OPTICAL REVIEW Vol. 13, No. 1 (2006) 29–33

Optical Glucose Monitoring Based on Femtosecond Two-Color Pulse Interferometry

Yasuaki HORI, Takeshi YASUI and Tsutomu ARAKI

Department of Mechanical Science and Bioengineering, Graduate School of Engineering Science, Osaka University, Toyonaka, Osaka 560-8531, Japan

(Received April 27, 2005; Accepted October 5, 2005)

We demonstrate the potential of femtosecond two-color pulse interferometry for *in vitro* optical glucose monitoring, by dispersion of the group refractive index in a glucose solution sample with respect to a red-color light and a blue-color light. By comparison with femtosecond one-color pulse interferometry, the basic performance of the present system with regard to sensitivity, quantitativeness, and tolerance to surrounding disturbances, is evaluated. The resulting accuracy and precision of glucose determination are 77 and 118 mg/dl for 10-mm-sample-thickness, respectively. This near-common-path configuration of the two-color pulse light provides good stability to fluctuations of sample temperature, which is important in clinical applications. Considering the performance of femtosecond two-color pulse interferometry as an optical glucose sensor, a suitable measurement site for *in vivo* optical glucose monitoring is discussed.

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

1. Introduction

One of the most challenging and valuable subjects in biomedical optics is optical glucose monitoring, which frees not only diabetic patients from the physical and mental burden accompanying finger puncture but also the medical staff from risk of a blood infection accident. Such the optical glucose monitoring also offers continuous and/or sensitive monitoring of glucose concentration in the blood. There are several reports on optical glucose monitoring: infrared absorption spectroscopy,[1] polarimetry of optical rotatory power,[2] Raman spectroscopy,[3] and so on. When these methods are applied for clinical examinations, selection of a measurement site is crucial. Considering direct measurement of blood glucose in blood vessel across the skin, the sensitivity, and quantitativeness of these methods do not satisfactorily meet the requirements for clinical examinations. One reason for the low sensitivity and quantitativeness is the coexistence of multiple-scattered light caused by the undesirable particles in blood such as cells, lipids, and proteins. Recent advance in optical technology, however provides effective techniques to extract the desired optical property from heavily-scattering biological tissue, such as coherence gate[4] or ultrafast time-resolved gate.[5] These techniques can detect the weak ballistic light while they reject the multiple-scattered light. We previously proposed the application of femtosecond one-color pulse interferometry (FOPI) to optical glucose monitoring.[6] The combined use of time-of-flight measurement and coherence gate achieved by FOPI gives quantitativeness to glucose concentration measurement even in a scattering medium similar to blood, resulting in the achievement of *in vitro* multiple-scattering-free optical glucose monitoring. To extend such an interferometry method to *in vivo* glucose monitoring, it is necessary to suppress the effects of surrounding disturbances: air turbulence, sound and mechanical vibrations, and temperature fluctuation of the sample, because they disturb the interference signal and hence decrease the measurement quantitativeness.

One attractive method to suppress such effects is use of common-path-configuration in interferometry. Combined use of an optical nonlinear effect [second-harmonic-generation (SHG)] induced by femtosecond pulse light with interferometry provides unique near-common-path pulse interferometry consisting of a red pulse light and a blue pulse light, namely femtosecond two-color pulse interferometry (FTPI).[7] The FTPI generates an interference signal arising from the difference of group refractive index between the two-color pulse lights and derives good stability to the surrounding disturbances described above.

In this paper, we apply the FTPI to optical glucose monitoring and evaluate its potential as an optical glucose sensor through comparison with the FOPI. We first evaluate its sensitivity to glucose concentration and quantitativeness of the glucose concentration measurement using an aqueous glucose solution sample. Next, we determine the stability to temperature change of sample in the glucose concentration measurement to evaluate tolerance to surrounding disturbances. Finally, based on the resulting performance of FTPI, suitable measurement sites for *in vivo* optical glucose monitoring are discussed.

