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Real-time multi-channel Fourier transform spectroscopy and its application to non-invasive blood fat measurement

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ABSTRACT

We developed a real-time multi-channel Fourier transform spectrometer with an area sensor to track biological sample motion. In order to decrease the time intervals between measurements, a simultaneous measurement system consisting of two interferograms with opposite phases was constructed by using an area sensor instead of the often-used linear sensor. Since this system requires no mechanical motion for measurement, the rate at which it outputs spectra depends only upon the frame rate of the area sensor. This spectrometer was employed in the near-infrared region at wavelengths of 900–1700 nm to realize non-invasive blood testing. Substances in blood can be measured at 20 Hz using this method, which is higher than the pulse rate of a human blood vessel. This high-frequency technique enables the extraction of only the blood spectra from samples obtained from fingertips. In this study, the concentration of neutral fat within the blood was determined by extracting the periodically changing signal from the 1200 nm peak.

1. Introduction

Absorption spectroscopy is difficult to employ for *in vivo* biomedical measurements. The incident light is attenuated immediately on the sample surface because the scattering coefficient is two orders of magnitude higher than the absorption coefficient. Therefore, the sample-penetrating light, which provides a wealth of information about the inside of the subject, must be collected effectively. Multi-channel Fourier transform (McFT) spectroscopy [11] can realize much higher optical throughput than a polychromator [3,15].

However, this technique has not been used yet because the mechanical rotating polarizer moves slowly. Therefore, we developed a real-time McFT spectrometer that can achieve high optical throughput and high-frequency measurement. In recent years, polychromators have been developed to realize high-throughput spectroscopy [10] and enable the measurement of blood *in vitro* [1]; however, they are inferior to McFT spectrometers in terms of optical throughput [4].

It is possible to perform McFT spectroscopy without using polarized measurements, although it is difficult to do so when the optical signals are weak. Thus, the noise reduction enabled by polarized measurement is advantageous, especially in near-infrared or infrared sensors.

In McFT spectroscopy, an interferogram can be obtained from twice-polarized measurements. Interferograms can be categorized as in-phase or anti-phase. In-phase interferograms are formed when the phases of two interfering waves are the same, while anti-phase interferograms are formed when the interfering waves are exactly out of phase. The type of interferogram that is obtained can be switched by rotating the polarizer by 90°. These two types of interferograms have opposite signals but equivalent noise levels, which result from the incoherent light from multiple reflections and detector noise. Therefore, by subtracting an anti-phase interferogram from an in-phase interferogram, a new interferogram with twice the signal level and drastically reduced noise can be obtained. This procedure results in slower performance but compensates for noise. Simultaneous detection of both phase interferograms was proposed using a Wollaston interferometer for astronomical spectroscopy [2]. However, for a biological sample, the light source throughput should be increased, which is only possible by using a lateral shearing interferometer. Therefore, we proposed a biologically compatible method based on a lateral shearing interferometer. In this method, the in-phase and anti-phase interferograms are detected in the upper and lower halves of the area sensor, respectively. This procedure enables real-time measurement without affecting the optical throughput, which is necessary for *in vivo* biomedical measurement.

This technique will be useful for health risk and dietary habit management and could prevent lifestyle-related diseases such as diabetes, arteriosclerosis, and hyperlipidemia, which can cause myocardial and brain infarctions.

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2. Methods

In order to realize the real-time spectrometer, we built a parallelized system using an area sensor instead of the often-used linear sensor. The optical setup is shown in Fig. 1. In this system, the polarization shearing interferometer has been modified by adding an area sensor and a combination polarizer. White light from a halogen lamp with an irradiation power density of 226 mW/cm^2 was guided through a 13-mm-diameter optical bundle fiber to illuminate a sample. The transmitted light was guided into the McFT spectrometer, which contains a lens, polarizer, Savart plate (Leysop, calcite, 10 mm thick), Fourier lens, and combination polarizer. After passing through the first polarizer, the linearly polarized beam is split into two equal-intensity parallel beams with orthogonal linear polarizations by the Savart plate. The Fourier lens reunites the two beams on the focal plane array of the detector. The upper half of the combination polarizer is parallel to the first polarizer and produces an in-phase interferogram, while the lower half is perpendicular to it and produces an anti-phase interferogram. The phase interferograms are detected simultaneously using an InGaAs area sensor with 640 pixels per line and 512 lines (NIRvana 640ST, Princeton Instruments, New Jersey, US). In this study, we used 190 lines out of the total 512 lines for each of the interferograms. However, the diffracted light from the joint region of the combination polarizer was detected several tens of lines around the center lines. Therefore, a boundary area of about 100 lines between in-phase and anti-phase interferograms was removed from the signal. The top 16 and bottom 16 lines of the area sensor were also removed because of the non-homogeneous light distribution.

