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Real-Time Blood Velocity Measurements in Human Retinal Vein Using the Laser Speckle Phenomenon

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Purpose: To measure the in vivo blood velocity in human retinal veins using a laser speckle system.

Methods: The system consists of a fundus camera, a diode laser, an image sensor, and a personal computer system. The fundus area, including a target retinal vein, is illuminated with a diode laser through a fundus camera and the laser speckle pattern is imaged onto the area sensor. From the time change of the contrast of the speckle pattern, the normalized blur (NB) value, a quantitative index of blood velocity, was calculated using a logic board.

Results: In an in vitro experiment, the NB obtained from blood flow in 50–300 μm internal diameter glass capillary tubes, used as an analogue of a retinal vein, correlated with the diameter of the tube, the actual blood flow rate, and the background NB value, which was used as an analogue of choroidal circulation. In the in vivo experiment, the blood velocity in human retinal veins of approximately 50 μm in diameter was estimated in 16 normal human eyes using nomograms based on the result of the in vitro experiment. Velocity averaged 11.1 ± 0.6 mm/s (mean ± SD, n = 16) in retinal veins 53 ± 6 μm in diameter. The coefficient of reproducibility of 5-minute interval measurements was 2.5 ± 0.9%, and it took 63 ± 15 seconds for one measurement.

Conclusions: The present methodology is clinically valid for measuring blood velocity in retinal veins. **Jpn J Ophthalmol 1999;43:186–195** © 1999 Japanese Ophthalmological Society

Key Words: Blood velocity, human retinal vein, laser speckle phenomenon.

Introduction

The evaluation of retinal blood flow in the human eye is essential for investigating the physiology and pathology of the retina. Noncontact evaluation of blood velocity in the retinal vessels is usually performed using either a dye dilution technique, such as fluorescein angiography with or without scanning laser ophthalmoscopy,[1–3] the blue field simulation technique,[4] or the laser Doppler technique.[5,6] The dye dilution technique[2,3] requires administration of exogenous substances and difficult repeated measurements. Moreover, analysis of the obtained data is rather time consuming. The blue field simulation technique[4] has the disadvantage that the measurable vessel is limited to the macular capillary and the result depends upon a subjective response. The laser Doppler velocimetry technique[5,6] has been considered the only truly noninvasive and clinically applicable method for measurement of the retinal blood velocity. Laser Doppler velocimetry, however, requires fine alignment of the laser beam and the retinal vessels. Furthermore, maintenance of strict eye fixation is critical, requiring very cooperative subjects.

We have recently constructed a new apparatus for noncontact, two-dimensional estimation of the tissue

Received: July 27, 1998

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Published by Elsevier Science Inc.

0021-5155/99/$–see front matter
PII S0021-5155(99)00005-2

circulation using the laser speckle phenomenon in the ocular fundus, including the retina, choroid, and optic nerve head tissue.[7–9] In the present study, the laser speckle method was applied to measure the blood velocity in retinal vessels. In vitro experiments were first performed to elucidate the relationship between normalized blur (NB) and actual blood flow velocity in glass capillary tubes, which were used as analogues of retinal vessels. The in vivo blood velocity was evaluated in human retinal veins based on the results of the in vitro experiment. A more peripheral portion of the vein, ie, distal to the second branching point, was used for the evaluation because it is probably more closely related to the local retinal physiology or pathology.

Materials and Methods

Laser Speckle Method

The laser speckle method, details of which have been described previously,[7–9] was used for the following experiments and is briefly described below. A fundus camera was equipped with a diode laser (wavelength 808 nm) and image sensor (100 × 100 pixels). The laser beam was focused on the fundus, which was observed using an infrared, closed circuit digital camera. The scattered laser light was imaged on an image sensor corresponding to a field of 1.06 × 1.06 mm (45-degree visual angle of the fundus camera) in the human fundus, on which a speckle pattern appeared. The difference between the average of the speckle intensity (I_{mean}) and the speckle intensity for successive scans of the image speckles at the pixels on the sensor plane was calculated, and the ratio of I_{mean} to this difference was defined as NB.[7] Normalized blur is nearly equivalent to the reciprocal of speckle contrast as defined by Fercher and Briers.[10,11] The NB was calculated using a logic board every 0.125 second successively for the maximum of 7 seconds and divided into 50 color-coded levels, which were displayed as color graphics on a color monitor showing the two-dimensional variation of the NB level over the field of interest. The average NB level (NB_{av}) in any rectangular field of interest on a displayed color map was calculated and the change of NB_{av} over a maximum of 7 seconds was monitored during one measurement. An electrocardiogram was monitored simultaneously with the NB measurement. In the human experiment, NB_{av} fluctuated synchronously with the cardiac pulse, and the NB_{av} averaged over two pulses was defined as mean NB_{av}. All NB data were digitally recorded for later analysis.

