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Multiple-Scattering-Free Optical Glucose Monitoring Based on Femtosecond Pulse Interferometry

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We have designed a novel multiple-scattering-free optical glucose monitoring system based on femtosecond pulse interferometry. The glucose concentration is determined by a time-of-flight method, while multiple-scattering light is rejected by a coherence gate. The resultant accuracy and precision were 94.7 and 24.5 mg/dl, respectively, for physiological-equivalent glucose concentrations in a scattering medium of 1.0% intralipid solution, while the accuracy and precision for a reference sample containing no scattering medium were 65.3 and 23.8 mg/dl, respectively. Comparing these results, we conclude that the proposed method is effective for glucose measurement in a scattering medium.

Key words: glucose, femtosecond pulse light, multiple scattering, coherence gate, interferometry, time-of-flight, group refractive index

1. Introduction

There are today a considerable number of diabetes mellitus patients worldwide. Among them, those suffering from serious diabetes must draw their own blood several times a day by puncturing their finger to monitor the glucose concentration. This blood drawing results in mental and physical stress for the patients. Furthermore, to carry out clinical diagnosis, medical staff members are subjected to the risk of blood infection when collecting blood.

Optical glucose monitoring is an attractive technique to achieve noninvasive and pain-free monitoring; however, its application to *in vivo* measurement has been limited. In *in vitro* measurement, studies using various techniques have been reported: (a) near-infrared absorption spectroscopy,[1] (b) polarimetry of optical rotatory power,[2] and (c) Raman spectroscopy.[3] However, the sensitivity and quantitativeness of these methods do not meet the requirements necessary for clinical examinations. One reason for the limited sensitivity and quantitativeness is the coexistence of multiple-scattered light caused by particles present in blood, such as cells, lipids, and proteins. For example, it is difficult to distinguish between absorption by the glucose and scattering by the particles in near-infrared absorption spectroscopy. In polarimetry, the multiple-scattering events depolarize the linear polarization of the incident light, resulting in reduced measurement precision. In Raman spectroscopy, multiple-scattering and reabsorption in biological tissue weaken the analytical Raman spectrum.[4] The weak Raman signal increases the measurement time required to gain a satisfactory signal-to-noise ratio (SNR) to at least a few minutes, resulting in limited potential for practical use in real-time glucose monitoring.

In optical glucose monitoring, because only non-multiple-scattered, ballistic light in the blood offers direct information on the glucose concentration, elimination of multiple-scattered light is essential to improve the reliability of the measurement. Several techniques for elimination of the multiple-scattered light in biological tissues have been reported, for example, using an ultrafast time-resolved gate[5,6] and a coherence gate,[7] the latter being attractive because of its simplicity. It extracts only the ballistic light as an interference signal using the coherence remaining in the ballistic light. Use of such a coherence gate offers the possibility of multiple-scattering-free optical glucose monitoring.[8,9]

Optical coherence tomography (OCT), which is one attractive application of the coherence gate technique, can be effectively applied to *in vivo* glucose monitoring.[10] OCT-based glucose monitoring uses the change of scattering coefficient depending on the glucose concentration in biological tissue. Although there is a correlation between glucose concentration and the scattering coefficient in tissue, the precision of the measurement does not meet the necessary requirements, even in *in vitro* measurement, because of the insensitive change of the scattering coefficient. To improve the measurement precision, we have designed a novel time-of-flight coherence gate system in combination with femtosecond pulse interferometry. Determination of the glucose concentration is based on the measurement of a group refractive index (n_g) of the glucose solution. Although measurement of the refractive index, using an Abbe refractometer, is one attractive method to determine the sugar content in fruits and drinks, it cannot be simply applied to optical glucose monitoring because of the poor resolution in the concentrations, necessity for blood drops, and the multiple scattering. To apply refractive index measurement to optical glucose monitoring, we propose determination of the glucose concentration via precise measurement of n_g by an interferometric technique. More precisely, the n_g value of the glucose sample is extracted as a time delay of femtosecond order by a time-of-flight method with femtosecond pulse interferometry. Simultaneously, multiple-scattered light, which disturbs the precise measurement of n_g, is rejected by the coherence gate.

