Research Paper

Image processing for functional transillumination imaging of animal body using near-infrared light

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Abstract: With a near-infrared (NIR) light, one can get a transillumination image of a living body. The transillumination images are seriously blurred by the light scattering at body tissue. A fundamental study has been conducted to visualize the functional change inside a living biological body. In this study, a technique was developed to visualize the attenuation change occurred in a diffuse scattering medium. Transillumination images are obtained before and after the physiological change. By taking the ratio of the transmitted intensities of these two images, one can obtain the spatial distribution of attenuation change while suppressing the effect of scattering. The effectiveness of this principle was verified in experiments. To examine the applicability of this principle to a biological body, localized physiological changes were made in the mouse abdomen and the rat brain. The hypoxia in one of the mouse kidneys was visualized selectively from another normal kidney. The local increase in the blood volume was visualized in the somatosensory area of a rat brain when its forelimb was electrically stimulated. The blood increase was detected in a symmetrical position with respect to the sagittal plane, when the forelimb of the opposite side was stimulated. Through these experiments, it was found that the changes in the tissue oxygenation and the blood volume could be detected noninvasively and that they are visualized in the transillumination images using the NIR light. In transillumination imaging, the physiological analysis has been limited with a few wavelengths. To advance this technique, we applied a hyperspectral camera to achieve the spectroscopy at each pixel of a transillumination image. The distribution of oxygen saturation was successfully visualized in the transillumination image of an adult hand. However, the transmitted light through animal body is generally weak and the image is greatly contaminated with noise. Moreover, the wavelength-scanning mechanism of the hyperspectral camera makes typical streak noise. To solve these problems, we analyzed the nature of the noise in Fourier domain, and constructed a new noise filter for image processing. In combination with the denoising filter using deep neural network, we succeeded to obtain clear transillumination image which can be used for the multi-spectral analysis of physiological functions.

Keywords: transillumination imaging, animal body, near-infrared, light scattering, optical absorption, visualization, noninvasive, tissue, brain, blood.

1. Introduction

Among the wide spectrum of visible and invisible light, near-infrared (NIR) light is known to have the high transmittance through a biological body [1]. In addition to the high transmission, the important chromophores such as hemoglobin show characteristic absorption spectra in this NIR range [2,3]. Since the spectrum is sensitive to the oxygenated state of the chromophores, we can obtain the functional change inside a living body in a transillumination image. We have conducted the fundamental study on the scattering in dense random media, and verified the effectiveness of the scattering suppression techniques [4-7].

This paper presents the experimental study for the image processing of transillumination images to visualize the physiological functions in an animal body using the NIR light.

2. Principle

The absorbance of hemoglobin (Hb) is generally dependent on its redox state except at the isosbestic wavelength around 800 nm. Thus, we can evaluate the change in the tissue oxygenation by measuring the absorption of Hb in the tissue. Further, we can measure the change in the amount of Hb, or the blood volume in tissue by measuring the absorption at this isosbestic wavelength.

This technique is based on the measurement of the transmitted light through a biological body. The light intensity transmitted through a non-scattering medium is given by,

$$I = I_0 \exp(-\varepsilon C D) \quad (1),$$

where I_0, I, ε, C and D are the intensities of incident and transmitted light, the attenuation coefficient, the concentration and the geometrical thickness of the object medium, respectively. Here we define the attenuation of light as the optical density OD, or

$$OD \equiv \log (I_0 / I) \quad (2).$$

Then it becomes,

$$OD = \varepsilon CD \quad (3).$$

This is the case when there is no scattering in the medium. If the medium has strong scattering property such as in the case of mammalian tissues, there is no analytical solution to obtain the transmitted light intensity. However, if the scattering characteristics of the medium do not change, it has been shown that the change of the transmitted intensity is proportional to the change in the attenuation parameters in the medium [8], i.e.,

$$\Delta OD = B\Delta(\varepsilon CD) \quad (4),$$

where B is the differential pathlength factor [*8*] which represents the increase of the optical pathlength due to the scattering process.