In Vitro Experiment Using Ground Glass

The first in vitro experiment was performed to confirm that the NB value obtained from a diffusing surface paralleled the speed of movement and to study the effect on the obtained NB value of the laser power used. A rotating ground glass (90 mm in diameter, 2 mm thick) polished with emery powder (gray, average size 600) was used as an in vitro model. The ground glass was placed in the focal plane of the fundus camera and rotated at various speeds. The average NB obtained over the whole area (NB_{av} for 100 × 100 pixels) was determined. Next, NB_{av} for 100 × 100 pixels was determined when the rotation of the ground glass was maintained at a constant speed. The diode laser power was varied from 1.5–4 mW, using a laser power meter (OPM-370 L; Sanwa, Tokyo) to measure laser power.

In Vitro Experiment Using a Model of the Human Eye

As a simple model of the human eye, an aspheric 60-diopter lens (60 DCC; Nikon, Tokyo) was placed between the apparatus and a 160-μm glass capillary tube used as an analogue of a retinal vein. The internal diameter of the tube was measured with a microscope equipped with a micrometer. The distance between the lens surface and the glass capillary tube was adjusted to 16 mm. A blood sample was taken from one of the authors (MN), a 32-year-old healthy male, and the sampled blood was mixed with heparin (5 IU/mL) to prevent clotting. The number of red blood cells and hematocrit of the blood sample were $444 \times 10^4/\mu L$ and 44%, respectively. During all experiments it was confirmed that hemolysis did not occur in the sampled blood. The velocity of blood flow was regulated using a peristaltic infusion pump (PST-100®; Iwaki Glass, Tokyo). Each measurement (duration = 0.125 second) was performed for 5.5 successive seconds and repeated three times to obtain an average value.

Effect of position of measurement point in capillary tube. The blood velocity in the glass capillary tube was set at 10 mm/s. The position of the measurement point corresponding to 1 pixel in the image sensor was located on the center line of the capillary tube and readings obtained from each pixel arranged perpendicular to the center line were recorded (Figure 1). In this model eye, one pixel corresponds to approximately 10 × 10 μm in the fundus.

Effect of laser power. The effects of laser power used for measurement of obtained NB value were

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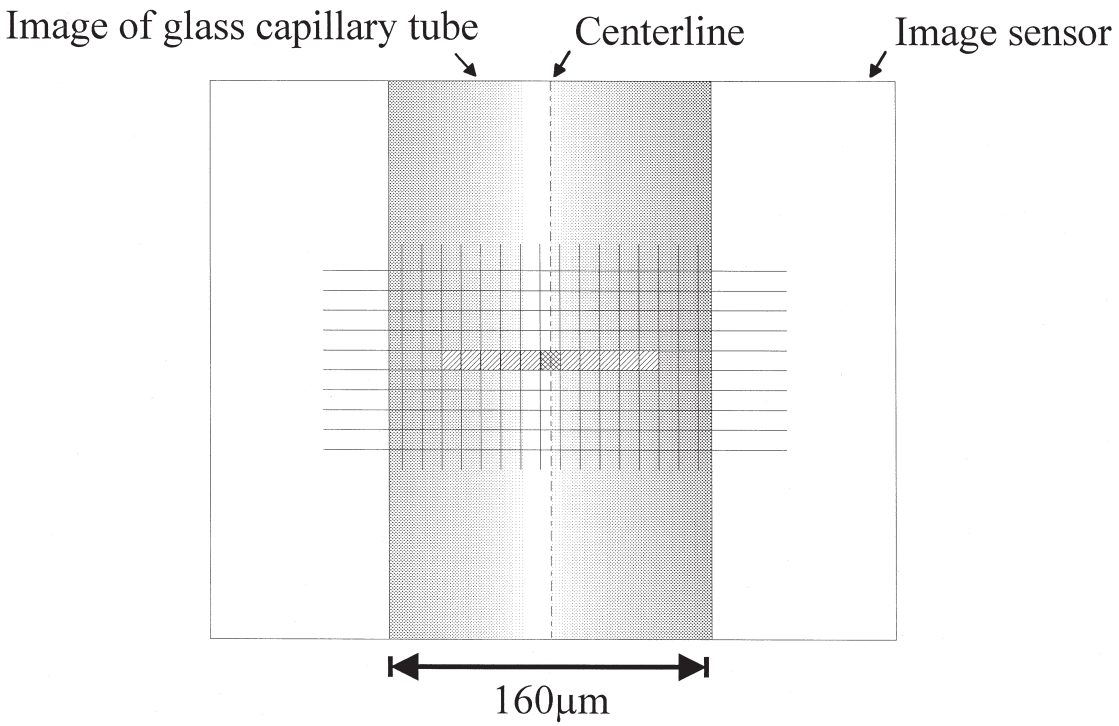

Figure 1. Position of measurement point in glass capillary tube.

studied in a simulated model of the human eye. The blood velocity in the glass capillary tube was set at 10 mm/s. The measurement point was placed on the centerline of the tube and the NB value was determined, varying the laser power from 1.5–4 mW.