After describing the principle and basic examination of our method, we will evaluate its ability to eliminate multiple-scattered light using a scattering medium. We will also demonstrate *in vitro* multiple-scattering-free optical glucose monitoring using a mixture of glucose and the scattering medium.

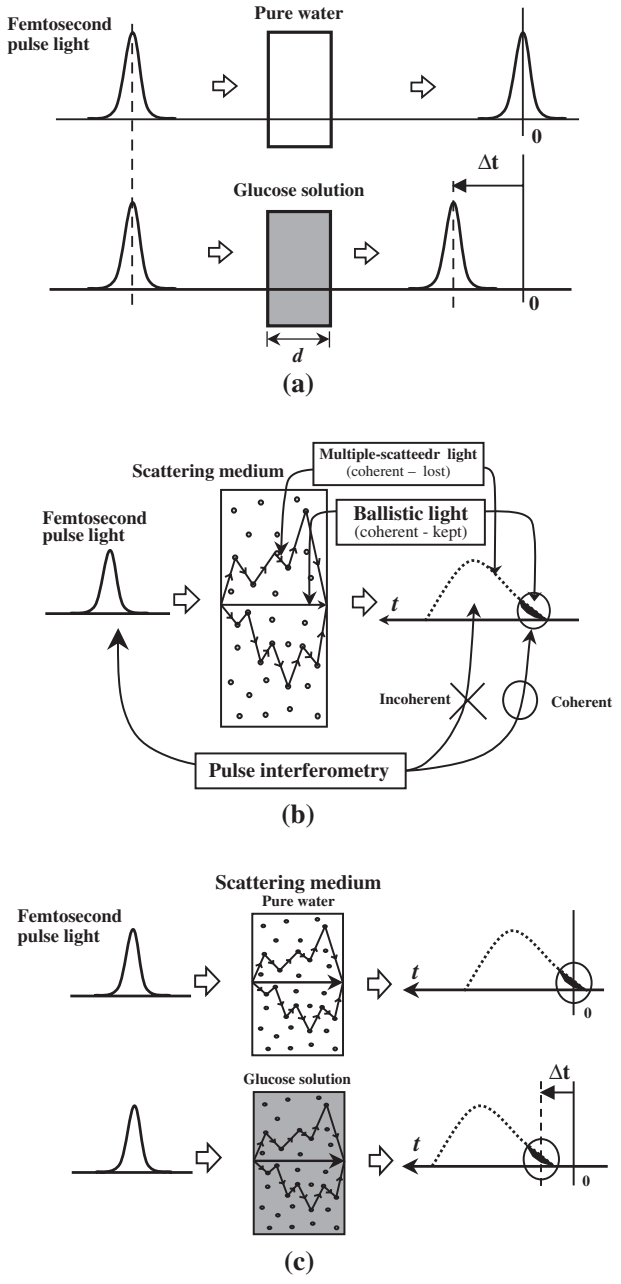

Fig. 1. (a) Principle of glucose concentration measurement. The temporal shift of femtosecond pulse light is caused by glucose addition, which is proportional to glucose concentration under a known geometric path length. (b) Principle of elimination of multiple-scattered light. Unscattered ballistic light is coherent with incident pulse light, resulting in generation of the interference signal in pulse interferometry. The coherent-lost, multiple-scattered light is rejected by the coherence gate with pulse interferometry. (c) Principle of multiple-scattering-free optical glucose monitoring. Temporal shift of ballistic light extracted by coherence gate gives glucose concentration.