2. Principle

2.1 Glucose concentration measurement

The principle of glucose concentration measurement is shown in Fig. 1. In general, when an ultrashort pulse light is incident to a glucose solution sample, the time required to pass through the sample depends on the group refractive index of the sample on the assumption of a fixed geometric path length. Since the group refractive index is proportional to the sample concentration, one can determine the glucose concentration by time delay of the interference signal based

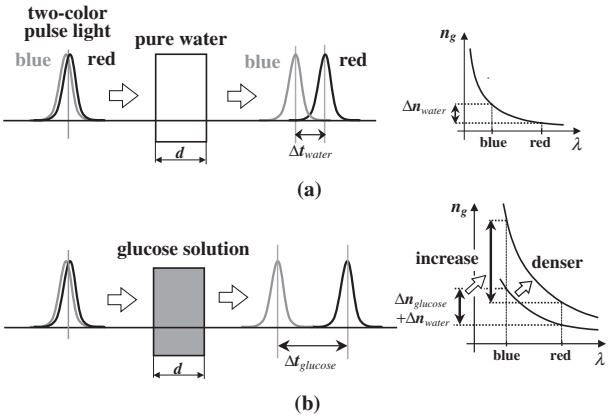

Fig. 1. Glucose concentration measurement based on FTPI: (a) pure water sample and (b) glucose solution sample. When temporally and spatially overlapped two-color (red and blue) pulse is incident to a glucose solution sample, the time delay between the two-color pulse varies depending on the glucose concentration.

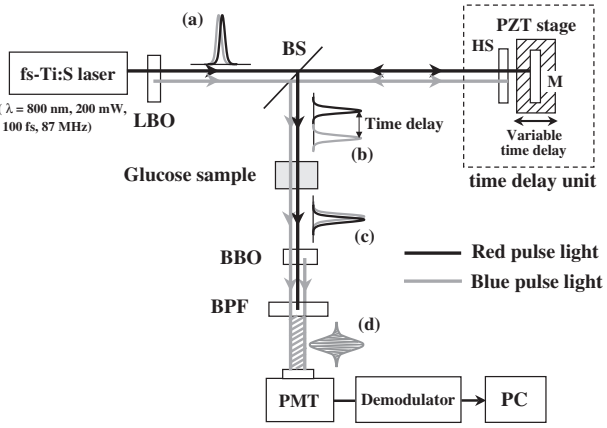

Fig. 2. Experimental setup of FTPI. LBO: frequency-doubling LiB_3O_5 crystal, BS: beam splitter, HS: harmonic separator, M: mirror, PZT stage: piezoelectric stage, BBO: frequency-doubling β-BaB_2O_4 crystal, BPF: blue-pass filter, PMT: photomultiplier, PC: personal computer.

on a time-of-flight method with femtosecond pulse interferometry.[6)]

Let us consider that spatially and temporally overlapped red and blue pulse light (namely, two-color pulse light) are incident to a glucose solution at the same time. First, we assume the sample is pure water without glucose [Fig. 1(a)]. After passing through the sample, the blue pulse light is delayed by $\Delta t_{\mathrm{water}}$ relative to the red pulse light because of positive dispersion of the group refractive index in the water. The value of $\Delta t_{\mathrm{water}}$ is given as follows:

$$\Delta t_{\mathrm{water}} = \frac{\Delta n_{\mathrm{water}} \cdot d}{c}, \tag{1}$$

where $\Delta n_{\mathrm{water}}$ is the difference of group refractive index between red and blue pulse light in the water, d is the geometric path length of the sample, and c is the light velocity in vacuum. Second, we consider a glucose solution as a sample [Fig. 1(b)]. The time delay in this sample ($\Delta t_{\mathrm{sample}}$) is given as follows:

$$\Delta t_{\mathrm{sample}} = \frac{(\Delta n_{\mathrm{glucose}} + \Delta n_{\mathrm{water}})d}{c}, \tag{2}$$