In the proposed technique, we measure the change in the transmitted intensity due to the changes in the tissue oxygenation and in the blood volume. When the oxygenation of the tissue (oxygenation of blood in the tissue, strictly speaking) changes without the change in blood volume (C), the difference between before and after the change is given by,

$$OD_2 - OD_1 = \log(I_1 / I_2) = (\varepsilon_2 - \varepsilon_1)BCD \quad (5),$$

where the subscripts 1 and 2 indicate before and after the change, respectively. This means that the difference of the optical density is linearly proportional to the change in the attenuation coefficient in the scattering medium. The attenuation coefficient ε consists of the attenuation caused by scattering and absorption of light. In the physiological change, the change in scattering is often much smaller than that in absorption. Therefore, through the image processing of Eq.(5), we can detect the absorption change caused by a functional change in the body. In other words, we can visualize the spatial distribution of this absorption change by calculating the ΔOD at each pixel of the transmitted image.

When the degree of Hb oxygenation does not change, or if we use the light of the isosbestic wavelength, the change in the optical distance due to the blood volume is given by,

$$OD_2 - OD_1 = \log(I_1 / I_2) = (C_2 - C_1)B\varepsilon D \quad (6).$$

This means that we can visualize the distribution of blood volume change in the transillumination image.

3. Imaging of Internal Structure

Figure1 shows the outline of an experimental setup schematically. A light source of infrared light was placed below a rat (Wistar, 100-150 g). The transmitted light across the rat was focused on a CCD camera placed above the rat. The image signal from the CCD camera was recorded in a video recorder, and processed by an image processor controlled by a microcomputer. As the light source, laser diodes and a Ti:Sapphire laser were used. The optical power of the diverged light from a fiber was about 100 mW. The hair of the rat surface was shaved off to eliminate unnecessary scattering and

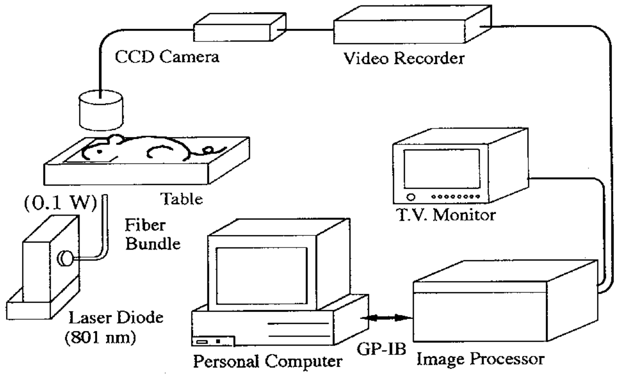


Fig. 1. System for transillumination imaging.

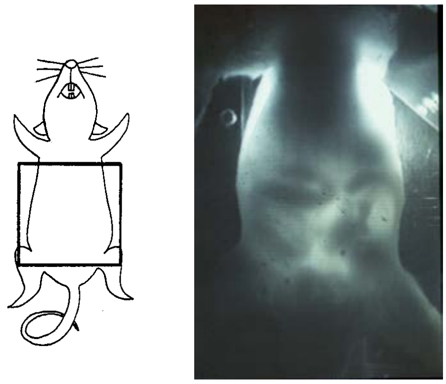


Fig. 2. Transillumination image of mouse abdomen.

to simulate the condition of the human skin. To check the vital sign of a rat, an ECG was kept monitored throughout the experiment.

Figure 2 shows the transillumination image of a mouse abdomen. The light source was a laser diode (wavelength λ=801 nm). Without any contrast media, the major structure could be observed such as the intestines, the spleen and the bladder. In the moving image, the peristaltic movement of intestines was clearly observed in a real time.

Figure 3 shows the transillumination image of a rat head and the position of the image. A main structure of the brain could be seen through the skin and the skull. We could see the sagittal vein running between the left and the right cerebral hemispheres and the transverse vein running between the cerebrum and the cerebellum. Further, the large olfactory bulb which is characteristic of a rat could be observed as well.