2. Principle

2.1 Glucose concentration measurement

The principle of the glucose concentration measurement is shown in Fig. 1(a). When femtosecond pulse light is incident on an aqueous solution sample, the time to pass through the sample depends on the group refractive index of the sample, assuming a known geometric path length. Here, we consider a temporal shift Δt of the femtosecond pulse light caused by glucose addition, where the time origin is defined as the time to pass through a sample of pure water. The Δt value is defined as follows:

$$\Delta t = \frac{\Delta n_g \cdot d}{v_c}, \tag{1}$$

where d is the geometric path length of the sample, v_c is the velocity of light in vacuum, and Δn_g is the change of group refractive index caused by the glucose addition. Since the Δn_g value is proportional to the glucose concentration, we can express Δt as follows:

$$\Delta t = \frac{k \cdot C \cdot d}{v_c}, \tag{2}$$

where C is the glucose concentration and k is a proportionality factor. If the k, d, and v_c values are known, we can determine the C value through precise measurement of Δt by time-of-flight measurement with femtosecond interferometry.

2.2 Elimination of multiple-scattered light

The principle of elimination of the multiple-scattered light is shown in Fig. 1(b). We consider the temporal behavior of ultrashort-pulse light propagation in the scattering medium. When ultrashort pulse light passes through the scattering medium, multiple-scattering events cause different effective optical path lengths between multiple-scattered light and unscattered light. As a result, the unscattered ballistic light arrives first while the multiple-scattered light is delayed. Hence, the ballistic light can be extracted by an ultrafast time-resolved gate, such as an optical Kerr gate.[5] In practice, however, the multiple-scattered light and ballistic light cannot be completely separated from each other, as shown in Fig. 1(b), resulting in limited extraction of the ballistic light.

Regarding the coherency of light in the scattering medium, the ballistic light maintains the coherency of the incident pulse light, whereas the multiple-scattered light loses the coherency during the scattering events. Hence, one can extract the ballistic light from the scattering medium as an interference signal by the coherence gate.

2.3 Multiple-scattering-free optical glucose monitoring

Combined use of the two ideas above provides a scheme for multiple-scattering-free optical glucose monitoring, as shown in Fig. 1(c). Here, we consider a mixture of glucose and scattering medium as the sample. Since the temporal characteristics of the ballistic light extracted by the coherence gate are directly related to the glucose concentration, one can determine the glucose concentration from the Δt value obtained by the time-of-flight measurement. The Δt value in Fig. 1(c) is determined by temporal shift of the maximum on the interference signal envelope of ballistic light with respect to glucose concentration. In this way, the femtosecond pulse interferometry provides the ability to achieve multiple-scattering-free optical glucose monitoring.

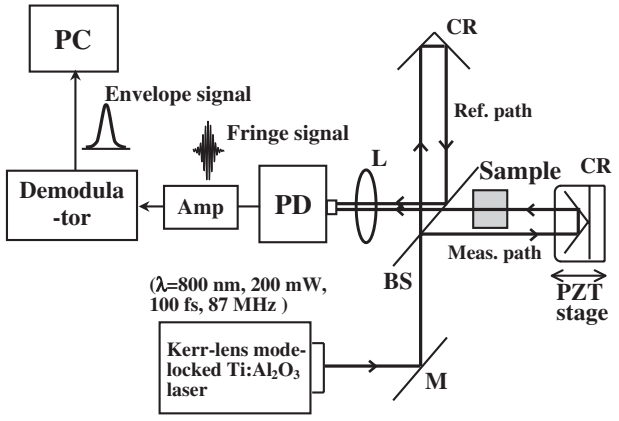

Fig. 2. Experimental setup of femtosecond pulse interferometry. M: mirror, BS: beam splitter, CR: corner reflector, PZT stage: piezoelectric stage, L: lens, PD: photodetector, Amp: amplifier, PC: personal computer.