where $\Delta n_{\mathrm{glucose}}$ is the difference of group refractive index between red and blue pulse light in the glucose. Using eqs. (1) and (2), the $\Delta n_{\mathrm{glucose}}$ value is determined as follows:

$$\Delta n_{\mathrm{glucose}} = \Delta n_{\mathrm{water}}\left(\frac{\Delta t_{\mathrm{sample}} - \Delta t_{\mathrm{water}}}{\Delta t_{\mathrm{water}}}\right), \tag{3}$$

where $\Delta n_{\mathrm{water}}$ and $\Delta t_{\mathrm{water}}$ are known values. The group refractive index at each wavelength increases proportionally with the glucose concentration, and the increment of the group refractive index at the shorter wavelength is larger than at the longer wavelength. Hence, the $\Delta n_{\mathrm{glucose}}$ value proportionally increases with the concentration of glucose solution. We determine the glucose concentration through precise measurement of the $\Delta t_{\mathrm{sample}}$ value by the time-of-flight measurement using FTPI.

3. Experimental Setup

Figure 2 shows an experimental setup of FTPI. The laser source is a Kerr-lens mode-locked Ti:Al_2O_3 laser (Avesta Project, TiF-Kit-100, central wavelength = 800 nm, pulse width = 80 fs, average power = 200 mW, repetition rate = 87 MHz). The SHG light is produced from the fundamental laser light by a frequency-doubling LiB_3O_5 crystal (LBO, thickness = 1 mm). The SHG light (blue pulse light, wavelength = 400 nm) and remaining fundamental light (red pulse light, wavelength = 800 nm) are introduced to the interferometer as two-color pulse light maintaining a temporal and spatial overlap [(a) in Fig. 2]. The red pulse light is separated and delayed compared to the blue pulse light by a time delay unit consisting of a fixed harmonic separator (HS; reflected wavelength = 400 nm) and a movable mirror mounted on a piezoelectric (PZT) stage (variable time delay, displacement stroke = 320 μm) with a capacitive positioning sensor [(b) in Fig. 2]. After adjusting the time delay between them, the two-color pulse light is incident to the glucose solution sample. The SHG light is again generated from the red pulse light by another frequency-doubling β-BaB_2O_4 crystal (BBO, thickness = 1 mm). The remaining red pulse light is eliminated by a blue-pass filter (BPF). The resulting two blue pulses are spatially superimposed and finally detected with a photomultiplier (PMT). Because dispersion of group refractive index of the sample results in different optical path lengths for the two-color pulse light, the interference between red and blue pulse light is extinguished without adjusting the time delay of the red pulse. However, when the time delay in the sample is well compensated by the time delay unit [(c) in Fig. 2], the blue interference fringe appears [(d) in Fig. 2]. The envelope component is electrically extracted from the interference fringe by a demodulator. A fringe signal and extracted envelope signal are shown in Fig. 3. The resulting envelope of the interference signal has 300 fs full-width at

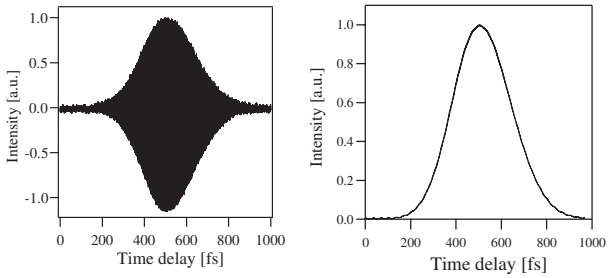

Fig. 3. Interference signal in FTPI: (a) interference fringe signal and (b) extracted envelope signal.

half-maximum (FWHM) width. The temporal shift of the maximum on the envelope signal is determined by displacement of the PZT stage in the time delay unit, which is read from the precise capacitive positioning sensor equipped with the stage (linearity = 5.3 nm, resettability = 35.1 nm).

