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Real-Time Measurement of Human Optic Nerve Head and Choroid Circulation, Using the Laser Speckle Phenomenon

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Abstract: A modification of a previously described instrument that used the laser speckle phenomenon for noncontact two-dimensional analysis of the fundus tissue circulation was devised so that tissue circulation in the optic nerve head (ONH) or choroid of the human eye could be measured on a real-time basis. The fundus was illuminated by a diode laser spot and the image speckle was recognized by an area sensor. A quantitative index of blood velocity, normalized blur (NB), was calculated by a logistic board every 0.125 seconds for 7 seconds. Using this modified device, the average NB of the measurement field in the temporal ONH, free of visible surface vessels (NB_{ONH}), and that in the posterior choroid (NB_{ch}) of normal human eyes were measured. The coefficients of reproducibility of 1-minute interval measurements were 11.7% for the NB_{ONH} and 8.7% for the NB_{ch} (each, an average of 5 pulses), and those of 24-hour interval measurements were 13.0% (NB_{ONH}) and 9.7% (NB_{ch}). The pulsatile component average of NB_{ONH} was 38.4% of mean NB_{ONH}; of NB_{ch}, 26.6% of the mean NB_{ch}. **Jpn J Ophthalmol 1997;41:49–54** © 1997 Japanese Ophthalmological Society

Key Words: Choroid, human eye, laser speckle phenomenon, optic nerve head, tissue circulation.

Introduction

Evaluation of tissue circulation in the human eye is essential for investigation of ocular physiology and pathology of ocular tissues. Noncontact evaluation of the tissue circulation in the optic nerve head (ONH) and choroid has been done with dye-dilution techniques, such as fluorescein angiography[1,2] or indocyanine green (ICG) angiography,[3] and with the laser-Doppler technique.[4–11] Dye-dilution techniques[1–3] require the use of exogenous substances and give a qualitative, rather than a quantitative, evaluation of fundus circulation. Noncontact and quantitative evaluation of ocular tissue circulation in the ONH was first made possible by laser-Doppler velocimetry.[4] This technique was originally used to estimate ONH blood velocity, but later modified to evaluate the ONH blood flow rate by the addition of reflectometry.[10,11] An alternative method involves combining a commercially available laser-Doppler flowmeter with a fundus camera.[5–7,9] These laser-Doppler techniques of velocimetry and flowmetry in the ONH[4–7,9–11] or choroid,[5,8] used mainly in animal experiments, have the disadvantage of being restricted to a small measurement site (approximately ≤ 180 μm in diameter) and results are subject to point-to-point variations of blood flow in the tissue.[12]

We have recently developed a new device for noncontact and two-dimensional estimation of the tissue circulation in the ocular fundus, including the retina,[13] choroid[14] and ONH tissue,[13,15] using the laser speckle phenomenon. The temporal resolution of the equipment currently used is approximately 10 seconds, significantly slower than that of a laser-Doppler flowmeter combined with a fundus camera.[5–8] Although measurement is completed within

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0.18 second, nearly 10 seconds are then needed to calculate and display the results, thereby making it difficult to detect changes in tissue blood flow rate occurring at a higher frequency, e.g., those of the cardiac cycle.[5,8] Our modification incorporates a logistic board so that tissue circulation in the human ONH and choroid, with much shorter intervals, can be measured on a real-time basis. In this study, we have assessed the pulsatility of the tissue blood velocity in the human ONH and choroid, and the reproducibility of the measurements made with the newly modified instrument.

Materials and Methods

Laser Speckle Tissue Circulation Analyzer

Laser speckle is an interference phenomenon observed when coherent light (such as laser light) is scattered by a diffusing surface. The speckle pattern is a random pattern with properties that can only be described statistically. One of the most useful statistics is the standard deviation of the intensity distribution in a speckle pattern (σ), which is equal to the mean intensity ($\langle I \rangle$) for the ideal speckle pattern. Under imperfect conditions, σ is less than $\langle I \rangle$, and the ratio, $\sigma/\langle I \rangle$ (the contrast of the speckle pattern), is:

$$\sigma/\langle I \rangle = [(\tau/2T)\{1-\exp(-2T/\tau)\}]^{1/2} \qquad (1)$$

where (T) is the laser exposure time and τ is the correlation time of the temporal fluctuations in the intensity. When the laser exposure time (T) is constant, the reciprocal of the speckle contrast, $\langle I \rangle/\sigma$, correlates with $1/\tau$, which is taken as the indicator of tissue blood velocity when living tissue is illuminated with laser beams.[16,17]

