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3-DIMENSIONAL SPECTROSCOPIC-TOMOGRAPHY OF BIOLOGICAL MEMBRANE BY THE IMAGING-TYPE 2-D FOURIER SPECTROSCOPY

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Abstract - We proposed the imaging-type 2-D Fourier spectroscopy that is the phase-shift interferometry between the objective lights. The proposed method can measure the 2-D spectral image on the focal plane. We construct the proposed method by the infrared radiation optical system to try to apply for the noninvasive measurement of the blood glucose level. In this report, we discuss about the spectroscopy method to measure the vessel area of the proximal skin surface that is little affected by the diffusion cased by the biological membrane. The proposed method is constructed with Fourier spectroscopy with high wavenumber resolution. Therefore, the proposed method can measure spectroscopy of greenhouse gases and noxious fumes with narrowband width of spectral adsorption index. In this report, we mention the principle of the imaging-type 2-D Fourier spectroscopy and the development of the 2-D spectroscopic measurement for unstructured environment.

Keywords : Fourier spectroscopy, phase-shift interferometry, infrared radiation

1. INTRODUCTION

We propose an imaging-type Fourier spectroscopic technology that enables 2-D spectroscopic images to be obtained within the focusing plane alone (for instance: [1]). This technology incorporates auto-correlational phase-shift interferometry that uses only object light generated by the bright points that optically make up the object.

We are currently involved in studies of non-invasive technologies used to measure blood components such as glucose and lipids, which are measured for use in daily living. Previous studies have investigated non-invasive technologies that measure blood glucose levels by utilizing near-infrared light that permeates the skin well. It has been confirmed that subtle changes in the concentration of a glucose solution, a sample used to measure the glucose level, can be measured by analyzing the spectroscopic characteristics of near-infrared light; however, when applied to a biomembrane, technology such as this is incapable of precisely measuring the glucose level because light diffusion within the skin disturbs the measurement. Our proposed technology enables 2-D spectroscopy to a limited depth below the skin covered by the measurement. Specifically, our technology concentrates only on the vascular territory near the skin surface, which is only minimally affected by light diffusion, as discussed previously; the spectroscopic characteristics of this territory are obtained and the glucose level can be measured with good sensitivity (for instance [2]-[4]).

In this paper we propose an imaging-type Fourier spectroscopy method that is used as the measuring technology in producing a 3-D spectroscopic image.

2. CONCEPTUAL DESIGN OF NON-INVASIVE BLOOD SUGAR SENSOR

We proposed the non-invasive blood sugar sensor. Fig. 1 shows the conceptual diagram of non-invasive blood sugar sensor.



Fig. 1. Conceptual diagram of non-invasive blood sugar sensor.

In other words, this proposed method can measure the 3-D spectral image of bio membrane. In recent years, the finger vein authentication reader system is known for personal identification. This system can verify the person only by holding the finger or palm over the sensor. This technology pays attention on the high transparency of skin to the near infrared light. The final product image of our proposed method is similar to this vein authentication reader system. By holding the finger or palm over the sensor, the near infrared 3-D spectroscopic image can be obtained. And, we can extract the vein area from the spectroscopic image. And then, we can get the spectral characteristics of vein area. From this measured results, the blood sugar level can be analytically acquired.

3. ISSUE FOR MEASURING THE SPECTRUM OF BIOLOGICAL MEMBRANE AFFECTED BY DIFFUSION

Conventionally, it is known that the glucose concentration can be measured as blood sugar level using near-infrared rays. Paying attention on the feature, many research projects have tried to develop the non-invasive blood sugar sensors. However, it was the main issue of conventional research approach that the sensitivity of conventional spectroscopy was unsatisfactory. Fig. 2 illustrates the issue for applying the conventional wavelength dispersive spectrometer to the biological membrane. Since the biologic membrane consists of various components, the distribution of refractive index is complicated. So, the near-infrared rays are diffused in the biologic membrane. By this diffusion of light, the spectral characteristics are affected by the area other than the blood vessel area. In addition, the conventional wavelength dispersive spectrometer converts all pencil of rays in a measuring area detected by the probe into spectrum. Therefore, biological membrane components outside the blood vessel area affect the spectral characteristics.



Fig. 2. Main issue for applying the conventional wavelength dispersive spectrometer to the biological membrane affected by diffusion.