This interferometer is known for having a pincushion-like distortion primarily due to the oblique ray incident upon the Savart plate [12]. Although it is not of much concern when using a line sensor, this distortion has a slightly negative effect on the interferogram images acquired by an area sensor. We corrected this issue using software that warps interferogram images to obtain patterns with parallel lines.

As a fast-moving and strong scattering sample, the fingertip of a test subject was used in this study. Non-invasive blood testing has been reported for several decades; however, no actual device to obtain blood samples non-invasively has been proposed yet. One of the main reasons that no such device has been developed is that it is difficult to obtain reliable blood spectra from *in vivo* measurements, because the thickness and content of skin vary appreciably from person to person. Certainly diffuse reflectance spectroscopy can be used to achieve sufficient light intensity for the measurement, but diffuse reflections are easily affected by the skin conditions. Hence, we decided to use transmitted light spectroscopy to reduce the measurement uncertainty. The problem of signal weakness was solved by employing McFT spectroscopy with a high optical throughput.

We measured blood fat as a specific example of non-invasive blood testing. Two blood components change shortly after a meal: neutral fat and glucose [17]. Although glucose can be monitored to obtain important medical information, the key technology for extracting glucose includes the use of an analysis algorithm, such as multicomponent analysis software, which is beyond the scope of the measuring equipment focused on in this paper [7]. Thus, to evaluate the proposed device, we measured neutral fat. Robust neutral fat detection with our developed device would be useful for the measurement of glucose.

We considered the absorption peak corresponding to a wavelength of 1200 nm to detect neutral fat. This wavelength is known to occur in molecular oscillations due to the second overtones of C–H vibrations, which occur predominantly in neutral fat and protein in the human body [16]. Unfortunately, most fat and protein absorption signals obtained from fingertip measurements are due to skin components, subcutaneous fat, and skin cell protein. However, real-time spectroscopy enables blood components to be distinguished from subcutaneous fat and skin cell protein by detecting the fingertip pulsations [8,9]. This kind of plethysmography is known to be useful for hemoglobin detection [13], because human blood is composed mostly of hemoglobin and water. Since lipids and glucose each only constitute 0.1 wt% of blood, optical non-invasive detection of these substances has not been realized directly.

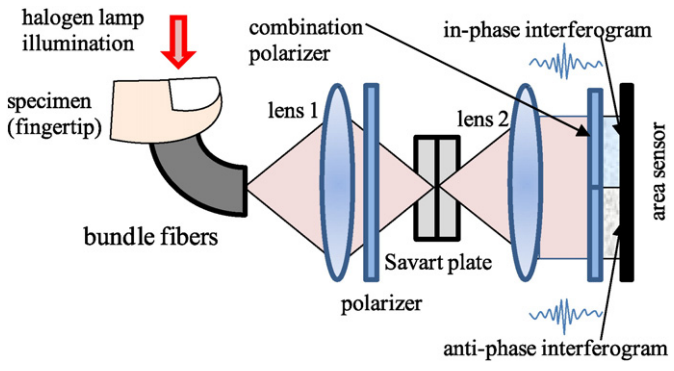

Fig. 1. Schematic of the developed real-time McFT spectrometer with high sensitivity.

3. Results and discussion

The in-phase and anti-phase interferograms were obtained using the upper and lower halves of the area sensor, respectively, and are shown in Fig. 2. These images were obtained after correcting the distortions using the geometrical warping transform. After the distortions were corrected, the in-phase and anti-phase interferograms, which each corresponded to 190 lines, were integrated into a single interferogram by subtracting the anti-phase interferogram from the in-phase