4. Results

4.1 Glucose concentration measurement

Before demonstration of optical glucose monitoring using FTPI, we evaluated the dependence of group refractive index on glucose concentration for a red pulse light (wavelength = 800 nm) and a blue pulse light (wavelength = 400 nm) using FOPI. A sample cell of 10-mm-thickness was 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). The temperatures of these samples were stable within $\pm 0.2^{\circ}$C and the measurements were repeated ten times at each concentration. The resulting relationships between glucose concentration and time delay for red and blue pulse lights are shown in Fig. 4, respectively. The solid lines are the slopes obtained by linear fitting of the data points. The slopes for red and blue pulse lights are 0.0435 and 0.0493 fs·dl/mg, respectively. This difference in slope between them is due to a positive dispersion of group refractive index in the glucose solution sample. Here, we defined accuracy as

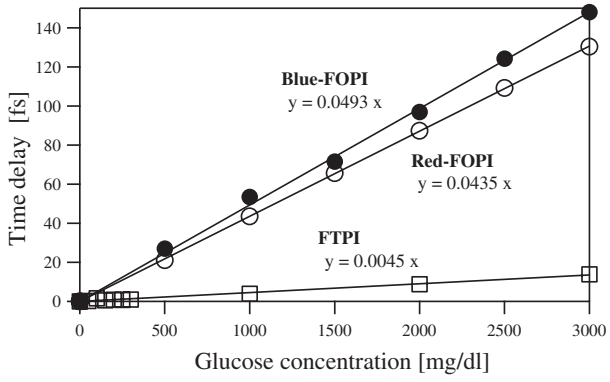

Fig. 4. Relationship between glucose concentration and time delay with respect to red-FOPI, blue-FOPI, and FTPI. Sample thickness is 10 mm. Solid lines are correlation curves obtained by a linear fitting.

the standard deviation of the difference of each measurement value from the value on the slope, and precision as the standard deviation of ten repetitive measurements at each concentration. For optical glucose measurement using red-FOPI, the accuracy and precision were 8.3 and 1.8 mg/dl, respectively;[6] the former was mainly due to temperature fluctuation of sample ($= \pm 0.2^{\circ}$C, corresponding error = 9.6 mg/dl), concentration precision in sample preparation (= 0.32%, corresponding error = 4.8 mg/dl), and/or linearity of PZT stage displacement (= 5.3 nm, corresponding error = 0.80 mg/dl) while the latter depended on resettability of PZT stage displacement (= 35.1 nm, corresponding error = 5.2 mg/dl).

Next, we examined the relationship between time delay of two-color pulse light (Δt_{sample}) and glucose concentration in optical glucose measurement using FTPI as also shown in Fig. 4. In this experiment, we used the same glucose samples as those used in FOPI measurement. The resulting slope of 0.0045 fs·dl/mg for FTPI was about 10 times smaller than those for FOPI because of reduced sensitivity due to use of dispersion of group refractive index. Although this value is in approximate agreement with difference of the gradient between red-FOPI and blue-FOPI (= 0.0058 fs·dl/mg), there is an error of 28.9% between them. The slope for red-FOPI and blue-FOPI may fluctuate within a range of 0.0435 ± 0.001 and 0.0493 ± 0.001 fs·dl/mg, respectively, depending on the resettability of the PZT stage displacement (= 35.1 nm) and the concentration precision of each glucose sample ($= \pm 37$ mg/dl at 3000 mg/dl). As a result, the difference of the two slopes in FOPI is expected to range from 0.0037 to 0.0079 fs·dl/mg. On the other hand, similar fluctuation in FTPI is estimated to be within a range of 0.0045 ± 0.0001 fs·dl/mg, in which the effect of the concentration precision compensated as a result of the near-common-path configuration. Therefore, the slope of 0.0045 fs·dl/mg for FTPI exists within tolerance limits. The accuracy and precision of the glucose determination with FTPI were 77 and 118 mg/dl, respectively. The accuracy is influenced by linearity of PZT stage displacement (estimated error = 7.9 mg/dl), and/or fluctuation of sample temperature (estimated error = 10.2 mg/dl), while the precision is mainly due to resettability of PZT stage displacement (estimated = 52 mg/dl). Furthermore, the actual accuracy and precision may be influenced by individual fluctuation of pulse width and its intensity in two-color pulse light.

