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Quantitative measurement of biological substances in daily-life environment with the little-finger-size one-shot spectroscopic tomography

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ABSTRACT

In daily-life environment, the quantitative measurement of biological substances, such as the blood glucose level in the human skin, is strongly required to realize the non-invasive healthcare apparatus. Fourier-spectroscopic-tomography of the little-finger-size with high time-resolution and with the strong robustness for mechanical vibrations is proposed. The proposed method is a kind of near-common-path interferometer with spatial phase-shift method. We install the transmission-type relative-inclined phase-shifter on the optical Fourier transform plane of the infinity corrected optical system. The phase shifter is constructed with the cuboid and wedge prisms to give the relative phase-shift spatially between each half-flux of the objective beams. The interferograms from each single-bright-point on an objective surface in a line are formed as fringe patterns on 2-dimensional imaging array devices. And because the proposed method is based on the imaging optics, only emitted rays from a focal plane can contribute forming of interferograms. Thus, the measurement plane can be limited onto the focal plane only. From the spectroscopic tomography, only at a localized vessel area in human skins, we can get the pinpointed near-infrared spectroscopic data. And we can expect the improvement of the determination precision, because a Fourier spectroscopic-character is acquired from multiple intensity data in accordance with amount of phase-shift. From the statistical point of view, the gradation of detector is improved with the square root of sample number, based on t-distribution. We constructed the statistical model to assure the determination accuracy, and demonstrated the feasibility of the glucose sensor using liquid cells.

Keywords: quantitative measurement, biological substances, Fourier spectroscopy, near-infrared light, common-path interferometer, spatial phase-shift, one-shot spectroscopic imaging, spectroscopic tomography

1. INTRODUCTION

We proposed the beans-sized one-shot Fourier spectroscopic tomography [1] that can be installed into smartphones. By developing into near-infrared light region, we are aiming at the realization of the quantitative measurement of biological substances, such as the non-invasive blood sugar sensor, that is used in daily life environment.

We install the wedge prism (inclination angle: around 1[deg.]) at the half flux of collimated objective rays on the optical Fourier transform plane of the infinity imaging-optics. The spatial phase-shift distribution is given to the half flux of objective rays by the installed wedge prism. Thus, the interferogram is formed as a fringe pattern that is interfered between objective beams by the spatial phase-shift method. If we use the 2 dimensional imaging device, the one-axis on the imaging device is assigned to phase-shift value. Another perpendicular axis on the imaging device is assigned to the imaging line. Thus, the spectral distribution within a line on objective planes can be obtained with one-shot (1 frame image data). Furthermore, the measurement depth can be limited into the focal plane, because only the rays from focal plane can contribute the forming of interferograms. On the contrary, rays from out-of-focus planes can't form the image, because the initial phases of these rays are random. Thus, these rays from out-of-focus planes can't contribute the forming of interferograms. By set ting the objective imaging-plane to inclined direction for surfaces of biological-tissues, we can obtain the spectroscopic cross-sectional view of biological membranes.

If the wedge prism is made by thin glasses (thickness: around 0.3[mm]) and inserted into smartphone's imaging optics, the beans-sized line-spectroscopic imager can be realized. The proposed method can obtain the spectroscopic line-

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tomography as fringe patterns by minute optical path differences of relative inclined beams. Thus, ultra-compact spectroscopic tomography will be realized. Conventional wavelength dispersive spectrometers need the long optical path length to secure wavelength resolutions and can't specify the measuring depth.