3. Experimental Setup

Figure 2 shows the experimental setup of the femtosecond pulse interferometry system. The laser source is a Kerr-lens mode-locked Ti:Al_2O_3 laser (Avesta Project, Ltd., TiF-Kit-100, central wavelength = 800 nm, pulse width = 100 fs, average power = 200 mW, repetition rate = 87 MHz). The laser light is divided into a reference path and a measuring path with a beamsplitter (BS). A sample cuvette is set in the measuring path. The two beams from both corner reflectors (CRs) are combined again by the BS. A PZT stage (Piezosystem Jena PX400CAP, stroke = 320 μm, with air-capacitive sensor) scans the measuring CR with a 1-Hz triangular wave. At the moment the two optical path lengths are equal to each other, a pulse interference signal (coherence time = 133 fs, full width at half maximum) is observed during the scanning procedure. The generated interference fringe signal is detected with a photodetector (PD). After amplification, the envelope signal of the fringe signal is electrically extracted using a demodulator. The temporal shift of the maximum on the envelope signal is determined by the PZT stage displacement.

Before demonstration of the optical glucose monitoring, we estimated the linearity and resettability of the PZT stage displacement using a Michelson interferometer equipped with a frequency-stabilized Zeeman laser. Here, the linearity is defined as the standard deviation of error between the measured displacement and the ideal value within a range used in the actual glucose measurement. The resettability is defined as the standard deviation of time origin fluctuation without the sample. To obtain the resettability value, we measured the fluctuation of the time origin every 2 min for 1 h. The resulting linearity and resettability were estimated as 5.3 and 35.1 nm, which correspondto a linearity of 0.04 fs and a resettability of 0.23 fs in time delay, respectively. These values limit accuracy and precision in glucose concentration measurement.

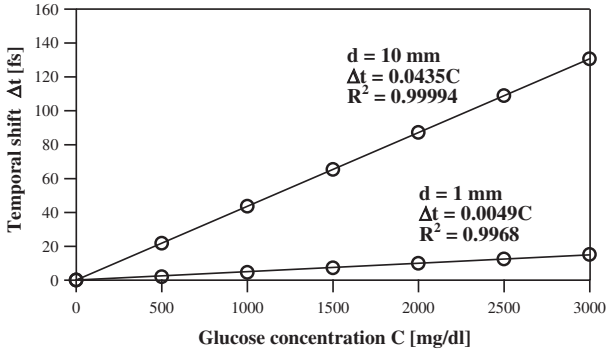

Fig. 3. Relationship between the glucose concentration and the temporal shift in 10-mm cell and 1-mm cell. Measurements were repeated 10 times at each concentration. Solid lines are correlation curve obtained by a linear fitting.

4. Results

4.1 Glucose concentration measurement

We investigated the relationship between the glucose concentration and the temporal shift of the envelope signal. Sample cells with 10-mm and 1-mm thickness were used. We prepared glucose solution samples with concentrations of 0, 500, 1000, 1500, 2000, 2500, and 3000 mg/dl, which covered the physiological range of blood glucose concentration (normal: 60–200 mg/dl, diabetic: 200–2000 mg/dl). In the measurement with the 10-mm cell and 1-mm cell, the temperatures of the glucose samples were stable within ±0.2°C. The measurements were repeated ten times at each concentration; Fig. 3 shows the results. The solid lines are the correlation curves obtained by linear fitting of the data points, equivalent to kd/v_c in eq. (2). As one can see, linear correlations were observed in both the 10-mm cell and the 1-mm cell. The ratio of the correlation slopes between the 10-mm cell and the 1-mm cell (= 8.9) was not completely consistent with the sample thickness ratio (= 10). The resettability of the PZT stage (= 0.23 fs) influences the measured value of time delay directly, resulting in variations of slopes for the two cells. Furthermore, the concentration error in the glucose sample also contributes to these slopes. Precision in an electronic balance used in sample preparation (= 0.3 mg) causes concentration error of ±37 mg/dl at 3000 mg/dl glucose concentration, resulting in variation of the slopes within 0.0435 ± 0.0005 for the 10-mm cell and 0.0049 ± 0.00006 for the 1-mm cell. Considering both effects, we estimate the minimum slope ratio is 8.76. Hence, we can conclude that the present slope ratio of 8.9 is within the range expected by resettability and concentration error.