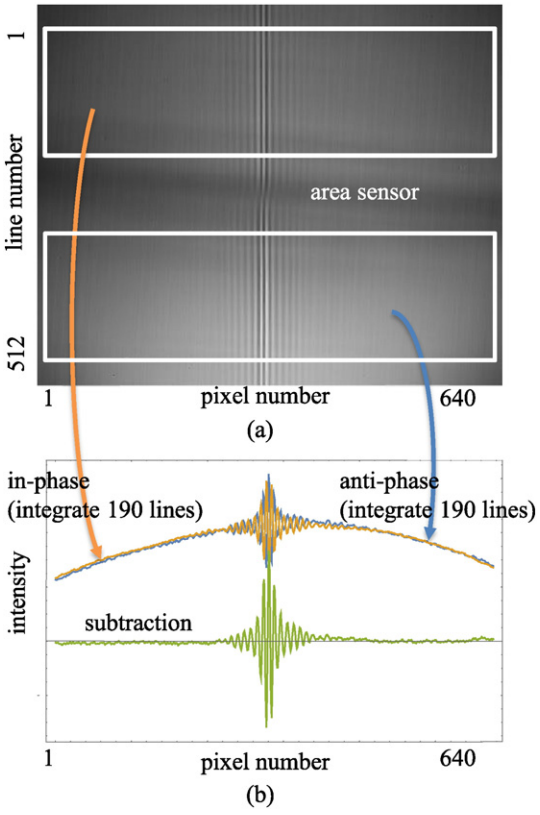

Fig. 2. Interferogram calculated from the InGaAs area sensor. (a) Detected interferogram on the area sensor corrected by warping calculation. The phase of interferograms is reversal between upper and lower halves. (b) Integrated interferograms from the upper half (in-phase) and lower half (anti-phase) of the sensor and the subtracted interferogram for removing the background noise.

interferogram to increase the signal-to-noise ratio. This procedure was performed at a sufficiently high speed so that it was completed within the frame rate of the area sensor.

We applied this technique to measure the absorbance of the fingertip. When calculating the sample absorbance, we use a neutral density filter with an absorbance of 3 as the reference. Fig. 3a shows one of the absorbance spectra, which was continuously measured at a scan rate of 20 Hz. The wavelength range was 900–1700 nm, which was limited by the area sensor used. The 1200 nm spectral peak, which is attributable to the second overtones of C–H vibrations, is clearly observable. The signals at wavelengths greater than 1500 nm were not usable for quantitative analysis because the irradiation power of the halogen lamp was insufficient.

Time-varying absorption was observed because of the peripheral arterial pulse, as shown in Fig. 3b. The high-frequency oscillation (approximately 80 bpm), which occurs once or twice per second, is due to peripheral vasodilation and vasoconstriction, whereas the low-frequency oscillation, which occurs approximately once every 8 s, is due to respiration. The concentration of neutral fat within blood can be determined by extracting the periodically varying signal from the 1200 nm peak. The calibration equation was obtained by *in vitro* triglyceride absorption spectroscopy (Wako Pure Chemical Industries Ltd., Japan). According to the calibration equation, absorbance is directly proportional to triglyceride weight with a proportionality constant of 0.012. Therefore, the observed absorption amplitude change 0.0045 ± 0.0010 was determined to correspond to 0.37 mg of triglyceride. To determine the corresponding triglyceride concentration, we estimated the blood volume obtained from a 133 mm^2 detection area on a fingertip. The blood volume was calculated to be 41 μL based on four arteries, which were assumed to be cylindrical tubes that had a 1-mm diameter and 13-mm length [14]. The volumetric change was then estimated to be one tenth of the total blood volume, 4.1 μL under systolic/diastolic pressure fluctuations [6], by neglecting the pulsations of the peripheral arteries. Thus, the 0.37 mg of triglyceride measured in 4.1 μL of blood yielded a concentration of 900 mg/dL. Considering that neutral fat is generally one sixth of the weight of albumin, its concentration was estimated to be 130 mg/dL, which may be in good agreement with the neutral fat content of normal human blood. However, it would be inaccurate to assume that the ratio of neutral fat to albumin is the same for everyone, as individuals have different blood component ratios. To avoid the effects of albumin concentration, it would be preferable to measure the time series variation after meals because the concentration of albumin remains unchanged, whereas the neutral fat concentration varies significantly after meals. Therefore, the index value of neutral fat in the blood was estimated by measuring the absorption changes after a meal. Fig. 4 shows an example blood fat index time series for a subject whose blood was sampled once every hour from 10:15 to 18:15. The subject ate uncontrolled meals at 7:30 and 12:30, and each measurement took less than 10 min. Peaks can be observed 4–5 h after each meal, which agrees with the results obtained in other invasive studies of blood fat concentration [5].