Conventionally, huge variety of challenges for non-invasive blood glucose sensors has been tried using infrared-radiation spectroscopy. Traditionally, it had been well known that chemical structures can be identified from mid-infrared spectral characteristics. But because water, that is a major component of biological tissues, absorb large volume of infrared light, for quantitative evaluation, the thickness of internal biological membrane remains at the skin surface layer (thickness: several hundred[nm]) using ATR (Attenuated Total Reflection) function of FTIR (Fourier Transform-Infrared Spectroscopy). Thus, near-infrared wavelength-dispersive spectroscopies that detect 2nd. or 3rd. harmonic of molecular vibrations had been studied well. But, there are complex distributions of refractive indices in internal biological tissues. The problem of complex optical-path caused from light diffractions is inevitable. Therefore, the minute change of glucose concentration (target accuracy: 10[mg/dl]) was buried in varied other biological components. Then, many inverse estimations using Monte Carlo method had been tried to identify optical path. But unfortunately these charges had not satisfied the target value yet. The confocal optical method that could specify the spatial 3-dimensional coordinate of internal biological-membranes was proposed [2]. Also, the confocal method was expected to reduce the optical diffraction. This confocal method that used monochromatic light (wavelength: 1600[nm]) measured the absorbance at a point area on biological membranes. 1600[nm] is well known as the wavelength of glucose-specific absorbance. In this monochromatic method, the disturbances of other components could not be eliminated by multiple classification analysis, so called chemometrics, using several absorbance data at multiple wavelengths. Moreover, backgrounds that are reflectance differences at each measurement point could not be corrected. But there are two essential issues to combine the confocal method and spectroscopy. One problem is that the appropriate diameter of pinhole can't be designed because spot diameters are difference depend on wavelengths. Second issue is that amount of light intensity that through a small pinhole is extremely low. Thus, wavelength dispersive spectrometers do not have enough detective sensitivity to detect these weak lights. The wavelength dispersive spectrometer that uses perpendicular polarized beams to improve the diffraction efficiency was proposed. But the commercially available product of this method was extremely large and expensive. Furthermore, theoretically it will be possible to combine the confocal method and FTIR. But the confocal method consumes long measuring time because of spatial scanning operation. Basically, FTIR itself consumes long measuring time for phase-shifting operation. The combination technology with confocal and FTIR will not be realistic approach of measuring for moving biological objects.

On the other hand, our proposed one-shot Fourier spectroscopic tomography utilizes the advantage of confocal effect that can limit the measuring depth into the focal plane. Moreover, the spectral line-imaging with high time resolution can be realized by one frame data without mechanical phase-shift operation. In the result, the proposed method is suitable for multi-components and moving biological-tissues. And, the beans-sized and low-price spectroscopic unit that can be installed into smartphones will be available as commodity.

In 3rd. chapter, we discuss that the imaging sensor whose number of gradation is no more than 256 can secure 0.001 quantization error. Because an interferogram is formed from many interference intensity data at several hundred pixels, the number of gradation is increased statistically in accordance with the square root of sample number based on t-distribution. Furthermore, we clarify that the theoretical gradation number of absorbance increases into 100,000, because of the spatial phase-shift method without mechanical fluctuation of phase-shift operation. In 4th. chapter, using glucose solutions in liquid cells, we verified that the accuracy corresponded to conventional monochromator. And the time resolution was reduced into $\frac{1}{3600}$. Furthermore, paying attention on high time resolution (measuring time=frame rate: $\frac{1}{60}$[sec.]), the mean of 60 spectral-data within 1 [sec.] reduces the error caused from light source fluctuations analytically. Finally, we demonstrated that the correlation coefficient between glucose concentrations and spectral absorbance was 0.92 using solutions with low glucose level (50[mg/dl]-200[mg/dl]). We demonstrated the feasibility of high accurate quantitative measurement with glucose solutions in liquid cells.

2. LITTLE-FINGER-SIZE ONE-SHOT SPECTROSCOPIC TOMOGRAPHY

The high-time-resolution measurement is indispensable for the spectroscopic tomography of moving biological tissues. So, the imaging type 2-D Fourier spectroscopy [3, 4] as the temporal phase-shift interferometer is developed into the one-shot spectroscopy as the spatial phase-shift method.

The proposed method obtains the 1-dimensional spectroscopic image from 1-frame data by the transmission-type relative-inclined phase-shifter without mechanical phase-shift operation. The transmission-type relative-inclined phase-shifter, that gives continuous spatial-phase-difference between objective beams, is configured with the wedge glass and the cuboid glass. As shown in figure 1, the horizontal axis on the imaging device in Fig.1 is assigned to phase-shift-value. And the vertical axis is assigned to image formation line. Thus, the distributions of light intensity at each pixel on a horizontal line form a interferogram. And the spectral characteristics can be obtained analytically by mathematical Fourier transform, such as FFT (Fast Fourier Transform). 2 beams that transmit through cuboid and wedge-glass interfered on the imaging device from relatively inclined angle. The coordinate value in a horizontal direction of light-receiving device corresponds to amount of phase-shift of temporal phase-shift. In addition, our proposed method can realize the 1-D spectroscopic measurement because the interferograms can be obtained simultaneously at each line on the light-receiving device. Therefore, because the proposed method has high time resolution with simple configuration, the bean-sized apparatus, that will be able to be introduced into the smartphone, can be realized.