Tissue circulation in the ONH and choroid was evaluated with a laser speckle tissue circulation analyzer, according to this theory.[15,18] A fundus camera equipped with a diode laser (808 nm wavelength) was used; and a halogen lamp illuminated the fundus area on which the laser beam was focused. The scattered laser light was reflected on an image sensor of 100 × 100 pixels (BASIS, Canon, Tokyo, Japan) from a 1.06 × 1.06 mm field (45° visual angle) in a human fundus, on which a speckle pattern appeared. The difference between the average speckle intensity (I_{mean}) and the intensity for successive scans of individual speckles at pixels on the sensor plane was calculated; the ratio of I_{mean} to this difference was defined as normalized blur (NB). NB is the approximate equivalent of the reciprocal of speckle contrast given by Briers and Fercher,[16,17] and indicates tissue blood velocity. The NB values were divided into 50 color-coded levels, with red indicating fast blood-flow velocity. Our logistic board calculated the NB every 0.125 second for 7 seconds and produced a color graphic display showing the two-dimensional variation of NB over the field of interest. The average NB (NB_{av}) in any rectangular field of interest displayed on a color map can be calculated, as well as the change in NB_{av} over 7 seconds, in one measurement. During NB measurement, an electrocardiogram (ECG) can also be done; both maximum NB_{av} during systole and minimum NB_{av} during diastole can be recorded and displayed by the software. A 5-pulse NB_{av} was calculated and defined as mean NB_{av}.

NB Measurement in ONH and Choroid

The current study was approved by the Ethics Committee of the University of Tokyo. Before admission to the study, a written consent was obtained from each subject after the nature of the study had been fully explained. Routine eye examinations were given to the 6 volunteers, 20–40 years old, who had no history of smoking, no systemic or ocular disease, and only mild refractive errors. Twenty minutes after dilating a pupil with one drop of 0.4% tropicamide (Mydrin M®, Santen Pharmaceutical, Osaka, Japan), image speckles from the field located in the temporal ONH, and free of visible surface vessels (NB_{ONH}, 0.72 × 0.72 mm, 30° visual angle), were recorded to measure the NB in the ONH tissue. Image speckles from a field located between the macula and the ONH, with no discrete vessels visible (NB_{ch}, 1.06 × 1.06 mm, 45° visual angle), were recorded for the NB value of the choroid (Figure 1). The NB_{ONH} was calculated as the average NB across the largest rectangular field free of visible surface vessels (Figure 2a). NB_{ch} was calculated as the average NB across the whole measurement field (Figure 2b). ECG monitoring was done throughout the NB measurement period.

Mean Value and Pulsatile Components of NB_{ONH} and NB_{ch}

The formula used for the pulsatile component of $NB_{ONH(ch)}$, $pNB_{ONH(ch)}$, is:

$$pNB_{ONH(ch)} = sysNB_{ONH(ch)} - diaNB_{ONH(ch)} \qquad (2)$$

where $sysNB_{ONH(ch)}$ and $diaNB_{ONH(ch)}$ are maximum $NB_{ONH(ch)}$ during systole, and minimum $NB_{ONH(ch)}$ during diastole. The $NB_{ONH(ch)}$ average of 5 pulses was defined as mean $NB_{ONH(ch)}$; the ratio of $pNB_{ONH(ch)}$/mean $NB_{ONH(ch)}$ was also found for the 12 eyes of the 6 subjects.

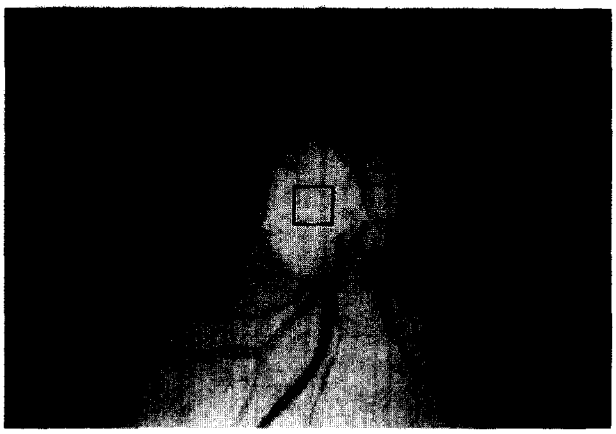

Figure 1. Measurement field of NB in optic nerve head and choroid. □ NB_{ONH}, 0.72 × 0.72 mm 30° visual angle: Speckles from field in the temporal site of ONH, free of visible surface vessels recorded to measure NB in ONH tissue. ▨ NB_{ch}, 1.06 × 1.06 mm, 45° visual angle: Speckles from field located between macula and ONH with no discrete retinal vessels recorded to measure NB in choroid.