Here, we consider a signal-to-noise ratio (SNR) in FOPI and FTPI. Intensity of the interference signal in FOPI (I_{FOPI}) is expressed as follows:

$$I_{\text{FOPI}} = 2(I_0 + \Delta I_0) + 2(I_0 + \Delta I_0)\beta(\delta_1)\gamma_1 \cos\delta_1, \quad (4)$$

where I_0 and ΔI_0 is an average and a fluctuation in intensity of laser pulse light, $\beta(\delta_1)$ is the degree of temporal overlap between the two pulse lights when a phase difference between a reference and a signal light is δ_1, and γ_1 is the degree of spatial overlap between the two lights. $2(I_0 + \Delta I_0)\beta(\delta_1)$ and $\cos\delta_1$ in the second term of eq. (4), respectively give an envelope component and a fringe component in the interference signal whereas the first term

indicates an offset component. In the present setup, since a bias component and high-frequency component in the interference signal are electrically eliminated by the demodulator, eq. (4) is modified as follows:

$$I_{\mathrm{FOPI}} = 2\Delta I_0 + 2(I_0 + \Delta I_0)\beta(\delta_1)\gamma_1. \tag{5}$$

When we define SNR as a ratio of the second term to the first term in eq. (5), SNR in FOPI (SNR_{FOPI}) is expressed as follows:

$$SNR_{\mathrm{FOPI}} = \frac{(I_0 + \Delta I_0)\beta(\delta_1)\gamma_1}{\Delta I_0}. \tag{6}$$

In contrast, intensity of interference signal in FTPI (I_{FTPI}) is given as follows:

$$\begin{aligned} I_{\mathrm{FTPI}} &= (\alpha_1 + \alpha_2)(I_0 + \Delta I_0)^2 \\ &\quad + 2\sqrt{\alpha_1\alpha_2}(I_0 + \Delta I_0)^2\beta(\delta_2)\gamma_2 \cos\delta_2, \end{aligned} \tag{7}$$

where α_1 and α_2 respectively indicate SHG conversion efficiency in the first and the second SHG crystals (LBO and BBO in Fig. 2), δ_2 is the phase difference between the red and blue pulse light and γ_2 is the degree of spatial overlap between the two pulse light. In eq. (7), we neglect loss of a red pulse light caused by the SHG process. After demodulation, the interference signal is modified as follows:

$$I_{\mathrm{FTPI}} = 2(\alpha_1 + \alpha_2)I_0\Delta I_0 + 2\sqrt{\alpha_1\alpha_2}(I_0 + \Delta I_0)^2\beta(\delta_2)\gamma_2, \tag{8}$$

here we made the approximation that $\Delta {I_0}^2 \approx 0$. The SNR in FTPI (SNR_{FTPI}) is determined by the following equation:

$$SNR_{\mathrm{FTPI}} = \frac{\sqrt{\alpha_1\alpha_2}(I_0 + \Delta I_0)^2\beta(\delta_2)\gamma_2}{(\alpha_1 + \alpha_2)I_0\Delta I_0} \tag{9}$$

The actual SNR in the present FOPI and FTPI were both nearly 2.0×10^3.