Effect of hematocrit on NB. The blood sample was divided into plasma and blood cells by centrifugation at 1,500 *g* and three blood samples with hematocrits of 31.0%, 37.0%, and 47.9% were prepared. The blood velocity in the glass capillary tube was set at 10 mm/s. The NB on the center line of the tube was measured.

Effect of background reflectance on NB. To examine the effect on the obtained NB of the reflectance from the retina or retinal pigment epithelium behind the retinal vessels, five chart papers with reflectances of 2.0%, 4.0%, 6.6%, 7.4%, and 12.8% (Gray Chart®; Murakami Color Research Laboratory, Tokyo) were placed in sequence behind the glass capillary tube having an internal diameter of 160 μm. The blood velocity in the glass tube was set at 10 mm/s. The NB on the center line of the tube was measured with a laser power of 2 mW.

Effect of internal diameter and blood velocity on the measured NB. As a model for the retina and retinal pigment epithelium, a film (thickness = 0.15 mm) with a reflectance of 8% and an absorbability of 20%, which were determined using a laser power meter (OPM-370 L; Sanwa, Tokyo), was placed just behind five glass capillary tubes with internal diameters of 50, 93, 125, 150, and 300 μm. The internal diameters of these capillary tubes were measured as above. Blood velocity was set at five different velocities between 0–70 mm/s in the five glass capillaries. The NB on the center line of the tube was measured with a laser power of 2 mW.

Effect of blood flow behind measured capillary tube. As a model for choroidal vessels behind the retina and retinal pigment epithelium tested in the previous experiment, a glass tube with an internal diameter of 300 μm was placed just behind the film (background glass tube). Five glass tubes with internal diameters of 50, 93, 125, 150, and 300 μm, in which the blood velocity was set at five different velocities, as described above, were used as analogues for the retinal vessels. The blood velocity in the background glass tube was set at five different velocities

between 0–70 mm/s. The NB was measured with a laser power of 2 mW on the center line of the background glass tube 30 μm from the outer wall of the front glass capillary tube (Figure 2A) and on the center line of the glass capillary tube where both glass tubes were crossed (Figure 2B).

In Vivo Experiments

Blood velocity determination in human retinal vein. The present study was approved by the Ethics Committee of Tokyo University. Before admission into the study, the nature of the study was fully explained, routine eye examinations were administered, and written consent was obtained from each subject. Ten young volunteers (20–34 years old), who had neither systemic nor ocular disease and only mild refractive errors, participated in the study. The pupil was dilated with one drop of Mydrin M® (0.4% tropicamide; Santen Pharmaceutical, Osaka). Vessel diameters were then measured from a fundus photograph taken by the laser speckle system and were corrected using the axial length, the refraction, and the corneal curvature of the individual eye, according to Littman's formula.[12] The image speckles from a pixel located on the center line of a retinal vein distal to the second branching point (approximately 50 μm in vessel diameter) and those from a pixel located about 30 μm from the retinal vein on both sides (Figure 3) were recorded to measure the NB value (NB_{vein} and $NB_{background}$, respectively) with a laser power of 2 mW. $NB_{background}$ consists of NB attributable retinal and choroidal circulation. NB_{vein} and $NB_{background}$ were averaged over two pulses when the fixation was satisfactory and was defined as mean NB_{vein} and mean $NB_{background}$, respectively. The movement of the subject's eye in any direction during the measurement period was checked by the method described previously.[7] Eye movement was also checked by inspecting the color map and the time course plot of NB taken every 0.125 second. When there was no eye movement during the measurement, visible surface vessels did not change position on the color map and the time course plot of NB exhibited periodic fluctuations synchronous to cardiac pulse.[9] On the other hand, when eye movement occurred, visible surface vessels in the color map changed position. A nomogram to calculate the blood velocity in retinal veins from the NB_{vein}, $NB_{background}$, and vessel diameter was constructed based on the results of the in vitro experiments. Using this nomogram, the in vivo blood velocities of the retinal vein were calculated in 16 eyes of the 10 subjects.

Reproducibility of measurement. Blood velocity in the retinal veins was measured in 16 eyes of 10 subjects as described above, twice, with a 5-minute interval between measurements, during which the subjects