4. FINGER VEIN AUTHENTICATION READER SYSTEM FOR PERSONAL IDENTIFICATION

Near-infrared rays are also used for the finger vein authentication reader system. So, the penetrated rays are diffused in biologic membrane. However, the finger vein authentication reader system uses this diffusion of light in a positive manner, as shown in Fig. 3. If near-infrared rays penetrate through a finger in a linear fashion, bone and various blood vessels are projected. This makes the image extremely complicated and prevents personal identification by simple image recognition. Since biologic membranes diffuse rays, however, an image deep inside a finger becomes vague as if seen through frosted glass. We can observe only blood vessels that are near the skin surface and not greatly affected by diffusion. An image becomes a comparatively simple blood vessel pattern that enables personal identification. So, we decided to focus on a layer close to the skin that is not greatly affected by diffusion. For this purpose, the 2-D spectroscopic imaging that can limit the measuring depth will make non-invasive blood sugar measurement come true. To obtain a spectral image of a specific layer, spectral characteristics must be measured by limiting the depth. And if 2-D spectroscopy limited in depth direction is realized, by scanning the measured area in depth direction, 3-D spectral image can be acquired. And we can discriminate the biological membrane components from the spectral cross-sectional image in high spatial resolution.



Fig. 3. Diffusion light path penetrating the finger in case of infrared radiation.

5. IMAGING-TYPE 2-D FOURIER SPECTROSCOPY

We proposed the imaging-type 2-D Fourier spectroscopy that can limit the measuring depth into the focal plane. Fig. 4 illustrates the principle of the proposed method. Fig. 4 shows the ordinary imaging optics. From the optical point of view, the surface of the object is covered by many bright points. From the single bright point, the rays are emitted to the multiple directions. These emitted rays are collimated by objective lens and are concentrated into single bright point on the imaging plane by the imaging lens. Therefore, the substantial bright point on the focal plane is optically formed as the conjugate bright-point image. We introduce the new optics that is called the variable phase filter. This variable phase filter can give the arbitrary phase difference to the half of objective rays. This allows a phase shift interferometer between object beams without reference beam. In the case of initial state, the phase of these object rays are same. Thus, these object rays are interfered and form the bright point as the interfere figure as same as the ordinary imaging optics. If a half-wavelength phase difference is given to the lower rays, the intensity of interfering light decreases. If a single-wavelength phase difference is generated, these two beams strengthen each other and form the bright point as same as the case of no phase difference. In this way, if the phase is shifted sequentially, the imaging intensity changes periodically with the amount of phase shift. As shown in Fig. 5 (a), if the monochromatic light is used as the light source, the changes

of imaging intensity form the simple cyclic sin wave. But for spectroscopy, polychromatic light is used. In the case of long wave length, the cycle of this imaging intensity becomes to be low frequency. And if the wavelength is short, the imaging intensity changes at high frequency. On the imaging plane, the sum of these cyclic imaging intensity changes of multiple wavelength is detected by CCD camera. As shown in Fig. 5 (b), it is called the interferogram in the field of Fourier spectroscopy. As I mentioned before, this interferogram is formed of multi cyclic sin wave. Thus, the spectral characteristics can be obtained analytically by mathematical Fourier transform, such as FFT, as shown in Fig. 5 (c). In this method, the rays from out of focus do not contribute the forming of interferogram. Because, the initial phase of rays is different. Thus, the proposed imaging-type 2-D Fourier spectroscopy can limit the depth into the focal plane. And by scanning the focal plane mechanically in the depth direction, we can acquire the 3-D spectral characteristics.

6. EVALUATION RESULT OF SPATIAL RESOLUTION OF IMAGING-TYPE 2-D FOURIER SPECTROSCOPY USING QUANTUM DOT

6.1. Fluorescently Dyed Single Cell with Quantum Dot as Theoretical Bright Point Light Source

To verify the spatial resolution of our proposed method, we used the fluorescently stained cells. Recently, in medical field, the multi-colored fluorescently staining method has been developed. Especially, the quantum dot is well known as the multi-colored fluorescently staining method. Currently, fluorescent indication using silicone particles called quantum dots is becoming popular. The particles whose diameter is around 10-20 nm generate the different colors according to the diameter. For example, large particles can generate the red color and small ones blue. Since various colors are available from quantum dots, we can measure the localization of specific components in a single cell whose diameter is 10miro meter. Thus, to discriminate the localization of color precisely, the 3-D spectroscopy with high spatial resolution was strongly required. Next, I explain about the experimental results to validate the proposed method about the spatial resolution. To examine the spatial resolution, we use the quantum dot as the theoretical point light source.