Here, we introduce an accuracy and a precision for the glucose measurement to evaluate the performance of the proposed system. Accuracy is defined as the standard deviation of the difference of each measurement value from the value on the correlation curve. Precision corresponds to the standard deviation of the ten repeated measurements at each concentration. The measurement accuracy and precision in the 10-mm cell were 8.34 and 1.75 mg/dl, respectively. We consider that the accuracy is limited by the

linearity of the PZT stage displacement, the concentration error in the glucose sample and/or the fluctuation of sample temperature, while the precision depends on the resettability of the PZT stage. The linearity of the PZT stage displacement (= 5.3 nm) and the concentration error in the glucose sample lead to the accuracy error of 0.79 and 4.78 mg/dl, respectively. Here, we estimated the concentration error using the standard deviation of sample concentration variance that arises from precision of a pipet (= 8.0 µl) for dilution of the glucose sample. On the other hand, the temperature instability of ±0.2°C causes an accuracy error of 9.63 mg/dl. Hence, the accuracy is mainly affected by the instability of the sample temperature. The precision is limited by the resettability of the PZT stage displacement. The resettability of the PZT stage (= 35.1 nm) is equivalent to a precision of 5.19 mg/dl. However, the actual precision value obtained (= 1.75 mg/dl) is better than the predicted value corresponding to the resettability of the PZT stage, because the measurement time (= 10 s) in the actual glucose monitoring was much shorter than that in the performance testing of the PZT stage (= 1 h).

For clinical applications, an optical path length of 1 mm is more realistic. The measurement accuracy and precision in the 1-mm cell were 65.3 and 23.8 mg/dl, respectively. These values are about 10 times larger than those in the 10-mm cell because of the lower dependence on glucose concentration due to the reduced sample thickness (see Fig. 3). In this way, there is an inherent trade-off between the optical path length and the measurement quantitativeness.

4.2 *Elimination of the multiple-scattered light*

We evaluated the ability to eliminate the multiple-scattered light using scattering solution samples of three different thicknesses. As the scattering samples, 1.0% intralipid solution, whose scattering coefficient is equivalent to that of biological tissue (= 1.0 mm^{-1}), was prepared. To examine the measurement dynamic range, we measured the maximum intensity level of the envelope signal at each sample thickness. Assuming that reliable glucose monitoring can be carried out when the SNR of the measurement is larger than 2, this system enables samples up to 3-mm thick to be used, as shown in Fig. 4. In the present system, multiple-scattered light collinear with the ballistic light contaminates the analytical signal and appears as an offset signal, resulting in an increase in the background noise level to −30 dB, though the shot noise level due to the detector and the electronic equipment is as low as −80 dB. If the background noise level is effectively reduced to the shot noise level (−80 dB), thicker samples, up to 8-mm thick, can be used.

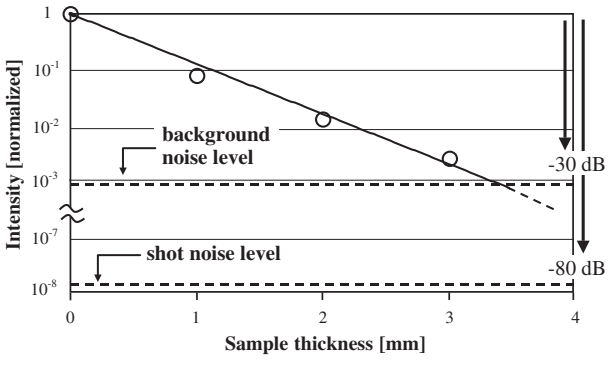

Fig. 4. Maximum intensity of envelope signal detected at each scattering sample thickness. A 1.0% intralipid solution was used as a scattering sample.