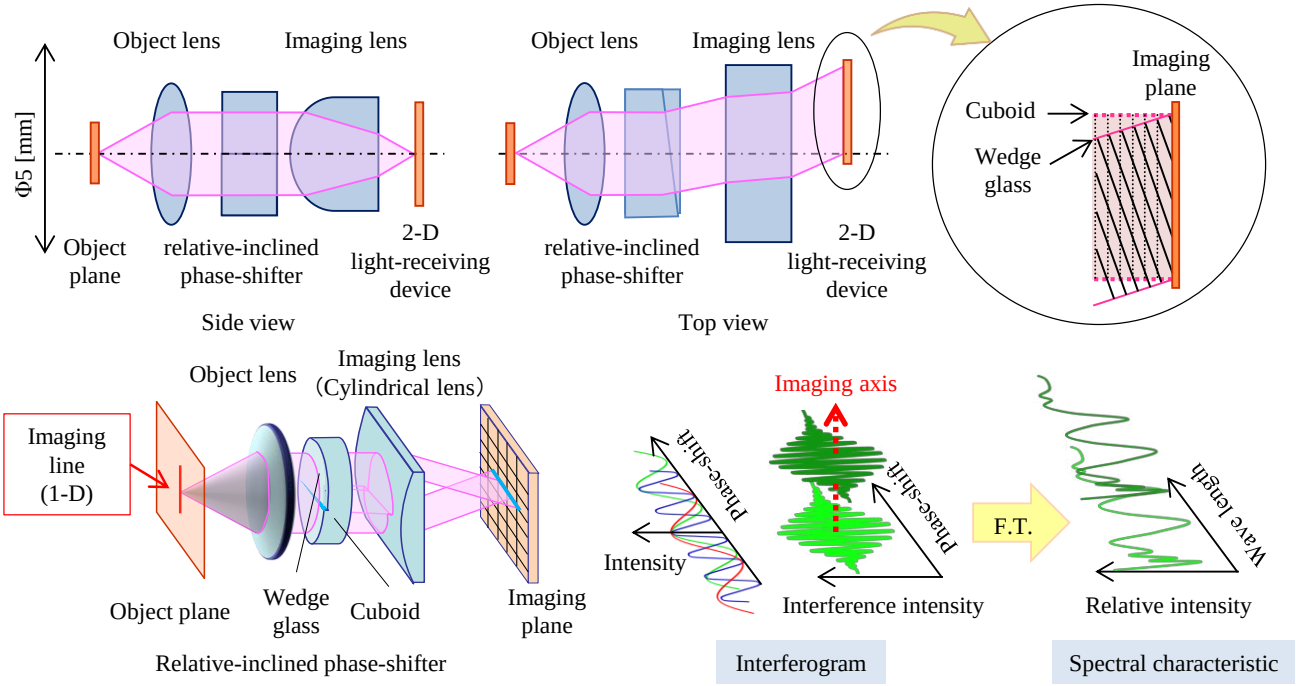


Fig.1 Schematic optical diagram of one-shot spectroscopic tomography.

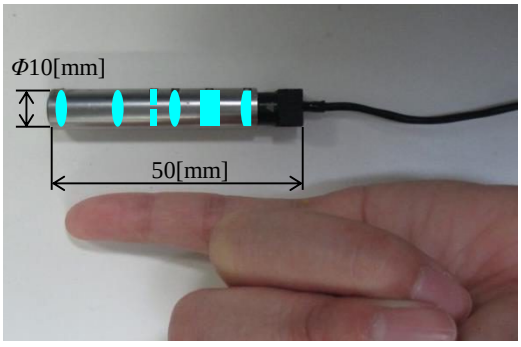


Fig.2 The trail product of the little-finger-size one-shot spectroscopic tomography.

3. EVALUATION OF MEASUREMENT ACCURACY OF ONE-SHOT SPECTROSCOPIC TOMOGRAPHY

3.1 Classification of quantification error causes

As shown in Table 1, we classified the quantification error causes of interferograms into two categories. One is the phase-shift fluctuation for horizontal axis of interferograms. For horizontal axis caused, there are two issues. One is the phase-shift fluctuation is derived from actuator movement accuracy. Another issue is the temperature changes of samples. Another error-cause is the interference-intensity error for vertical axis of interferograms. The interference-intensity error includes three issues that are the quantization errors, the light-source temporal-fluctuations, and humidity changes. For vertical axis, even if 2-D array devices have only 256 number of gradations, Fourier spectroscopy can reduce the quantification error. Because the interferogram is formed from many interference intensity data, the quantification error is reduced into $\frac{1}{\sqrt{N}}$, based on t-distribution. From the numerical analysis, we clarified that 10[nm] fluctuation of phase-shift value corresponds to 0.1[%] quantification error.