4.2 Dependence of temperature change in sample

Because of very high sensitivity for change of the refractive index and/or optical path length, usual interferometry is easily influenced by surrounding disturbances such as air turbulence, sound and mechanical vibrations, or temperature change. This results in a limitation of applications to the measurement under unstable environments such as clinical examination. For example, temperature of the human body fluctuates within $\pm 0.5^\circ$C during a day, physiologically. Such temperature change in a sample causes changes of refractive index (or time delay) even if the glucose concentration is constant. Stability to the temperature change can be gained by FTPI because near-common-path configuration of the two-color pulse light can compensates the effect in the interferometer. Here, we compare stability to the change in sample temperature in FTPI to that in red-FOPI. The sample is pure water with a thickness of 10 mm, and the sample temperature is continuously changed from 24 to 40°C. Figure 5 shows dependence of glucose concentration on the sample temperature in FTPI, which is compared with that in red-FOPI. The glucose concentration value is calculated from the slopes (see Fig. 4) and fluctuation of time delay with respect to the sample temperature. In Fig. 5, the value at 40°C is shown as the origin of fluctuation in glucose concentration. The resulting temperature-dependent slope is -1.3×10^2 mg/(dl·°C) for FTPI and -228 mg/(dl·°C) for red-FOPI, respectively. For example, temperature fluctuation of $\pm 0.5^\circ$C, which is the physiological value in the human body in a day, leads to instability of ± 114 and ± 65 mg/dl in glucose concentration for red-FOPI and FTPI, respectively. One can confirm that the effect of temperature fluctuation in the sample is reduced to 57% by FTPI, although there is still fluctuation of time delay due to temperature change in FTPI. The remaining fluctuation results from different temperature dependence of the group refractive index for red and blue pulse lights. From the comparison, we can conclude that the FTPI is effective for glucose measurement of a temperature-fluctuant sample such as the human body.

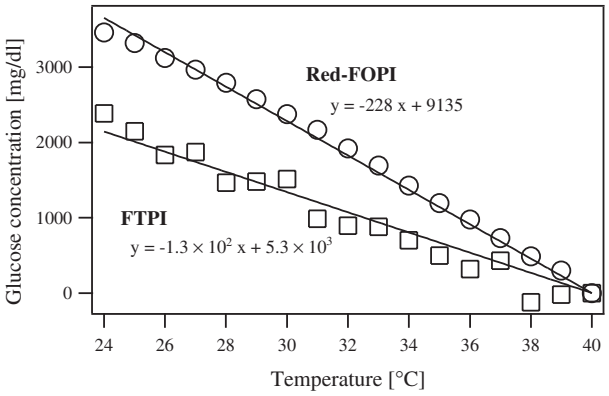

Fig. 5. Dependence of glucose concentration on sample temperature for red-FOPI and FTPI. Sample is pure water with 10 mm thickness. Time delay at 40°C is defined as origin of time delay fluctuation.

5. Discussion

From the comparison of performance between the FTPI and FOPI for optical glucose monitoring *in vitro*, the near-common-path FTPI shows superiority in tolerance to fluctuations in sample temperature. Besides the temperature fluctuations in a sample, the near-common-path configuration is inherently tolerant of surrounding disturbance of air, mechanical vibration, or sound vibration. Good stability in these disturbances in FTPI will provide a powerful tool to achieve clinical application. For application of the optical glucose monitoring to clinical use, selection of a measurement site to which transdermal measurement at a transmittance configuration can be achieved is most important. Skin and vessel are heavily scattering media to visible and infrared lights. Although the FTPI possesses the elimination power of multiple-scattering light using the coherence gate,[7] the power is rather smaller than that in red-FOPI because of double scattering of the two-color pulse lights, severe scattering of a blue light, and decreased detection sensitivity due to the two SHG processes. Hence, it is difficult to find a transdermal measurement site possible for the optical beam to pass through the skin and vessel overall. Use of deep-penetrative femtosecond laser with longer wavelength, such