Fig. 4. Imaging-type 2-D Fourier spectroscopy by phase-shift interference between object beam.



Fig. 5. Procedure of measuring Spectrum from interferogram in Fourier spectroscopy.



Fig. 6. The acquired 2-D spectral characteristics of monochromatic stained cell with quantum dot.

6.2. The Acquired 2-D Spectral Characteristics of Monochromatic Stained Cells

Using the proposed imaging-type 2-D Fourier spectroscopy, we successfully measured the 3-D spectral distribution in a cell stained with quantum dots. Fig. 6 shows the evaluation result using a breast cancer cell about 10 microns in diameter. The sugar protein on the surface of the cell membrane is stained fluorescently with quantum dots. Since the measured cross-section of a cell is limited into the focal plane, the fluorescent indicators are observed in a ring form, as shown in the left hand side image of Fig. 6. We can confirm the change of imaging intensity in accordance with the phase shift, as shown in the upper right hand side graph of Fig. 6.

At an arbitrary pixel, we can get the interferogram as same as the graph. And from this acquired interferogram, we can get the spectral characteristic analytically in each pixel, as shown in the lower right hand side graph in Fig. 6. In this experimental result, we can confirm the peak value of quantum dot around 550nm. Thus, we can verify the proposed imaging-type Fourier spectroscopy.

7. 3-D SPECTROSCOPIC TOMOGRAPHY OF THE STAINED CELLS

Fig. 7 shows the evaluation results of 3-D spectroscopic tomography of the stained cell with quantum dot. To verify the feature that can limit the 2-D measuring plane into the focal plane, the objective lens is made to be scanned mechanically in the depth direction. In each measuring plane, we acquired the interferogram and then move the objective lens in the depth direction sequentially. The bright point that is circled on the right-hand image in Fig. 7 indicates a quantum dot of fluorescent indication taken from the surface of the cell membrane. The spectral characteristics of the bright-point ultrafine particle no larger than about 10 nm could be obtained. We also proved that these spectral characteristics could be obtained from any pixel in an arbitrary image using visible light.



Fig. 7. Evaluation result of 3-D spectroscopic tomography of the stained cell with quantum dot.

8. NEW CHALLENGE FOR DEVELOPING THE 2-D SPECTROSCOPIC IMAGING INTO UNSTRUCTURED ENVIRONMENT MEASUREMENT

We assume the fixed star as the stable light source, and decide to construct the astronomical spectroscopic optical system for observing absorption spectrum of transmitted light through the greenhouse gases from the grand. And we are able to observe the greenhouse gases such as the ozone, the carbon dioxide and methane on the grand. This application utilizes the advantages of the proposed spectroscopy that have the robustness for mechanical oscillation and the capability of the absorption spectrum measurement of gases with high wavenumber resolution. And the proposed method can realize the 2-D spectroscopic astronomical telescope with the reflective optical system.

In this report, we discuss the two preliminary experimental results of the 2-D spectroscopic astronomical telescope with the reflective optical system. One is the feasibility evaluation of the 2-D spectroscopic imaging with the reflecting telescope equipped with the imaging-type 2-D Fourier spectroscopy. Second one is the robustness for the blurring of an astronomical image due to the distorting effects of the atmosphere that is simulated by the dry ice smoke.

9. FEASIBILITY STUDY OF FOURIER SPECTROSCOPIC IMAGING BY THE REFLECTING TELESCOPE

We evaluated feasibility of 2-D spectroscopic imaging by the reflecting telescope equipped with the imaging-type 2-D Fourier spectroscopy. Fig. 8 shows the experimental reflecting telescope that is equipped with the imaging-type 2-D Fourier spectroscopy. In this experiment, we simulated the fixed star with the metal halide lamp (Manufacturer: SIGMA KOKI CO., LTD., Type: IMH) as the light source. After the rays emitted from the light source transmitted though the red filter, the rays were collimated by lens and entered into the reflecting telescope. After the ray that were reflected from the primary and the secondary mirror, the rays collimated by relay lens. The collimated rays were reflected by the variable phase filter installed at 45 deg. with respect to the light axis. The variable phase filter operated the phase-shift between beams of light. And the ray reflected from the variable phase filter were focused onto the Electron-Multiplying CCD camera (EMCCD, Manufacturer: ANDOR CO., Ltd, Type: DU-897) by the imaging lens. From Fig. 9 the image taken by the EMCCD camera indicates the iris behind the light source at 2.2-fold magnification. The 2-D spectral characteristics was obtained by calculating the change of imaging intensity at each pixel based on Fourier transform. Fig. 10 shows the change of imaging intensity at arbitrary pixel on EMCCD camera, whose waveform is called interferogram. As shown in Fig. 11, the spectral characteristics, that is the relative intensity at each frequency, is analytically calculated from the acquired interferogram by the Fourier transform algorithm. In Fig. 11 the red full line shows the spectral