Table 1 Classification of quantification error cause

	Spectroscopic factor	**Environmental factor**
Vertical axis	The quantization error The fluctuation of the light source	Humidity
Horizontal axis	The actuator movement	Temperature change of the samples

3.2 Quantization error of 2-dimensional array device

For most commercially available 2-dimensional array device, the number of gradation is only 256. Therefore, resolution of detection sensitivity for 2-D array device is only around 0.4% that is calculated from $\frac{1}{256}$. The quantization error of 2-D array device causes the light intensity error of interferograms, that is one of error causes for vertical axis. The number of gradation is increased in accordance with sample number by statistical effect, so called t-distribution, because the Fourier spectroscopic character is acquired analytically from multiple interference intensities. The detective sensitivity S is expressed as equation (1).

$$S = \frac{1}{P} \times 100\ [\%] \tag{1}$$

p is the number of gradation. The resolution of relative intensity for Fourier spectroscopy is expressed as equation (2).

$$S' = \frac{1}{P} \times 100 \times \frac{1}{\sqrt{N}}\ [\%] \tag{2}$$

N is number of images. Approximately, using the 2-D detective device whose number of gradation is 100, the resolution of relative intensity is equivalent to 1[%]. From equation (2), if 100 is substituted to image-number N, the resolution of relative intensity becomes to be 0.1[%]. Thus, even if the number of gradation for 2-D array device is only 100, the resolution of relative intensity becomes to be $\frac{1}{10}$ and can be improved into 0.1[%].

3.3 Fluctuation of amount of phase-shift

Because of the temporal phase-shift method, the fluctuation that is caused from actuator movement error with phase-shift operation deteriorates the phase-accuracy of interferograms. From the estimation results of the numerical analysis, the fluctuation error of translational phase-shift movement affects the forming of interferograms. As the simplified numerical model, we define the monochromatic light as a light source. In this case, the interferogram is formed as a cyclic cosine wave. When fluctuation α is given to each sampling points of cosine wave. The error of relative intensity was estimated by subtraction the fluctuation waveform from the ideal waveform. Table 1 shows the parameters for the numerical analysis. We confirmed that the relative intensities were proportionally increasing in accordance with

fluctuation value α. If the fluctuation α was 10[nm], the relative intensity error was 0.1[%]. The relative intensity error 0.1[%] corresponds to the quantization error.

But because the one-shot spectroscopic tomography is a spectral phase-sift interferometer, 1-dimensional spectral images can be obtained from 1-frame data without phase-sift operation. Therefore, the coordinate value of horizontal axis of light-receiving device corresponds to the amount of temporal phase-shift. 10[nm] of temporal phase-shift fluctuation is converted into 1.3[μm] of pixel-pitch error. But preliminarily calibration is enough for the phase-shift correction at each pixel, because phase-shift errors of pixel pitches are invariant at every measurement.