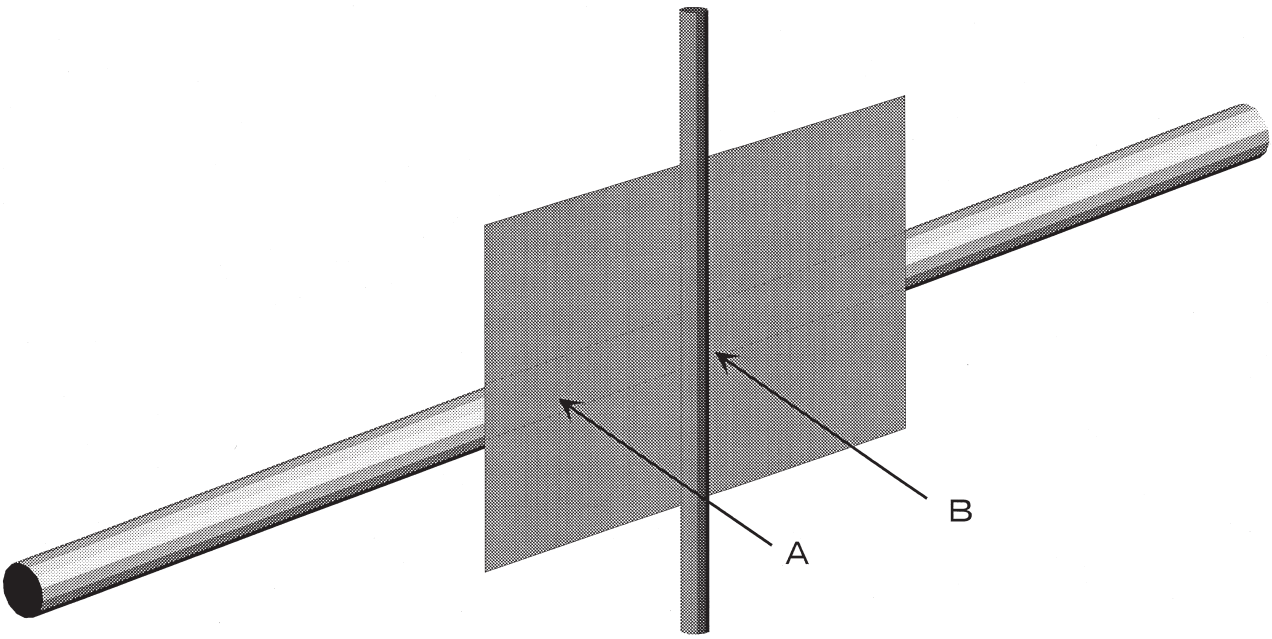

Figure 2. Measurement pixel in the in vitro experiments. (**A**) Background glass tube. (**B**) Glass capillary tube where background glass tube was crossed.

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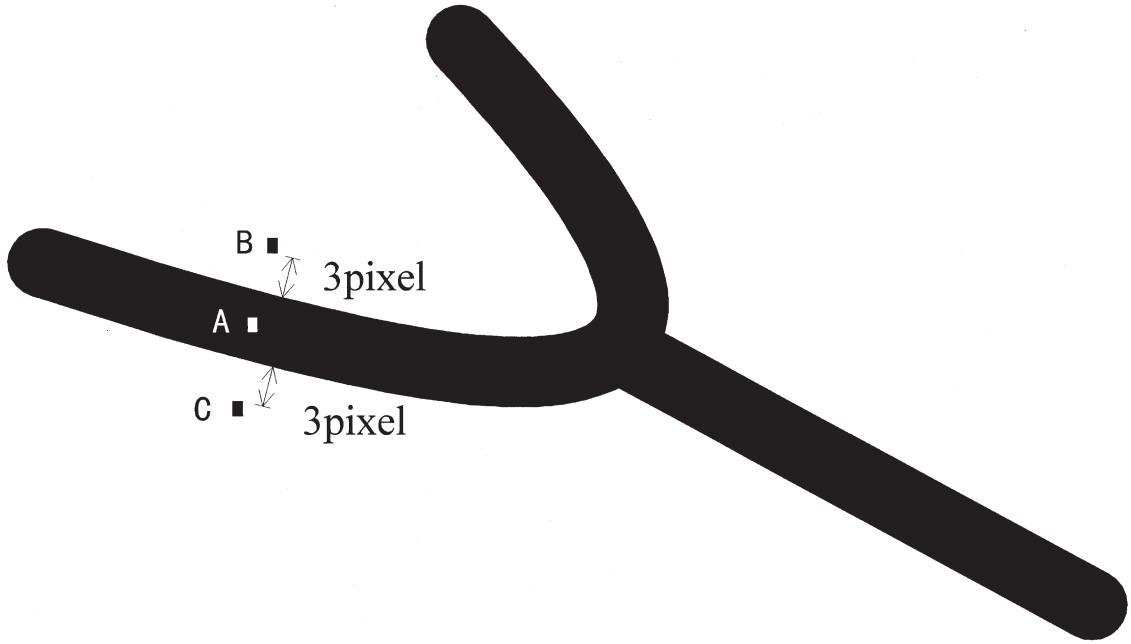

Figure 3. Measurement pixel in the in vivo experiments. (**A**) Retinal vein. (**B**,**C**) Field located 3 pixels apart from retinal vein on both sides (choroid).

rested. The coefficient of the reproducibility of measurements was determined as described previously[9] using the following equation:

$$\frac{V_1 + V_2}{(V_1 + V_2)/2} \quad (1)$$

where V_1 and V_2 represent blood velocity in the retinal vein during the first and second measurement.