Reproducibility of Mean $NB_{ONH(ch)}$ Measurements

Mean $NB_{ONH(ch)}$ was measured in all eyes, as above, twice, with a one-minute interval between measurements. After photographing the fundus to record the measured field, mean $NB_{ONH(ch)}$ was measured again after 24 hours.

The equation for the coefficient of the reproducibility of measurements, as previously described[13]:

$$\frac{|V_1 - V_{2(3)}|}{(V_1 + V_{2(3)})/2} \qquad (3)$$

where V_1, V_2, and V_3 are the mean $NB_{ONH(ch)}$ in the first, second, and third measurements.

Results

Mean Value and Pulsatile Component of NB_{ONH} or NB_{ch}

Figure 3 shows a representative 7-second pattern of $NB_{ONH(ch)}$ exhibiting periodic fluctuations synchronized with the cardiac rhythm. The ratio of $pNB_{ONH(ch)}$/mean $NB_{ONH(ch)}$ was 38.4 ± 2.5%/26.6 ± 2.6% (mean ± SEM, n = 12).

Reproducibility of Mean $NB_{ONH(ch)}$ Measurements

Tables 1 and 2 show the coefficients of reproducibility of 1-minute and 24-hour interval measurements of mean NB_{ONH} as 11.7 ± 3.3% and 13.0 ± 3.0%; those of mean NB_{ch} were 8.7 ± 1.5% and 9.7 ± 2.5% (mean ± SEM, n = 12).

Discussion

The temporal resolution of the instrument previously reported was approximately 10 seconds, sig-

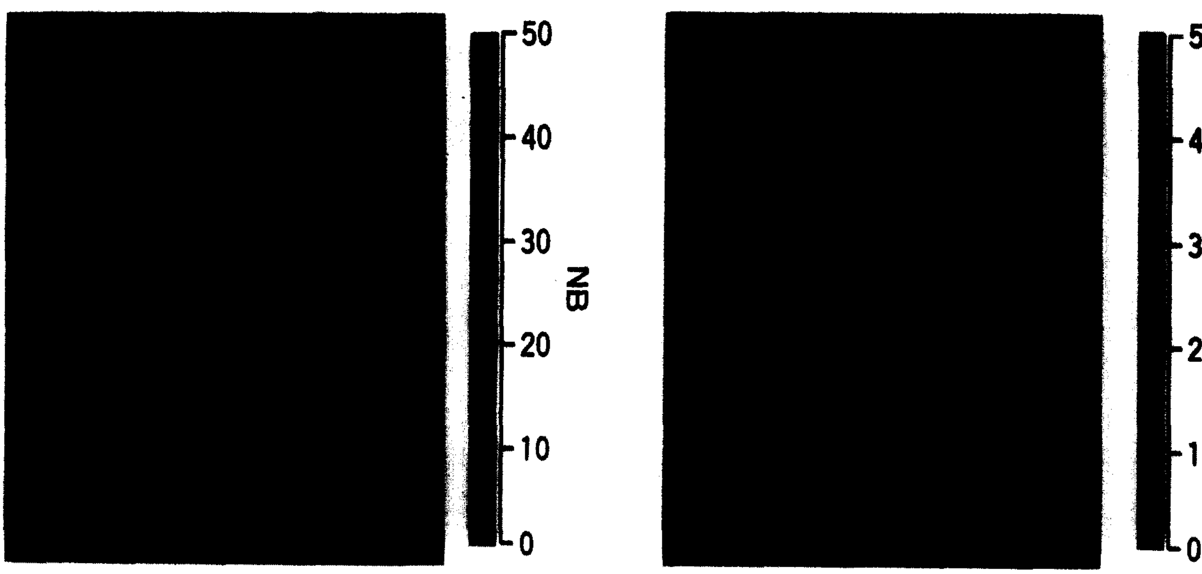

Figure 2. Representative color mapping of human optic nerve head (**A**: left) or choroid (**B**: right). Red areas correspond to areas of high flow. (**A**) NB_{ONH}: Average NB of largest rectangular field free of visible surface vessels (□). (**B**) Average NB of entire choroid measurement field.