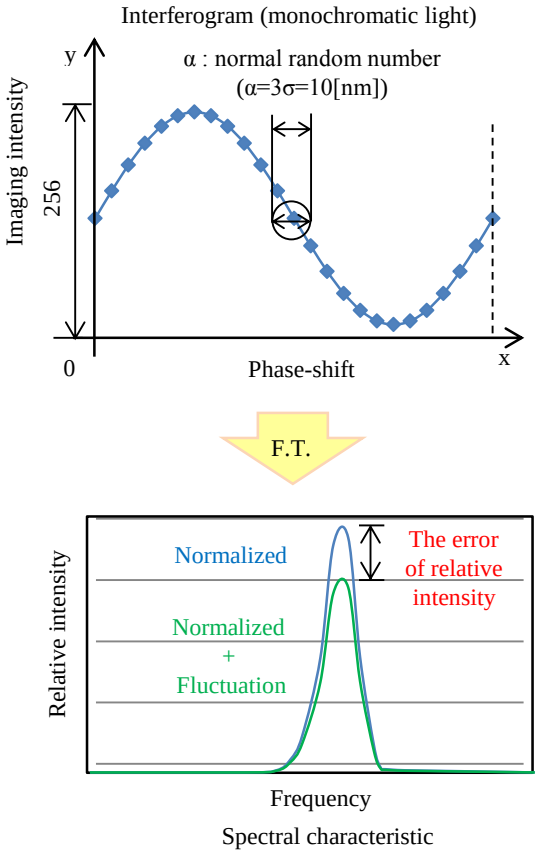


Table.2 The parameters for the numerical analysis

Wavelength	2[μm]
Sample number	n=100
Fluctuation (normal random number)	α=3σ

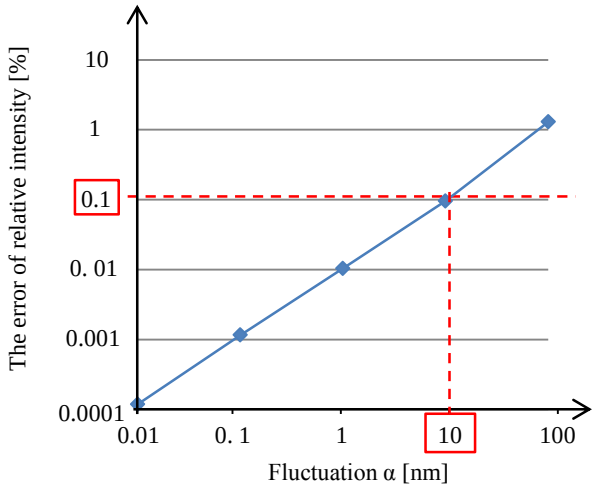


Fig.3 The relative intensity error caused from translational movement accuracy of mechanical phase-shift operation.

4. EXPERIMENTAL RESULTS OF QUANTITATIVE ONE-SHOT FOURIER-SPECTROSCOPY

4.1 Construction of quantitative one-shot Fourier spectroscopy

As shown in figure 4, we constructed the one-shot Fourier spectroscopic apparatus for evaluating the measurement accuracy of glucose concentration in liquid cells. The primary specification is shown in table 2. The transmitted lights through liquid cell are collimated by objective lens. And by the relative inclined phase-shifter (inclination angle: 1.6[deg.], maximum optical path difference: 382[nm], wavelength resolution: 13[nm]), the spatial phase-shift distribution is given to half flux of collimated objective beam. The beams through cuboid glass and wedge prism are concentrated into line shape by cylindrical lens and interfered each other. To keep the measurement condition constant, Peltier devices control the temperature within 36±0.1[℃]under a dried nitrogen purge.

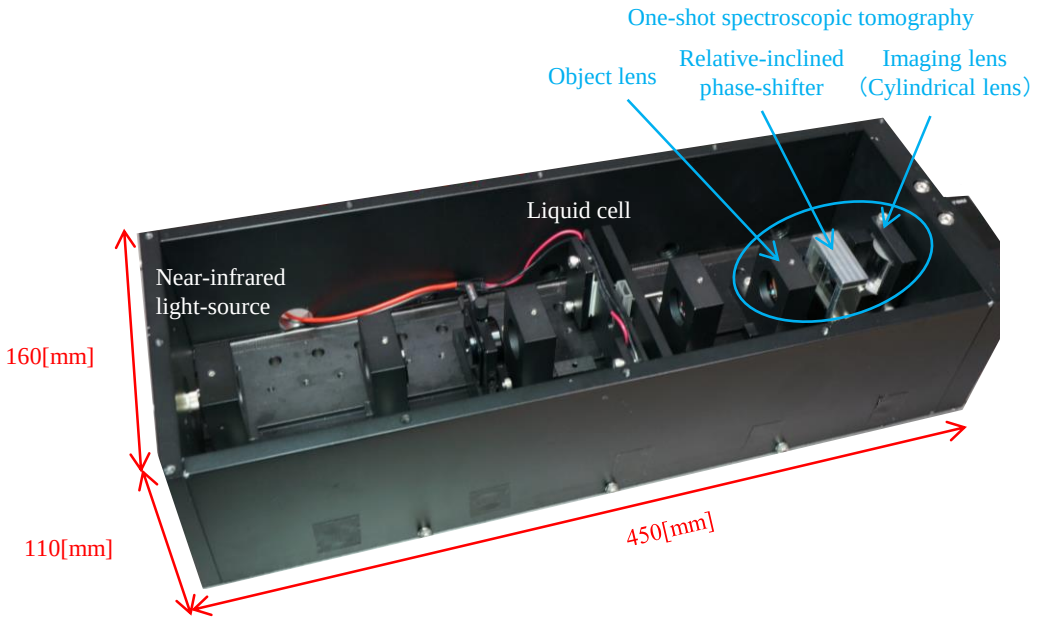


Fig.4 (a) Quantification Infrared Fourier Spectroscopic-tomography Apparatus

Table 3 Primary Specification

Light source wavelength region	1520 -1620 [nm]
Liquid Cell Thickness	3 [nm]
Optical Magnification	m = 1
Sample Temperature	36±0.1 [℃]
Apparatus Dimension [W×D×H]	160×450×110 [mm]
Light-receiving device Wavelength region	900 -1700 [nm]
Light-receiving device pixel pitch / effective pixels	30 [μm] / 320×256