Optical detection has been thought incapable of measuring absolute concentrations of blood components. Therefore, most researchers have employed multicomponent analysis techniques, such as partial least squares or principal component analysis. However, these analysis methods have central contradiction of the quantity measurement and therefore require daily calibration based on invasive blood monitoring. However, the proposed real-time spectroscope provides difference detection in the near-infrared range and can realize quantitative measurements *in vivo*.

4. Conclusions

We proposed a real-time near-infrared absorption spectroscopy method applicable to biological samples with strong scattering. In this technique, the in-phase and anti-phase interferograms are detected simultaneously to reduce the background noise. The proposed method can solve the problems associated with near-infrared spectroscopy, which involves a lengthy spectral measurement procedure because of the low detection efficiency and the weak light source intensity. The effectiveness of the developed system was demonstrated by performing non-invasive measurements of neutral lipids in blood. Substances in blood can be measured at 20 Hz, which is higher than human pulse

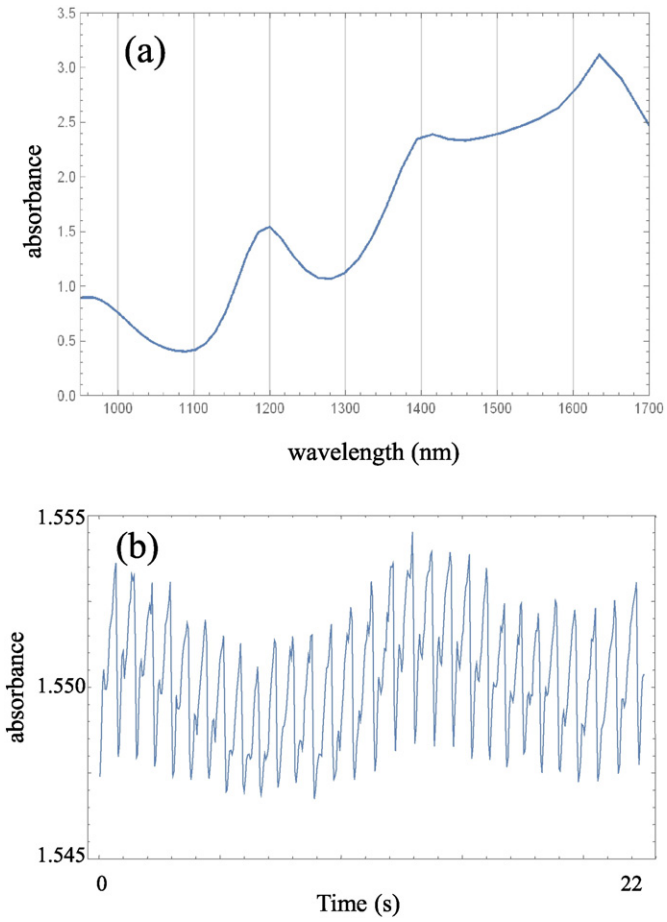

Fig. 3. Absorbance spectrum of transmitted light for a fingertip detected at 20 Hz. (a) Near-infrared spectrum of a fingertip. (b) Changing absorbance of 1200-nm peak due to peripheral arterial pulse.

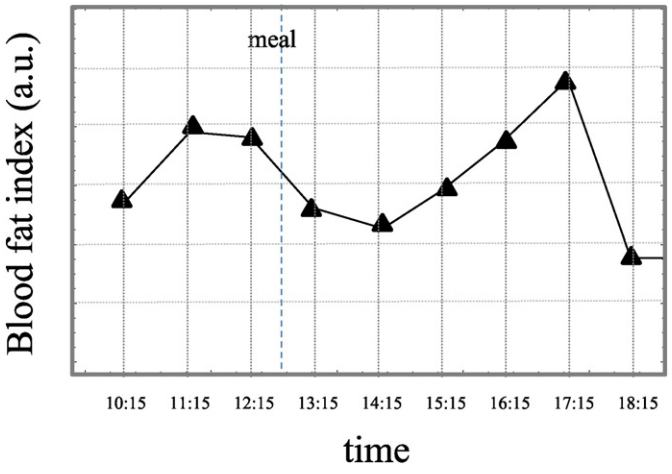

Fig. 4. Blood fat index changes after meals measured by proposed non-invasive method. Subject ate meals at 7:30 and 12:30.

rates. This non-invasive measurement technique, which extracts only the blood spectrum from the body, was determined to be effective, stable, and safe.

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