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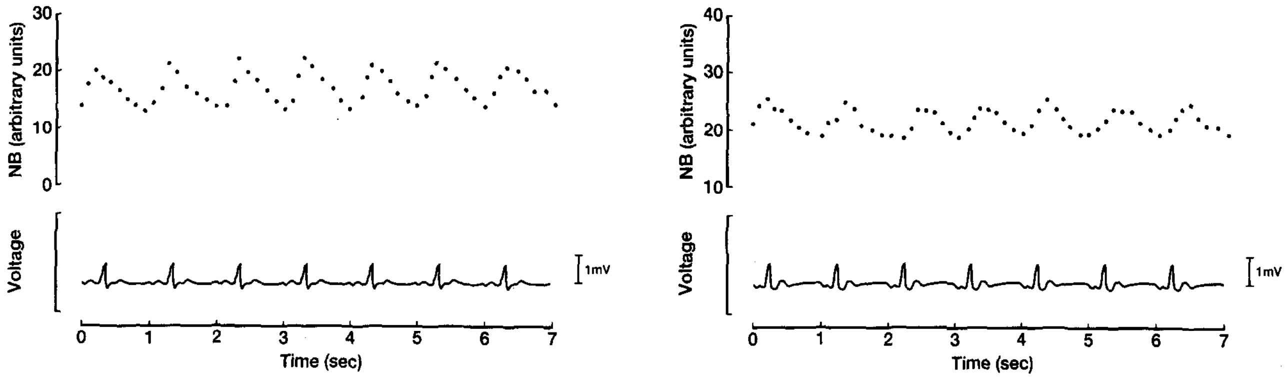

Figure 3. Representative NB pattern in optic nerve head (**A**: left) or choroid (**B**: right) compared with electrocardiogram.

nificantly slower than that of a fundus-camera-equipped laser-Doppler flowmeter.[5–9] Although the measurement was completed within 0.18 second, nearly 10 seconds were needed for calculating and displaying the results, making it difficult to detect rhythmic changes in the blood flow rate having a higher frequency, as when synchronized with the cardiac cycle.[5,8] The pulse rate of the rabbit is approximately 300/minute,[16,19] or 1 every 0.2 second, and time needed for measurement of NB was 0.18 second. Therefore, rhythmic change in the tissue blood flow rate caused by the cardiac pulse was probably an average, in previous reports.[13,18] Since the normal human pulse rate is 65–85/minute,[20] rhythmic changes in the blood flow of the human fundus related to pulse could not easily be evaluated with that equipment. With our modification, the NB is calculated every 0.125 second for 7 seconds, allowing detection of those rhythmic blood flow changes in the human fundus caused by the pulse. The measurement field of our equipment is 1.06 × 1.06 mm, about 40 times greater than previous laser-Doppler methods (180 μm diameter).[4–7,9–11] This is an improvement for assessment of site-to-site variations of human fundus tissue circulation when laser scattering shows no significant variation, and also for showing the circulatory status of the target tissue. In the current study, the coefficients of reproducibility of 1-minute and 24-hour interval measurements of mean $NB_{ONH(ch)}$ were 11.7% and 13.0%; those of mean

Table 1. Reproducibility of Mean NB_{ONH} Measurements

Subject No. Right, Left Eye	V_1[a]	V_2[a]	V_3[a]	$\frac{\lvert V_1 - V_2 \rvert}{(V_1 + V_2)/2}$	$\frac{\lvert V_1 - V_3 \rvert}{(V_1 + V_3)/2}$
1 R	11.9	11.6	12.8	0.026	0.073
L	11.5	14.7	13.2	0.244	0.138
2 R	18.2	13.1	14.1	0.326	0.254
L	12.7	16.4	10.9	0.254	0.153
3 R	13.4	13.7	13.2	0.022	0.015
L	15.3	15.5	15.4	0.013	0.007
4 R	14.2	18.5	14.6	0.263	0.028
L	13.9	12.6	13.8	0.098	0.007
5 R	15.6	14.7	11.7	0.059	0.286
L	12.8	13.5	15.3	0.053	0.178
6 R	16.2	15.6	21.8	0.038	0.295
L	16.8	16.9	14.7	0.006	0.133
Mean	14.4	14.7	14.3	0.117	0.130
SEM	0.6	0.5	0.8	0.033	0.030

V_1 and V_2 were obtained at 1-minute intervals, while V_1 and V_3 were obtained at 24-hour intervals.
[a]Arbitrary units.

