Introduction

Since measurement of the oxygen saturation in the fundus of the eye is assumed to provide an important parameter for early detection of retinal vascular disorders such as diabetic retinopathy and retinal ischemia, it is important to develop a noninvasive measuring equipment that can be used for medical checkups aimed at early detection of such diseases. Therefore, we attempted to create an oxygen saturation map for the blood vessels at the fundus by applying the spectroscopic imaging technology to the fundus.

At present, the reliable method of measurement of the oxygen saturation in the fundus still uses a Swan-Ganz catheter, which accompanies invasion. The first attempt of noninvasive optical measurement ever done was the measurement by Hicham et al. in 1963 using bandwidth filters of two different wavelengths, 640 nm and 800 nm, although it was a qualitative measurement using bandwidths of over 100 nm.

Since then, the technique has been improved by Cohen and Laing, Delori, Tiedeman et al., and Smith et al., etc. who used four different wavelengths for enhanced precision. While the method based on the use of wavelength band filters is simple and convenient, its disadvantage is that the data is widely varied depending on the examinee. Although our method, because it uses spectrometric measurement, requires more complicated equipment than the conventional method, its general versatility is higher.

The difficulty in the application of spectrometric measurement to funduscopy was significant lack of light intensity. If a high wavelength resolution is used in spectroscopic analysis, fast measurement is not possible due to lack of detected light intensity. Since constant eyeball movement occurs during a funduscopy, it is not appropriate to sacrifice the speed of measurement. We solved this problem by illuminating the fundus with pre-dispersed light and conducting an absorbance determination to avoid eye damage and eyestrain from injection of unnecessary light while maintaining a sufficient light intensity.

Retinal oxymetry

Because the conventional method which is based on the use of wavelength band filters normally uses two different wavelengths, the choice of wavelengths used is important. The absorbance spectrum in the visible to far-red range is different between deoxygenated hemoglobin and deoxygenated hemoglobin, but there are several equivalence points. One or two wavelengths based on these is selected. As for the other wavelength, the oxygen saturation is calculated by selecting a portion where the difference between HbO2 and Hb is large and measuring the difference between that and the base wavelength.

In this method, because the hemoglobin absorbance has drastically changed between the base wavelength and the different wavelength, it is difficult to keep the dynamic range of the detector within the optimal range of both wavelengths. Because the light intensity that transmits to the fundus differs from individual to individual, especially for someone with a cataract, the light intensity must be set differently depending on the person. While wavelengths in the vicinity of 530-580 nm can be measured relatively easily as the absorbance variation is not so large, it is necessary to increase the light intensity of irradiation due to the large absorbance. Therefore, this is assumed to be a range where the examinee is greatly strained and measurement with a good SN is difficult from the viewpoint of detection range.

Our method does not choose any particular base wavelength, which allows measurement in any arbitrary dynamic range and mitigates restraints placed on the light intensity of illumination and the detector, thus preventing variation of measurement. Although far-red light somewhere around the isosbestic point of 800 nm is generally used in pulse oxymetry, we determined that this was not appropriate for our measurement because retinal vessels had a wall and far-red light with low absorbance did not provide sufficient accuracy of measurement.

The following is the measurement method we employed. Fig. 1 shows a pattern diagram of absorbance spectrum of the measured vessel. The oxygen saturation of this vessel is x ($0 < x < 1$), and the absorbance spectral curve when this is satisfied is $f(x, \lambda)$. The dotted lines indicate the absorbance spectrum of oxygenated hemoglobin (HbO2, oxygen saturation 100%) and deoxygenated hemoglobin (DeoxyHb, oxygen saturation 0%) in the visual light range, represented as $f(1, \lambda)$ and $f(0, \lambda)$ respectively.

Fig. 1 Absorbance spectrum of oxygenated hemoglobin (HbO2) and deoxygenated hemoglobin (DeoxyHb) in the visual light range

In this case, the relationship shown in formula (1) exists.

Formula (1)

If the wavelength of the measured vessel and HbO2 when the absorbance is A is λa and λm respectively, formula (2) is satisfied because the absorbance at this position equal to each other.

Formula (2)

x is obtained by assigning formula (1).

Formula (3)
Consequently
Formula (4)

Oxygen saturation x is obtained because absorbance curves f(1, λ) and f(0, λ) are standard values, and wavelengths λa and λm are observed values.

