different from those measured for the glucose solution, as shown in Fig. 5. There were random changes in the amplitude of the absorption peaks at 1080 and 1035 cm\(^{-1}\). As described above, these peaks originate from C-O bonds of compounds other than glucose, such as phospholipids in oral mucosa [29] that reflects compounds in ingested foods. In addition, carbon hydrate residues attached to collagen show absorption peaks at 1032 and 1086 cm\(^{-1}\) [23]. We hence focused on the peak arising from the C-C and C-O combination at 1155 cm\(^{-1}\) because it originates from the pyranose ring structure of glucose. Figure 6 shows spectra around 1155 cm\(^{-1}\) for different blood glucose levels. Here, baselines drawn from 1173 to 1140 cm\(^{-1}\) were subtracted from the measured spectra. We found that the peak at 1155 cm\(^{-1}\) was not affected by random factors such as saliva and proteins and that the peak amplitude changed with the blood glucose level.

Figure 7 shows the change in differential absorption at 1155 cm\(^{-1}\) from the fasting stage of the experiment to after the glucose solution was ingested. A least-squares spectrum fitting was done using the solution spectrum as the base spectrum. Blood glucose levels measured by blood sampling performed at the same time as the optical measurement are also shown for comparison. The results of the optical measurement closely follow the changes in blood glucose level. Figure 8 shows a calibration plot drawn on a Clarke error grid. The reference values are blood glucose levels measured by blood sampling, and the measured values are calculated from the differential glucose absorption around 1155 cm\(^{-1}\) explained above. The figure shows that the certainty is improved and that the coefficient of determination \(R^2\) is 0.75. The standard error in the glucose level is 12 mg/dl, and all the measured values are in Region A, where the measured values are within 20% of the reference values.
3. Conclusion

To measure blood glucose levels, we developed a mid-infrared ATR spectroscopy system that consists of hollow optical fibers for infrared light delivery, a trapezoidal multi-reflection ATR prism, and a conventional FT-IR spectrometer. Owing to the low transmission loss and high flexibility of the hollow optical fibers, the system can be used anywhere blood capillaries are close to the surface, such as inner lip mucosa. Using a multi-reflection prism brought about higher sensitivity, and the flat and wide contact surface of the prism resulted in higher measurement reproducibility. In the analysis of in vivo measurement data, difference spectra between the fasting state and states of higher glucose levels were calculated and clear signatures of glucose absorption were evident in them. Owing to the distinct absorption spectra of glucose found in the difference spectra, we found that absorption peaks assigned to C-O bonds of glucose tend to be randomly affected by other components such as phospholipids and proteins in tissues. We also found that although the peak at 1155 cm$^{-1}$ that originates from the pyranose ring structure of glucose was smaller than those originating from C-O bonds, it was clearly evident and its amplitude followed changes in blood glucose level. By applying a least-squares fitting of the glucose reference spectrum to the measured ones at 1155 cm$^{-1}$, we obtained a calibration plot with measurement errors less than 20%.

The proposed system still has some difficulties. One of the difficulties is that examinees need to become accustomed to the measurement if fluctuation in the pressure applied by their lips is to be avoided. This method detects glucose in interstitial fluid that reflects the blood glucose level because the penetration depth of mid-infrared light is limited to a few microns, and a small change in contact pressure causes fluctuation in the measured depth and results in measurement errors. The error of 20% that is reported in this paper is not good enough and smaller error with real time measurement is necessary. We are therefore trying to measure blood glucose at sites where a small clamp that provides stable pressure can be used, such as an ear lobe. Access to the ear lobe is easy because of the flexibility of the hollow optical fibers. In order to show the feasibility of the method, we are now working on in vivo experiments with more volunteers that will involve measuring at both the lips and the ear lobes.

The uncertainty of the measurement should be reduced by improving the signal-to-noise ratio of the system, and this could be done by using stronger light sources such as quantum cascade lasers (QCLs). Our findings suggest that blood glucose could be measured accurately and rapidly by using QCLs with a wavelength of 1155 cm$^{-1}$ and another wavelength at which glucose shows no absorption.