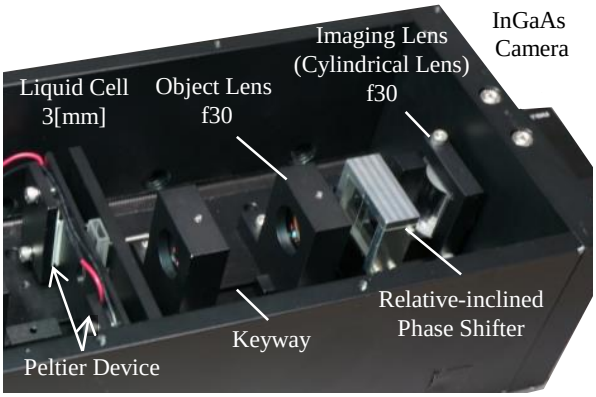


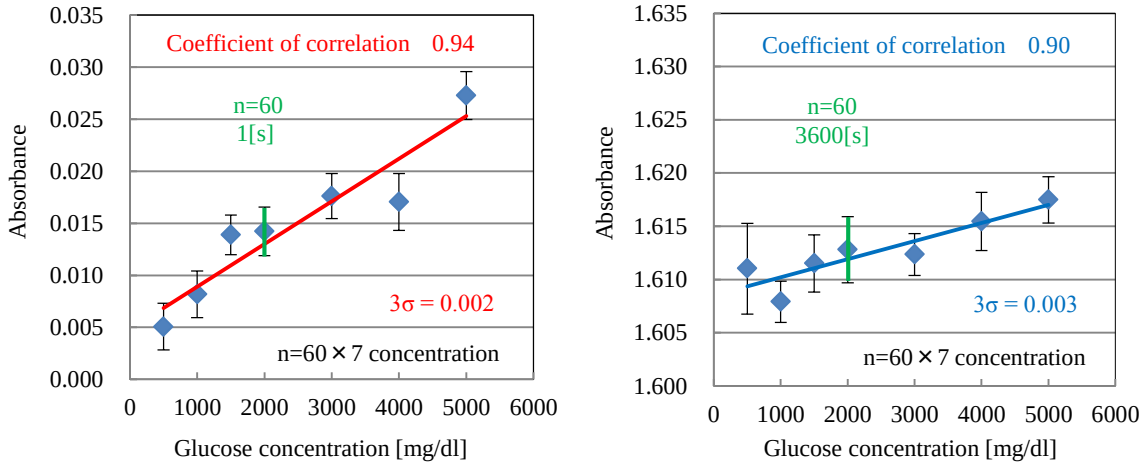
Fig.4 (b) Quantification Infrared Fourier Spectroscopic-tomography Apparatus

4.2 Quantification evaluation results of glucose concentration

In near-infrared region (wavelength: 0.9[μm] -1.7[μm]), we verified the dispersion by measuring 7 kinds of glucose-concentration (range: 500[mg/dl]-5000[mg/dl]) for 60 times in each condition. ASE (Maker: FiberLabs Type: ASE-FL-7004) light-source whose wavelength region 1520[nm]-1620[nm] was used, because absorbance at 1540[nm] is weak for water and significant for glucose. For other measurement conditions, humidity was 26[%], thickness of liquid cell was 3[mm], InGaAs camera (Maker: Hamamatsu Photonics K.K., Type: C10633-13, Detective wavelength region: 900[nm]-1700[nm]).

Figure 5 (a) shows the relationship between absorbance at 1540[nm] and glucose concentrations. We obtained the high correlation-coefficient 0.94. Furthermore, to compare with conventional method, we measured the same samples using the monochrometer (Maker: Shimadzu Corp., Type: SolidSpec-3700) as shown in Fig.5 (b). To measure the spectroscopic character between 1520[nm] to 1620[nm], it took 3600[sec.] for measurement time. The proposed method took only 1[sec.] to measure 60 data by 60 frame rate camera. Comparing with monochrometer and the proposed method, the measurement time was reduced into $\frac{1}{3600}$. Monochrometers are high sensitive spectrometers. Moreover, because 3σ was approximately equivalent between two methods, the proposed method can measure with same accuracy of the conventional monochrometer.

As mentioned above, we could demonstrate the feasibility of the high-accurate quantitative-measurement with high time resolution by the proposed one-shot Fourier spectroscopy.