Results

In Vitro Experiment Using Ground Glass

Figure 4 shows the relationship between the speed of rotation of the ground glass and NB_{av} for 100 × 100 pixels. NB_{av} had a significant linear correlation with the speed of rotation in the range of 5–200 mm/s ($r = 0.99$, $P = .00$). NB_{av} showed little change when a ground glass was rotated at a constant speed and laser power for measurements was varied from 1.5–4 mW (Figure 5).

In Vitro Experiments Using a Model of the Human Eye

Effect of position of a measurement point. Figure 6 shows the relationship between the location of the pixel and its distance from the centerline of the capillary tube. The NB value given by the pixel located on the centerline of the capillary tube was highest. The NB gradually decreased as the distance between the pixel and the centerline increased.

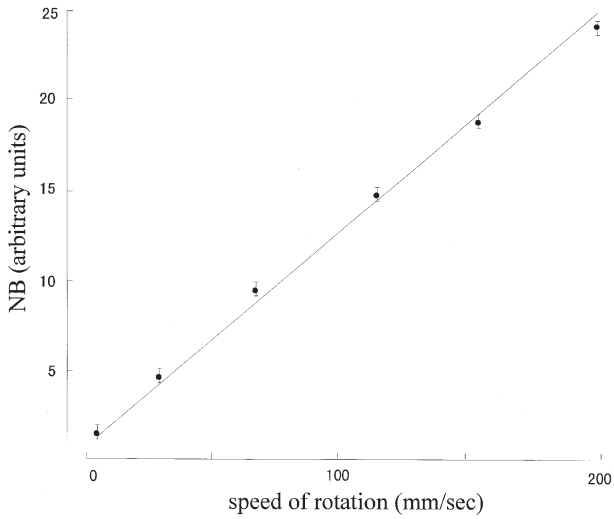

Figure 4. Speed of rotation of ground glass and average of normalized blur (NB) values over whole measurement field. Each plot represents average of NB with SD (error bars) in three measurements.

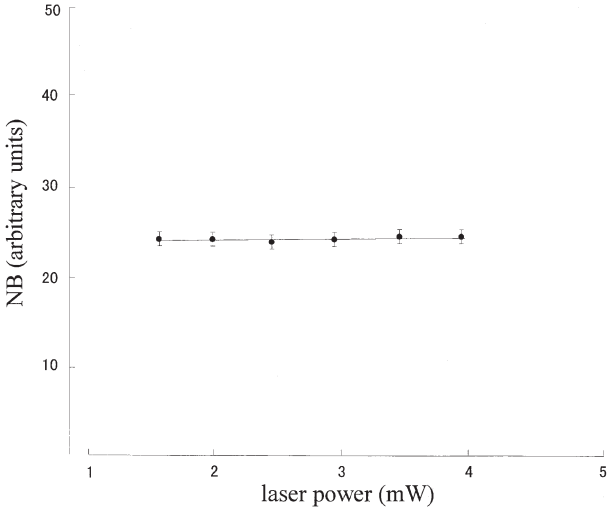

Figure 5. Laser power for measurements and average of normalized blur (NB) values over whole measurement field in the in vitro experiment using ground glass. Each plot represents average of NB with SD (error bars) in three measurements.

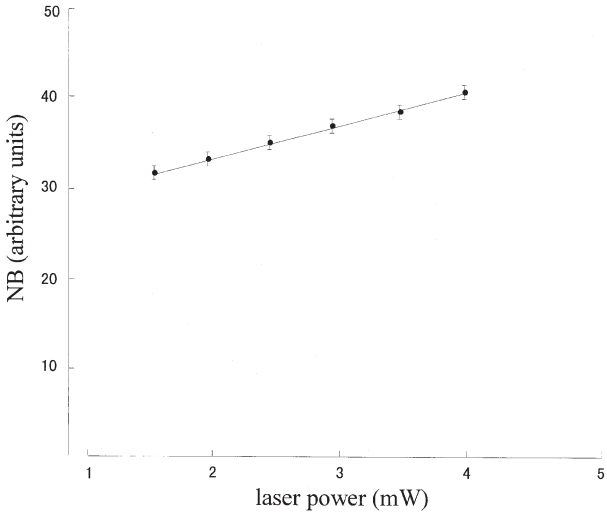

Figure 7. Laser power for measurements and normalized blur (NB) in the in vitro experiment using a human eye model. Each plot represents average of NB with SD (error bars) in three measurements.

Effect of laser power. Figure 7 shows the relationship between the laser power and NB value on the centerline of the capillary tube when the blood velocity was 10 mm/s. The NB increased by 12% with each 1 mW increase in laser power, within the range of 1.5–4 mW.