Table 2. Reproducibility of Mean NB_{ch} Measurements

Subject No. Right, Left Eye	V_1[a]	V_2[a]	V_3[a]	$\frac{\lvert V_1 - V_2 \rvert}{(V_1 + V_2)/2}$	$\frac{\lvert V_1 - V_3 \rvert}{(V_1 + V_3)/2}$
1 R	22.6	23.9	22.7	0.056	0.004
L	22.5	20.9	22.9	0.073	0.018
2 R	22.1	26.1	29.3	0.166	0.280
L	24.9	27.2	26.7	0.088	0.070
3 R	28.2	34.5	28.5	0.201	0.011
L	28.7	25.4	24.6	0.122	0.154
4 R	16.9	18.7	17.8	0.101	0.052
L	22.9	21.4	28.2	0.068	0.207
5 R	27.1	26.7	25.0	0.015	0.080
L	21.9	20.0	21.8	0.091	0.004
6 R	31.9	32.9	27.5	0.031	0.155
L	32.3	31.1	28.2	0.038	0.136
Mean	25.2	25.7	25.3	0.087	0.097
SEM	1.3	1.4	1.0	0.015	0.025

V_1 and V_2 were obtained at 1-minute intervals, while V_1 and V_3 were obtained at 24-hour intervals.
[a]Arbitrary units.

NB_{ch} were 8.7% and 9.7%. These are an index of the physiologic fluctuations in fundus tissue circulation as well as of the error of measurement. The current results indicate the stability of the measured NB and suggests that our combination of equipment is well suited for monitoring changes in the fundus tissue circulation on a long-term basis.

Pigment in the human retinal pigment epithelium interferes with light transmission, unlike in the albino rabbit.[14] The NB obtained from the posterior fundus will be affected by both retinal and choroidal blood flow in human eyes. Since the retinal blood flow rate is only 5% of that in the choroid in monkeys,[20] the retinal contribution to the NB value also may be considered to be negligible. And if we use diode laser as the laser source, we find no significant difference between the NB from the medullary field and from the extramedullary field of Dutch rabbits.[13] This indicates that retinal blood flow has little effect on the speckle image obtained with our equipment. According to Koelle et al,[21] the penetration depth of near-IR laser (811 nm wavelength) in the cat optic nerve exceeds 1 mm, suggesting that, in our study, the effective depth of sampling in the ONH tissue may be > 1 mm, and that there is some contribution to the measured NB from the retrolaminal region, in addition to that from the prelaminal region. The NB_{ONH}, therefore, is believed to represent the microcirculatory status of the ONH tissue around the lamina, where injury occurs in glaucoma or anterior ischemic optic neuropathy.

We found that blood velocity in the human ONH tissue and choroid exhibited periodic fluctuations synchronized with the cardiac rhythm. This resembles the change in red blood cell flux of various microcirculatory beds, such as the cat choroid,[8] or pig ONH and choroid,[5] measured with laser-Doppler flowmetry. The ratio of $pNB_{ONH(ch)}$/mean $NB_{ONH(ch)}$ was approximately 38%/27%, much smaller than those of the human ophthalmic artery measured with color-Doppler imaging (approximately 120%–150%).[15,22] This suggests that the measured NB value reflects the peripheral circulatory status more than the ophthalmic artery flow. Further, the ratio of pNB_{ONH}/mean NB_{ONH} was greater than pNB_{ch}/mean NB_{ch}, suggesting that blood flow in relatively larger vessels derived from the posterior ciliary arteries may contribute more to NB_{ONH} than to NB_{ch}.

The potential hazards to human eyes of measurement with the present equipment must be considered. The maximum permissible exposure of the retina to a diffuse reflection from a diode laser is 460 mW/cm^2 for 10-second exposures (American National Standards Institute).[23] The maximum retinal exposure with our modified equipment in this study was 90 mW/cm^2 for 10 seconds, both well below the permissible parameters.

In conclusion, our results indicate that the newly modified instrument combination can evaluate the tissue circulation in the human ONH and choroid with sufficient reproducibility, and is useful for in vivo study of the effects of various factors on human fundus-tissue circulation.

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