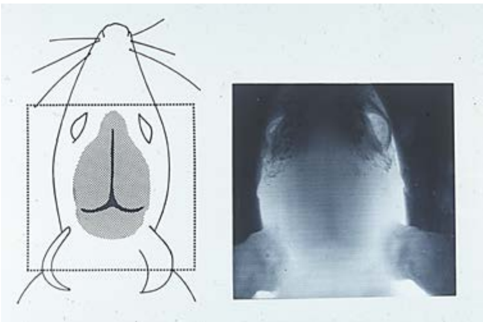


Fig. 3. Transillumination image of rat head.

These results show that the imaging of internal structure is possible to some extent, and that the image provides the information different from that of X-rays.

4. Imaging of Physiological Functions

The significance of the optical trans-body imaging is the capability of imaging the internal physiological functions. First, the feasibility to detect the internal functional change was examined. As the functional change, one of the mouse kidneys was made hypoxic. The blood circulation of the right kidney was blocked by tying the renal arteries and veins to make the kidney hypoxic. As a light source, Ti:Sapphire laser (λ=775 nm) was used.

In the transillumination image itself, it was difficult to observe any distinct changes caused by the hypoxia. Fig.4 shows the image of the transmittance ratio between the images before and after the hypoxia. By taking the ratio, the part of local hypoxia appeared distinctively. The left bright area seemed to be the artifact due to the movement of the intestine. This result shows that we can detect the change in the oxygenation of the internal tissue.

Next, we attempted to detect the physiological change caused by natural biological functions. To induce such a change, a sensory stimulation was applied to cause the change in blood circulation in the rat head. Fig.5 shows the results of functional imaging of rat brain. It is the spatial distribution of the transmittance ratio between before and after the functional change. The change was caused by the electric stimulation on the right forelimb (Fig.5(a)) and on the left forelimb (Fig.5(b)) of the rat. Since the laser diode of the isosbestic wavelength (λ=800 nm) was used as a light source, the image corresponds to the distribution of the blood volume change. The localized increase of blood volume was well detected in the somato-sensory area of the rat brain.

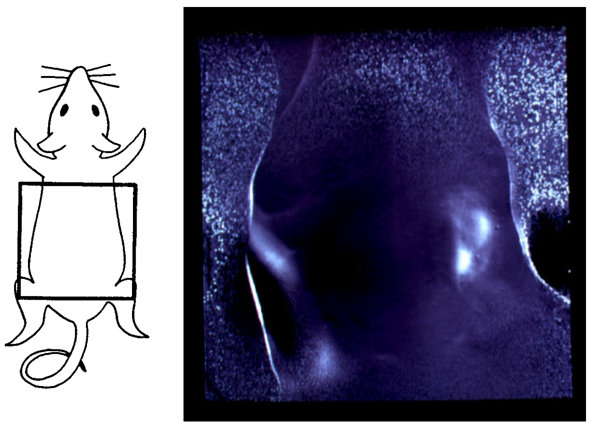
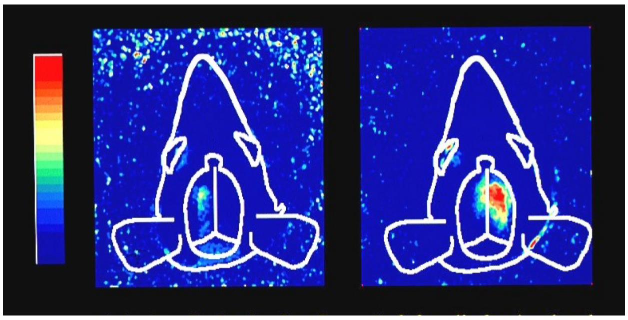


Fig. 4. Imaging of local hypoxic part in the body.