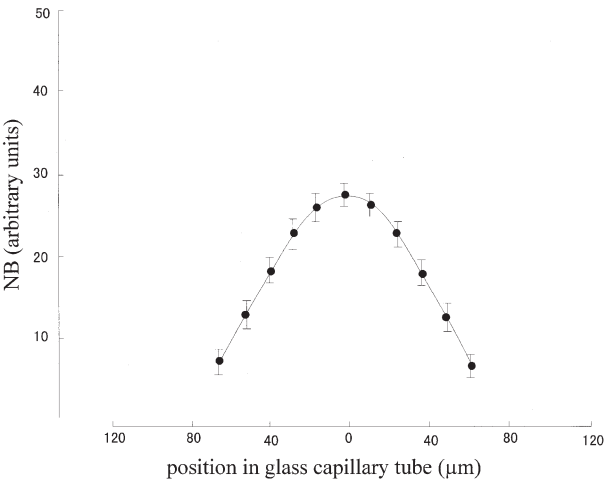

Figure 6. Position on glass capillary tube where measurement field was located and normalized blur (NB). Each plot represents average of NB with SD (error bars) in three measurements.

Effect of hematocrit. The variation in hematocrit in the range presently examined induced little change in the NB value in the center of the glass capillary tube.

Effect of background reflectance on NB. Figure 8 shows the effect of background reflectance on the NB value obtained on the centerline of the glass capillary tube. When the background reflectance was less than 6.6%, the NB increased along with the increase in background reflectance. There was little effect on NB, however, when the background reflectance was over 6.6%.

Effect of internal diameter and blood velocity on the measured NB. When the blood velocity in the glass tube was 0 mm/s, the NB was not equal to 0. This was probably due to the random movement of blood cells in the glass tube when the peristaltic pump was switched off. The NB value obtained when the peristaltic pump was switched off was subtracted from the measured NB value as a blank in the following experiments.

There was a strong correlation between NB and blood velocity in the glass tube. Given a constant blood velocity, the NB increased with the increased internal diameter of the glass tube (Figure 9). In a glass tube with an internal diameter of 50 μm, NB had a linear relationship with blood velocity at levels below 33 mm/s. Above 33 mm/s, NB had a quadratic

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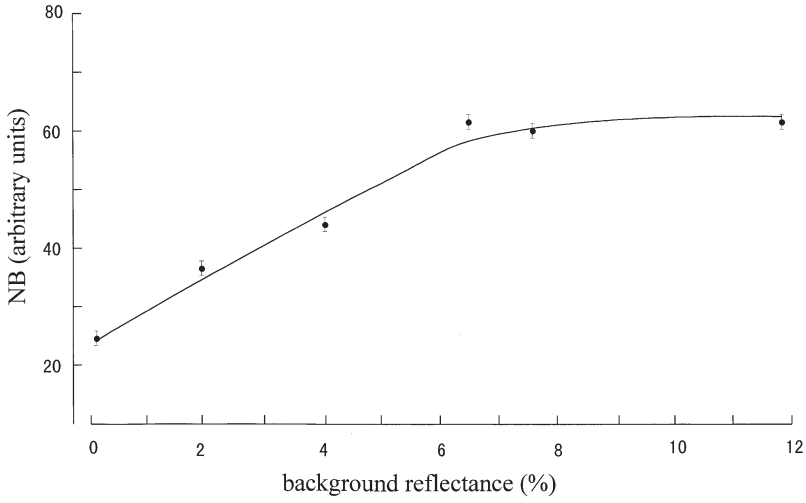

Figure 8. Background reflectance and normalized blur (NB) in the in vitro experiment using the human eye model. Each plot represents average of NB with SD (error bars) in three measurements.

relationship with blood velocity. The linear dynamic range of NB measurements are between a blood velocity of 0–20 mm/s for the internal diameter of 93 μm, 0–10 mm/s for 150 μm, and 0–5 mm/s for 300 μm, etc.

Effect of blood flow behind the measured capillary tube. The NB with a background flow of 10 mm/s was higher than that without a background flow. The NB was more affected by background flow when the internal diameter of the glass capillary tube was smaller (Figure 10). A nomogram was produced that illustrates the relationship between the NB obtained from a glass capillary tube as analogue of a retinal vessel, its internal diameter, actual blood velocity, and the NB obtained from the background glass capillary tube. Figure 11 shows an example of the nomogram for a glass capillary tube with an internal diameter of 50 μm.

Blood Velocity Determination in Human Retinal Vein and Its Reproducibility

The average diameter of the retinal veins was 53 ± 6 μm (mean ± SD, n = 16). The nomogram obtained from a glass tube with an internal diameter of