Since this method only requires observation of the wavelength corresponding to one absorbance, calculation is possible without depending on the dynamic range of the detection system. While the absorbance differs because the hematocrit value varies depending on the individual, but, in this method, λa which is the base for absorbance can vary for each measurement. The table below shows an example of oxygen saturation for observed wavelengths λa and λm obtained from formula (4). After the isosbestic point of 585 nm, the oxygen saturation accuracy becomes higher if λa is as deep on the long-wavelength side as possible.

Table 1　Oxygen saturation for observed wavelengths λa and λm

Experiment
The optical system shown Fig. 2 was used for the measurement of fundus spectrum. A spectroscopic image measurement system requires a white light source that is stronger than that used in normal fundus cameras, and it is also necessary to place a wavelength-variable filter for light dispersion at an appropriate location. For this reason, we configured the system by arranging elements on an optical surface plate that is 30 centimeters square in order to secure freedom of optical system design. Similar to an ordinary fundus camera, the basic geometrical-optical configuration consists of a illumination system with a structure for suppressing specular reflection from the corneal surface and an image formation system that leads specular light from the fundus to the imaging system.

We used a 300 W xenon lamp for the light source, a VariSpec wavelength-variable filter, a wavelength range of 500-700 nm, and a wavelength resolution of 7 nm. Wavelengths outside the measured range were cut with a colored glass filter. The light exposure used was less than that used with an ordinary fundus camera because the fundus was exposed to dispersed light. These conditions satisfied the eye safety

standards of JIS C 6802 and IEC 60825-1.

Fig. 2　Optical system

In Fig. 2, the illumination system is arranged in such a way that the illumination from the xenon lamp is evenly dispersed by the use of a diffuser plate and a ring slit image is formed on the cornea. The illuminated light passes through the cornea and expands to illuminate the retina uniformly. The ring slit is used to divert the illuminated light on the cornea from the optical axis of the imaging system. If the illumination spot in on the optical axis of the imaging system, the light reflection on the cornea decreases the image quality of the imaging system as flare. Handling of flare is important especially in fundus imaging because there is little reflection from the fundus.

As formula (4) shows, intravascular oxygen saturation x is obtained from λa and λm, which are the wavelendgths when the absorbance of the measured vessel and that of the vessel with 100% oxygen saturation equal to each other. Multiple pairs of wavelengths λa and λm can be obtained depending on the absorbance selected. However, the measurement deviation can be minimized if wavelength λm is as deep on the long-wavelength side as possible. Although the absorbance becomes small on the long-wave side, measurement is possible in the 590-600 nm range without the use of a high-sensitive CCD. Because measurement not dependent on the CCD dynamic range is possible in our method, the absorbance was determined at the lower detection limit of the CCD used, and the λa-λm wavelength pair was obtained as deep on the long-wave side as possible.

The measured results are as shown below. Fig. 3 shows the retinal images measured at an interval of 2 nm in the 596-600 nm range. Fig. 4 shows the detected brightness of the arteria-vena pair with the same diameter plotted from this data set.

Fig. 3　Retinal images measured for each 2 nm in the 596-600 nm range

Fig. 4　Absorbance in the arteria and vena (550-650 nm)

The dotted lines show the absorbance determined as close to the lower detection limit of the CCD as possible. Since the wavelengths in the arteria and vena that achieve this

absorbance are λa=597 nm and λm=612 nm, by assigning these values to formula (4), the oxygen saturation at the measured point of 49% is obtained.

Because absorption in the fundus is assumed to take place both when the light enters the retina and when the reflection on the retinal pigment epithelium returns in the direction of the detector, the light path length of the absorbed light has to be previously determined in accordance with the Lambert-Beer Law before comparing the molar absorbance coefficients of the arteria and vena. As Fig. ?? shows, the molar absorbance coefficient is stable with wavelength 584 nm regardless of the oxygen saturation. The image for this wavelength should be proportional to the light path length, and is assumed to represent the thickness of the blood vessel in the depth direction. For this reason, the point where the brightness at 584 nm was standardized, and the effect of the vessel diameter was compensated.

In this study, we measured the band of wavelengths ranging from 550 nm to 650 nm was measured at each 2 nm. The measurement time for one wavelength band was approximately 150 ms, and the total measurement time was approximately 7.5 sec. Because the fundus image kept shifting due to eye ball movement during this measurement time, each spectrum image was aligned by image matching.

In this paper, we have proposed a measurement method that solves difficulties in hemoglobin photometry, especially problems in quantitative measurement in a field accompanied by sudden change of absorbance, and that allows accurate measurement by the use of widely available low-cost measuring equipment.