characteristics that is obtained by the proposed method and the blue line shows the spectral characteristics that is obtained by the conventional spectroscopy (Manufacturer: Hamamatsu Photonics K.K., Type: PMA-11) Because both spectrums had transmittance maximum of the red filter around 700nm, we confirmed the feasibility of the 2-D imaging spectroscopy by the reflecting telescope. These experimental results verify the feasibility of the 2-D imaging spectroscopy by the reflecting telescope equipped with the imaging-type 2-D Fourier spectroscopy.



Fig. 8. Image of the experimental optical system equipped with the imaging-type 2-dimentsional Fourier spectroscopy.



Fig. 9. Image of the iris on the EMCCD camera.



Fig. 10. Interferogram detected by the EMCCD camera.



Fig. 11. Experimental results of the calculated spectrum of the red filter.

10. PRELIMINARY ADAPTATION EXPERIMENT FOR ATMOSPHERIC FLUCTUATION

The problem of the atmospheric fluctuation should be solved to develop this proposed method into astronomical spectroscopic measurement. The fluctuation is caused by the change of the refractive index derived from the wind and the temperature difference. As the simulation of the atmosphere fluctuation, the change of the refractive index of air was induced by the dry ice smoke. Fig. 12 shows the optical system of adaptation experiment that simulated the change of the refractive index of air by the dry ice smoke. In this experiment, the argon (Ar) laser (Manufacturer: Showa Optronics Co., Type: GLG3110) whose wavelength was 488nm was used as the light source, because the change of the spectrum was easily estimated. We installed the dry ice between the light source and the variable phase filter to generate the irregular light blinking by the dry ice. The collimated rays of the Ar laser transmitted the dry ice smoke and were reflected by the variable phase filter installed at 45 deg. with respect to the light axis. The variable phase filter generated the phase-shift interference within the objective beam. The rays reflected from variable phase filter were focused onto the EMCCD camera by the imaging lens. Fig. 13 shows the interferogram with or without the dry ice. The interferogram with the dry ice indicated the irregular intensity change. Fig. 14 shows the spectral characteristics whose relative intensity at each frequency is calculated by Fourier transform algorism from the acquired interferogram. The wavelength of Ar laser at 488nm is confirmed from the red full line and blue full line. But when the dry ice smoke induced the atmospheric fluctuation, the disturbed spectral components were also confirmed at wavelength other than 488nm. The relative intensity at 488nm of Ar laser is derived from the high-frequency components that are illustrated in the full circle line. The dry ice smoke induced the low-frequency components in the interferogram that is illustrated the dotted circle line. And in the long-wavelength region, the noise was accumulated on the spectral characteristic. The disturbance of the dry ice smoke affected the spectral characteristics. In the future work, we will try to solve the atmospheric-fluctuation problem using such as the adaptive optics.



Fig. 12. The experiment optical system to estimate the influence of the fluctuation caused by the dry ice.



Fig. 13. Experiment results of interferogram with or without the dry ice.



Fig. 14. The acquired spectral characteristics with or without the dry ice.

11. CONCLUSION

We are carrying out study on imaging-type 2-D Fourier Spectroscopy that can limit the measurement plane into the focal plane and obtain the spectral characteristics at arbitrary point in the measurement plane. In this report, we explain the approaches to the development of the noninvasive measurement of blood sugar level. With evaluating the feature of the proposed method, that is the high spatial resolution and can limit the depth into focal plane, we've got the good experimental results using the quantum dots as the theoretical point light source. Furthermore, the proposed method can measure spectroscopy of greenhouse gases and noxious fumes with narrowband width of spectral adsorption index. We mention the development of the 2-D spectroscopic measurement for unstructured environment.

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