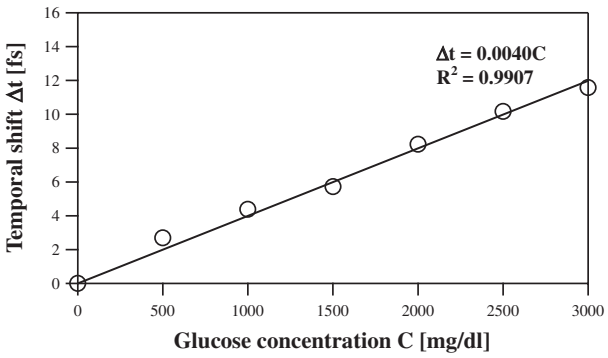

Fig. 5. Relationship between the glucose concentration and the temporal shift in a mixture of a 1.0% intralipid solution and glucose. Thickness of a sample cell is 1 mm. Measurements were repeated 10 times at each concentration. Solid line is a correlation curve obtained by a linear fitting.

4.3 *Glucose concentration measurement in scattering medium*

We demonstrated the measurement of the glucose concentration in intralipid solutions using the proposed system. Mixtures of glucose and a 1.0% intralipid solution were measured ten times at each concentration (= 0, 500, 1000, 1500, 2000, 2500, and 3000 mg/dl). The sample thickness was 1 mm considering the clinical measurement. Figure 5 shows the relationship between the glucose concentration and the temporal shift. One can find a linear correlation between the concentration and the shift value similar to that for the 1-mm thickness in Fig. 3. The measurement accuracy and precision were 94.7 and 24.5 mg/dl, respectively. These values are in good agreement with those of the 1-mm thick aqueous glucose solution. This result indicates the possibility of multiple-scattering-free optical glucose monitoring.

5. Discussion

We have accomplished a measurement accuracy in glucose concentrations of 8 and 65 mg/dl for the 10-mm cell and 1-mm cell, respectively. These values are satisfactory for *in vitro* glucose monitoring. However, several problems for clinical application remain unresolved in our system. Since usual interferometric methods are very sensitive to environmental disturbances (air turbulence, mechanical and sound vibrations, and changes in sample temperature), the proposed method needs a very stable, controlled environment, such as a laboratory. We are now investigating an optical glucose monitoring method using

femtosecond two-color pulse interferometry as a common-path interferometric method to overcome the problems concerning environmental disturbances.[11)]

Although the present method was demonstrated under the assumption of known sample thickness, unknown sample thickness is more realistic in clinical applications. In that case, the temporal shift Δt is dependent on both the glucose concentration and the sample thickness. Simultaneous measurement of two independent optical parameters sensitive to glucose concentration (for example, group refractive index and optical rotatory power) can determine the glucose concentration and sample thickness, respectively.

Selection of the measurement site is a troublesome problem common to various optical glucose monitoring methods. The power penetrating 3 mm into a scattering medium is not necessarily sufficient for optical glucose monitoring through skin. One possibility is to use another femtosecond pulse laser, such as a mode-locked Cr:Forsterite laser (center wavelength = 1300 nm), which possesses deeper penetration power into biological tissues. However, optical glucose monitoring through the skin is strongly influenced by the condition of the skin surface even if such a laser is applied. Another possibility is to use the aqueous humor in the eye as a direct window for the optical glucose monitoring; this technique is conventionally used in polarimetry of optical rotatory power[12)] and Raman spectroscopy.[13)]

6. Conclusion

We proposed a multiple-scattering-free optical glucose monitoring method based on femtosecond pulse interferometry. For determination of the glucose concentration, we used the temporal shift of femtosecond pulse light caused by glucose concentration dependence of the group refractive index. The resulting accuracy and precision of the glucose concentration measurement were 8.34 and 1.75 mg/dl in a 10-mm cell, and 65.3 and 23.8 mg/dl in a 1-mm cell. The ballistic light component was effectively extracted in a scattering medium having a scattering coefficient equivalent to that of biological tissue ($= 1.0\,\mathrm{mm}^{-1}$) by the proposed method. Furthermore, we demonstrated multiple-scattering-free optical glucose measurement using a mixture of glucose and scattering medium.

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