(a) (b)

Fig. 5. Imaging of functional change in rat brain: (a) right foreleg stimulated, (b) left foreleg stimulated.

5. Fourier Filtering of Hyper-spectral image

We can observe the functional change in a living body noninvasively in the transillumination images with NIR light. This advantage is greatly enhanced using multiple wavelengths of light. For example, Fig.6 shows the absorption spectra of hemoglobin for different oxygen saturation. Using this characteristic change, we can visualize the oxygenation distribution in a transillumination image of a human body.

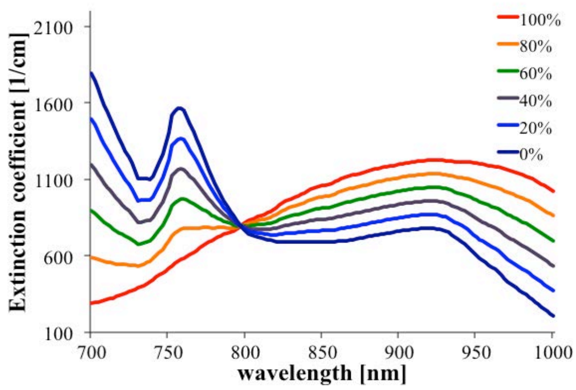


Fig. 6. Absorption spectra of Hb for different oxygen saturation.

To realize this method, we introduced a hyper-spectral camera to transillumination imaging. If we use a hyper-spectral camera, we can obtain trans-illumination images in a wide range of continuous wavelength. This makes the accurate visualization of various internal physiological functions possible. The validity of this principle was examined in experiments. Fig. 7 shows the outline of the measurement system. It consists of a halogen lamp (λ=700-2500 nm), a band pass filter (λ=691-1225 nm) and hyper-spectral camera (λ=500-900 nm, NH-W, Eba Japan Co.,Ltd.) and a PC.

First, we obtained the spectrum of the light source by measuring the diffuse reflectance from a white paper. Then, the full spectral transillumination images were taken as shown in Fig.7. The images were normalized by the source spectrum. Finally, we apply the curve fitting technique with the spectra shown in Fig.6, and obtained the oxygen saturation at each pixel. The result was presented in a pseudo-color image to show the oxygen saturation mapping.

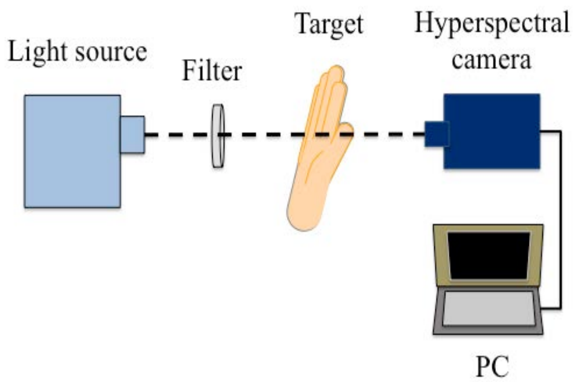


Fig. 7. Measurement system for transillumination imaging with hyper-spectral camera.

Fig.8 shows an example of the results. The 3 images are the representative 3 frames of continuous video picture. The temporal change of oxygen saturation (SaO_2) in a human hand was captured successfully.

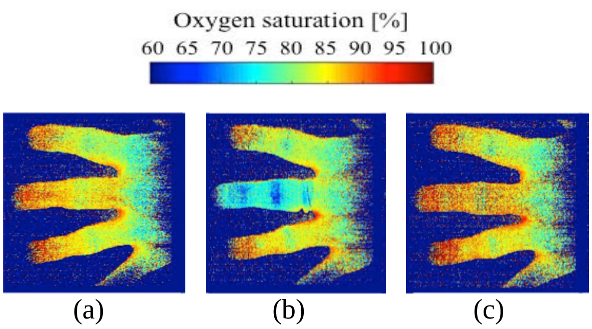


Fig.8. Temporal change of oxygen saturation in transillumination image in middle-finger constriction: (a) before constriction, (b) 11 minutes after constriction, (c) 11 minutes after release of constriction.