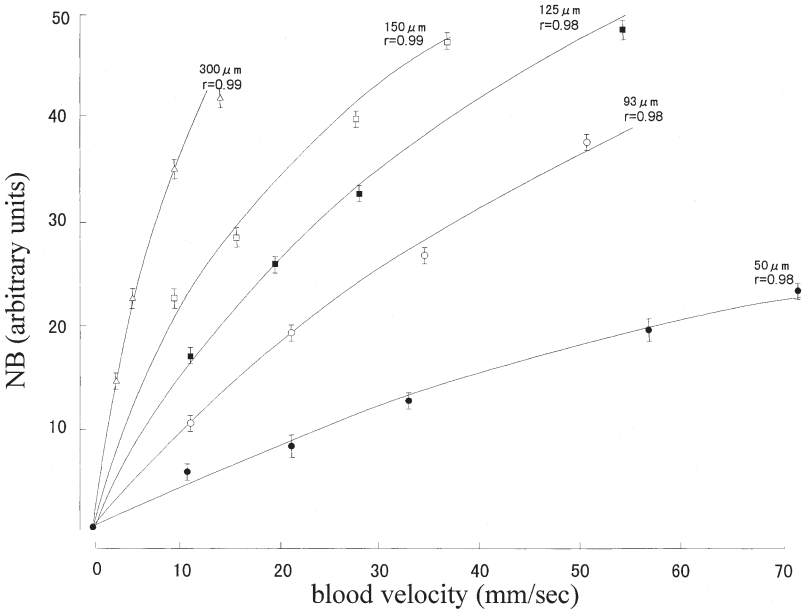

Figure 9. Blood velocity in glass capillary tubes of various internal diameters and normalized blur (NB) without background flow. Each plot represents average of NB with SD (error bars) in three measurements.

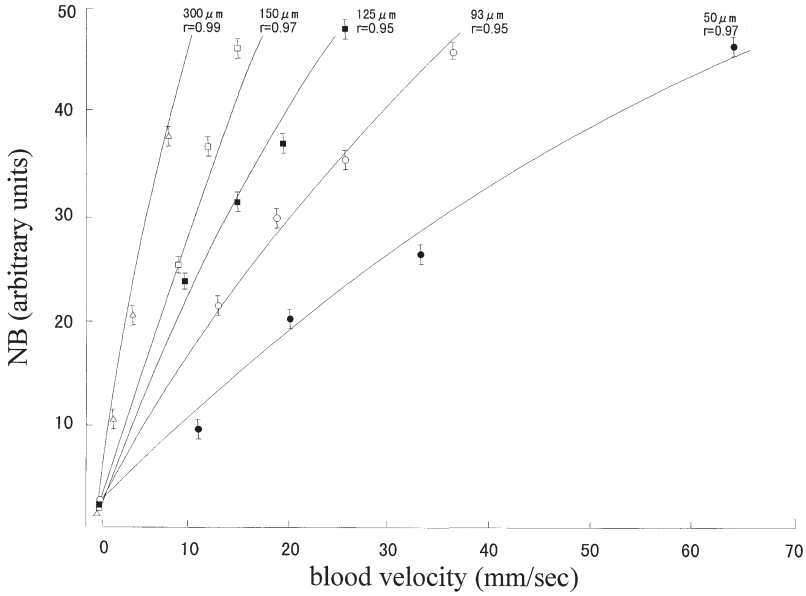

Figure 10. Blood velocity in glass capillary tubes of various internal diameters and normalized blur (NB) with blood velocity of background glass tube of 10 mm/s. Each plot represents average of NB with SD (error bars) in three measurements.

50 μm was used for all subjects. Both NB_{vein} and $NB_{background}$ exhibited periodic fluctuations synchronous to cardiac pulse, but the blood velocity in the retinal vein, calculated using the nomogram, showed little fluctuation (Figure 12). Average velocity in retinal veins determined by mean NB_{vein} and mean $NB_{background}$ was 11.1 ± 0.6 mm/s (10.4–11.9 mm/s).

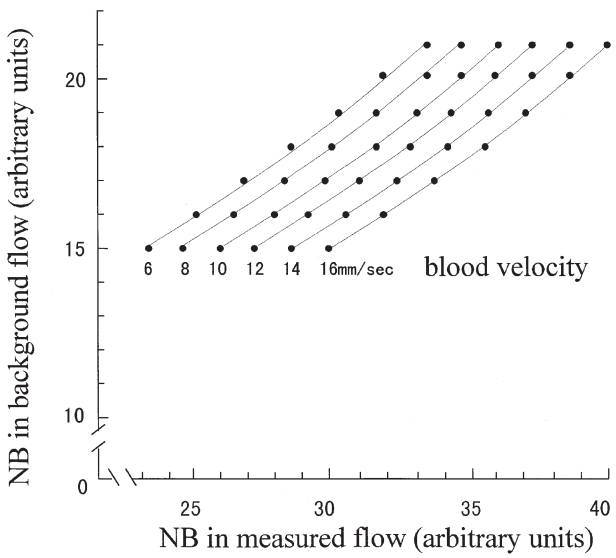

Figure 11. Nomogram that calculates blood velocity by normalized blur in both measured and background glass tube with internal diameter of 50 μm.

The coefficient of reproducibility of the two measurements of blood velocity in the retinal vein was 2.5 ± 0.9%. It took 63 ± 15 seconds to complete one measurement of blood velocity.