as a mode-locked Cr:Forsterite laser (fundamental wavelength = 1250 nm, SHG wavelength = 625 nm) or a mode-locked fiber laser (fundamental wavelength = 1500 nm, SHG wavelength = 750 nm), may improve the elimination power of multiple scattering in FTPI because the scattering is less efficient at these wavelengths. However, the sensitivity of FTPI to glucose concentration is reduced because of a gradual decrease in the dispersion of group refractive index in the longer wavelength region. Furthermore, such transdermal glucose monitoring is often affected by the condition of skin surface such as drying and sunburn. On the other hand, the aqueous humor in the eye at which multiple scattering hardly occurs is interesting as a measurement window for the optical glucose monitoring because there is high correlation between the blood glucose and the aqueous glucose.[8] In general, the blood is comprised of glucose and other components (cells, lipids, and proteins). In optical glucose measurement based on the group refractive index and its dispersion in such a multi-component system, fluctuation of the concentration of other components interferes with that of the glucose, resulting in difficulty of quantitativeness in the glucose concentration measurement. Another advantage in using the aqueous humor as the measurement site is to suppress the interference caused by other components because the aqueous protein has very low concentration (0.013 g/dl) in aqueous humor.[9] The sensitivity of FTPI to glucose concentration can be gained by increased optical thickness of the aqueous humor. Considering *in vivo* optical glucose monitoring via the eye, use of an eye-safe laser has to be considered although there are few femtosecond lasers which generate a femtosecond two-color pulse light in the eye-safe region (wavelength = 1.4–2.6 μm). From these considerations, we expect FTPI to be suitable for *in vivo* optical glucose monitoring of anterior humor.

6. Conclusion

We propose optical glucose monitoring using FTPI and evaluate its performance through comparison with FOPI. Linear dependence of glucose concentration on dispersion of the group refractive index gives quantitativeness in the *in vitro* optical glucose monitoring. Accuracy and precision in the glucose concentration measurement is seriously affected by the performance of the PZT stage positioning for time delay because of the gentle dependence on dispersion of the group refractive index. The precision of this measurement is also limited by lengthy coherent time (= 300 fs) of the present FTPI signal as compared with the temporal shift (< 10 fs) caused by the glucose concentration. Near-common-path configuration of the two-color pulse light in FTPI provides good stability to fluctuations of the sample temperature. This feature is significant of reproducibility in *in vivo* optical glucose monitoring based on the interferometric method because the temperature of the human body fluctuates within ±0.5°C during a day. Furthermore, the near-common-path FTPI will effectively suppress the influence of air disturbance or vibrations on the interferometry, which is encountered in unstable environments such as a clinic. This tolerance to surrounding disturbances allows the FTPI to be used in a clinical setting. Based on the present investigations of performance of the FTPI as an optical glucose sensor, the aqueous humor is proposed as a non-transdermal measurement site for *in vivo* optical glucose monitoring based on the FTPI. The proposed FTPI will be a powerful tool for stable optical glucose monitoring *in vivo* in clinical applications.

Acknowledgements

This work was supported by a Grants-in-Aid for scientific research Nos. 16300155 and 17200032 from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and the Tateishi Science and Technology Foundation. We also thank Dr. M. Hashimoto at Osaka University for many insightful explanations of the measurement results.

References

1) H. Zeller, P. Novak and R. Landgraf: Int. J. Artif. Organs **12** (1989) 129.
2) G. L. Coté and B. D. Cameron: J. Biomed. Opt. **2** (1997) 275.
3) S. Y. Wang, C. E. Hasty, P. A. Watson, J. P. Wicksted, R. D. Stith and W. F. March: Appl. Opt. **32** (1993) 925.
4) D. Huang, E. A. Swanson, C. P. Lin, J. S. Schuman, W. G. Stinson, W. Chang, M. R. Hee, T. Flotte, K. Gregory, C. A. Puliafito and J. G. Fujimoto: Science **254** (1991) 1178.
5) K. M. Yoo, Q. Zing and R. R. Alfano: Opt. Lett. **16** (1991) 1019.
6) Y. Hori, T. Yasui and T. Araki: Opt. Rev. **12** (2005) 202.
7) K. Minoshima and H. Matsumoto: Opt. Commun. **138** (1997) 6.
8) W. F. March, B. Rabinovitch and R. L. Adams: Diabetes Care **5** (1982) 259.
9) C. Chou, C. Y. Han, W. C. Kuo, Y. C. Huang, C. M. Feng and J. C. Shyu: Appl. Opt. **37** (1998) 3553.