The validity of the proposed technique was verified, but the transillumination image taken with the hyper-spectral camera is commonly contaminated with different kinds of noise, which makes the image quality low and has limited the application of this technique. To solve this problem, we analyzed the characteristics of the typical noise of the hyper-spectral camera, and attempted the functional imaging with noise-suppression.

Figure 9 (a) shows an example of oxygen saturation (SaO_2) map obtained in transillumination imaging. The noise appears as horizontal/vertical streaks and granular dots. The former comes from the wavelength-scanning mechanism in a hyper-spectral camera, and the latter from low signal to noise ratio in transillumination imaging. In image analysis in the spatial-frequency domain, the streak noise components were identified along x- and y-axes of the two-dimensional Fourier transform. The granular noise was in the high frequency region of the transform. The noises were eliminated by masking the corresponding area in the spatial-frequency domain. However, the masking the high-frequency component resulted in blurring the hand image itself. To control this effect, we applied the deep-learning filter [9]. Fig.9 (b) shows the result of the filtering of both components. The streak and granular noises were eliminated without blurring the hand image.

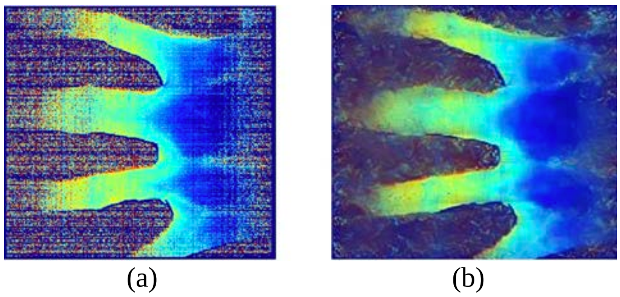


Fig.9. Elimination of streak and granular noise in transillumination image of human hand: (a) before filtering, (b) after filtering.

Using the denoised hyper-spectral transillumination images, functional imaging was attempted. Fig.10 shows the temporal change in SaO_2 in an adult hand after stopping the blood circulation with a wrist belt. This result suggests the effectiveness of the proposed technique for the functional transillumination imaging with a hyper-spectral camera.

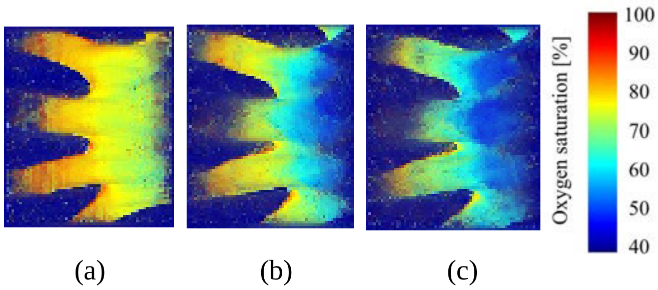


Fig.10 Temporal change of SaO_2 in human hand in wrist constriction: (a) before constriction, (b) 4 minutes after constriction, (c) 8 minutes after constriction.

6. Conclusions

For the functional imaging of animal body with near-infrared light, we have developed some techniques to suppress the scattering effect in diffuse medium such as a human body. Using these techniques, we can visualize the physiological change inside the body noninvasively. With appropriate image processing, we could suppress the blur and the noise effectively.

These techniques provide useful tools to suppress the scattering effect in the imaging through diffuse medium. Particularly, they are useful for better observation of optical images through the skin. They can be used to improve noninvasive diagnostics in medicine. In animal experiments, they can reduce the necessity of open surgery because it obviates the need to remove surface tissue for clear observation. Consequently, it will contribute to reducing the number of sacrificed animals in such experiments. Lack of necessity of operations also allows long-term experiments, such as during growth periods of the animal.

Acknowledgements

This research was supported by a Grant-in Aid for Scientific Research from the Japan Society for the Promotion of Science.

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