(a) Quantification Infrared Fourier Spectroscopic-tomography Apparatus

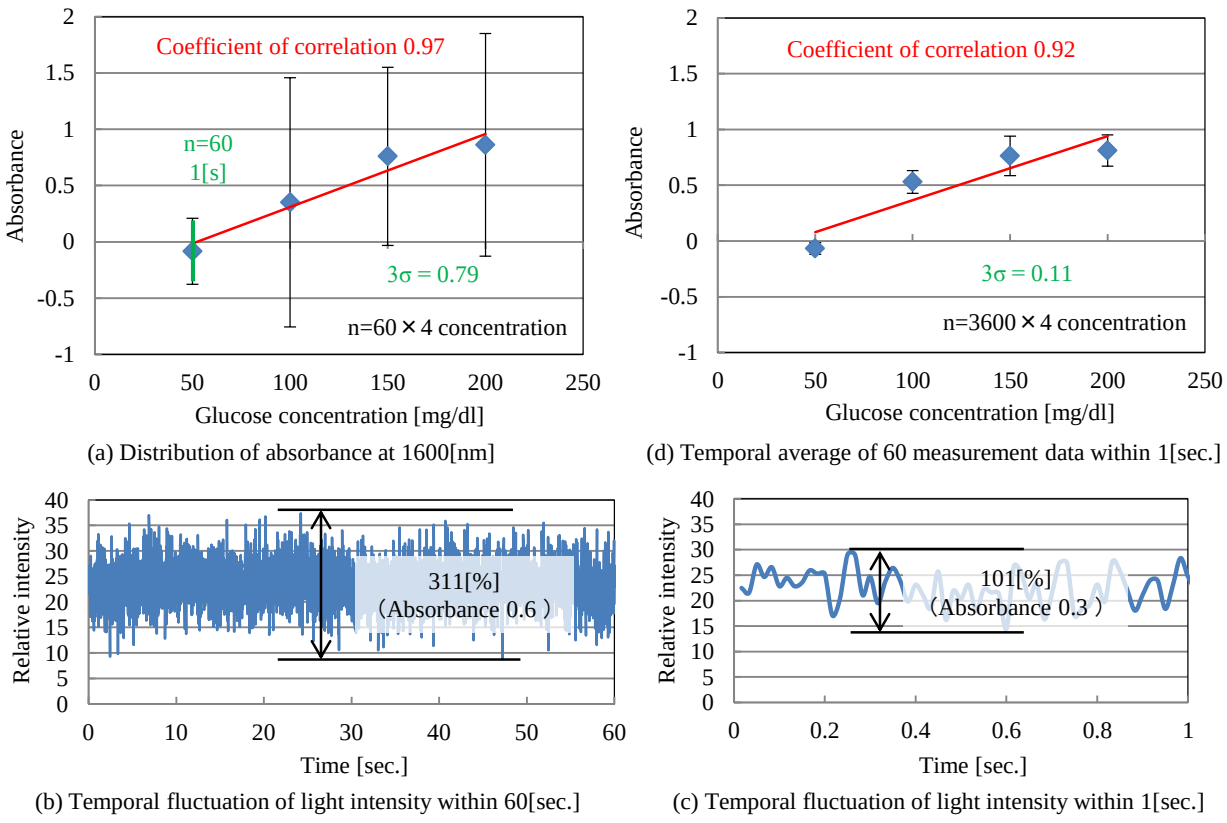
(b) Monochrometer (Maker: Shimadzu Corp., Type: SolidSpec-3700)

Fig.5 Quantification evaluation results of glucose concentration

4.3 Temporal averaging effect of one-shot Fourier spectroscopy with high time resolution

Because the proposed method can measure the line distribution of spectroscopic characters with one frame image with high time resolution, we can obtain 60 spectra in 1[sec.] using camera with 60[fps]. Thus, we can expect the accuracy improvement by the temporal averaging effect. For evaluation of the quantitative accuracy, we measured the extremely low glucose concentrations that corresponded to human blood-sugar level. We used 4 kinds of glucose water solution (concentration range: 50[mg/dl]～200[mg/dl]). The super continuum (Maker: Fianium, Type: WhiteLase SC480-2, Wavelength range: 480-2400[nm]) was used as broadband near-infrared light-source. Other experimental conditions were InGaAs camera (Maker: Hamamatsu Photonics K.K., Type: C10633-13, Detective wavelength region: 900[nm]-1700[nm]).

As shown in figure 6(a), we could obtain the high correlation coefficient 0.97 between absorbance at 1600[nm] and glucose concentration. But as show in Fig.6 (b), because the temporal light-intensity fluctuation of the super continuum were big, the average of 3 σ for 60 measurement data was 0.79. We could not identify each glucose concentration. Then, we calculated the temporal average of 60 measurement data within 1[sec.]. The dispersion of temporal averaged data is shown in Fig.6 (d). From this evaluation results, we could reduce 3 σ into $\frac{1}{7}$ and discriminate each glucose concentration with correlation coefficient 0.92. Therefore, we verified the temporal averaging effect with high time resolution measurement and demonstrated the feasibility of high accuracy quantitative measurement.



(a) Distribution of absorbance at 1600[nm]

(d) Temporal average of 60 measurement data within 1[sec.]

(b) Temporal fluctuation of light intensity within 60[sec.]

(c) Temporal fluctuation of light intensity within 1[sec.]

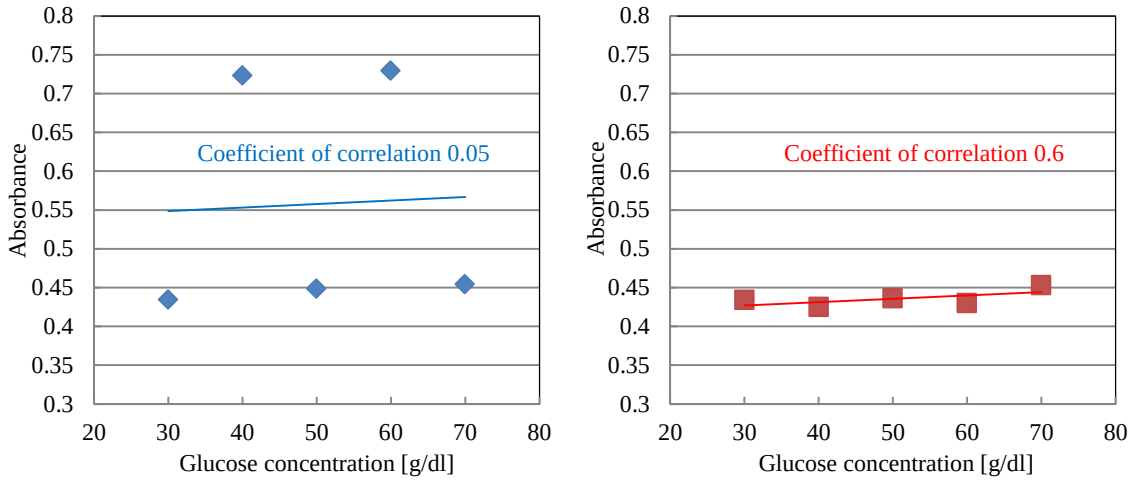
Fig.6 Temporal averaging effect of one-shot Fourier spectroscopy with high time resolution

5. SIMULATION FOR UNEVENNESS OF REFRECTANCE AND LIGHT-INTENSITY TEMPORAL-FLUCTUATION OF LIGHT SOURCE

To adapt to biological tissues, the unevenness of reflectance at each measuring area and the light-intensity temporal-fluctuation of light source are inevitable issues. In this chapter, we tried to correct the unevenness of reflectance at each measuring area by calculating the absorbance ratio between hemoglobin and glucose. The concentration of hemoglobin is stabilized in biological substances. Thus, the relative absorbance between stable component's absorbance and glucose absorbance will improve the difference of light reflection. For hemoglobin, the spectral absorbance is used at wavelength 1000[nm]. And for glucose, the spectral absorbance is used at wavelength 1600[nm].

Then, using the conventional dispersive monochrometer (Maker: Shimadzu Corp., Type: SolidSpec-3700), we simulated the difference of light reflectance by installing 50[%] ND filter. The sample solutions consisted the glucose concentrations 30, 40, 50, 60, 70[g/dl] mixed with 13[g/dl] hemoglobin. And thickness of liquid cell was 1[mm]. Temperature was 24.1[℃]. Humidity was 46.0[%].

In this experiment, we inserted 50[%] ND filter for 40[g/dl] and 60[g/dl] glucose solution to simulate the reflectance differences. As results, when ND filter was inserted for reducing luminance, the absorbance became to be increased. And then by calculating the relative absorbance between 1000[nm] and 1600[nm], the correlation coefficient was improved from 0.05 to 0.6. We could verify the effectiveness of correction method for the unevenness of reflectance at each measuring area.



(a) Distribution of absorbance at 1600[nm]

(b) Calculated the relative absorbance between 1000[nm] and 1600[nm]

Fig.7 Experimental results of the correction method for the unevenness of reflectance at each measuring area.

6. CONCLUSION

We evaluated the accuracy of the proposed one-shot Fourier spectroscopy for glucose concentration. Because Fourier spectroscopy uses many data as interferogram, 2-dimensional imaging array device with 256 gradations can secure 10 times quantization resolution based on t-distribution. And we clarify that the spatial phase-shift interferometer has advantage to secure the high quantitative accuracy without mechanical phase-shift fluctuation. We demonstrated the feasibility of the high accurate measurement using extremely low concentration glucose solution in liquid cells.

7. ACKNOWLEDGEMENT

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