Discussion

The in vitro experiment using the ground glass revealed a linear correlation between the velocity of rotation and NB_{av} for 100 × 100 pixels between 5 and 200 mm/s, indicating that the NB currently used as an index of blurring of a speckle pattern parallels the velocity of the diffusing substance. Further, NB_{av} showed little change when the ground glass, the scattering particles of which existed only on its surface, was rotated at a constant speed and laser power for measurements was varied from 1.5–4 mW.

The in vitro experiment using a model of the human eye indicated that the NB value increased as the position of the measurement pixel changed from the periphery to the center of the capillary tube and NB also increased with increasing laser power. The effective penetration depth of the laser in the sampled blood was thought to increase as the laser power increased, which resulted in an increase of the sampling volume and the number of moving blood cells contributing to the speckle pattern formation. This is probably the reason why the NB increased with increasing laser power, because NB obtained from a ground glass showed little change when laser power was changed.

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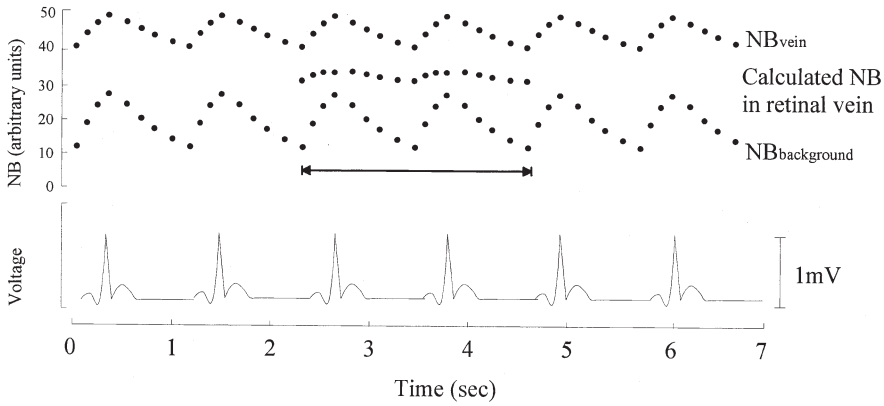

Figure 12. Representative normalized blur (NB) pattern in NB_{vein}, $NB_{background}$, and blood velocity in retinal veins calculated using nomogram.

The sampling volume also increased as the position of the measurement pixel was changed from the periphery to the centerline of the capillary tube. This might partly explain why the NB increased as the position of the measurement pixel was changed from the periphery to the centerline of the capillary tube. Further, this result might be partly attributed to the fact that fluid in the tube generally flows faster in the center than in the periphery.[13] Thus, the measurement point was always placed on the centerline of the glass capillary tube and the laser power was fixed at 2 mW in later experiments.

The variation in hematocrit of the blood sample induced little change in NB value. When hematocrit is lower, the effective penetration depth of the laser would probably be deeper; this mechanism is thought to result in little change in the number of moving blood cells in the sampling volume. Variation in hematocrit values within the physiologic range did not seem to be a factor that effects the NB value. The possibility that the hematocrit value affects the NB when it is much lower than physiologic value, however, cannot be excluded.

According to Van Norren and Tiemeijer,[14] the reflectance of human retinal tissue in response to a laser with a 711-nm wavelength is 7.73%. When the background reflectance was over 6.6%, there was little effect on NB in the present study. Thus, the reflectance of the film behind the glass tube was fixed at 8% in the later in vitro experiments, and the in vivo blood velocity in retinal veins was also calculated based on the in vitro results obtained with the background reflectance of 8%. There was a good correlation between NB and blood velocity in a glass capillary tube and, with a constant blood velocity, NB increased with the increasing internal diameter of the glass capillary tube. The NB-increasing effects of increasing internal diameter is thought to be partly attributable to the increase in the sampling volume. Further, the NB with a background flow was higher than that without a background flow, and more affected by background flow in a glass capillary tube with a smaller internal diameter. This result probably indicates that the diode laser penetrated beyond the glass capillary tube and that the laser was also scattered by the background flow, which contributed to the NB. Further, the present result indicates that the contribution of both choroidal and deep layer retinal circulation to NB should be taken into account when calculating the in vivo blood velocity in the retinal vein. Thus, the nomogram that illustrates the relationship between the NB obtained from the surface glass capillary tube measured (an analogue of the retinal vein), its internal diameter, the actual blood velocity and the NB obtained from background glass tube (an analogue of choroidal circulation). The nomogram was constructed for each internal diameter of the surface glass capillary tube and used to evaluate in vivo blood velocity.

In the in vivo experiments, both NB_{vein} and $NB_{background}$ exhibited periodic fluctuations synchronous with the cardiac pulse. Blood velocity in the retinal vein calculated using the nomogram, however, had no periodic fluctuations, which suggests that periodic fluctuations in NB_{vein} were not due to periodic fluctuations in blood velocity, but rather to the superimposition of the fluctuating $NB_{background}$ on the periodic fluctuations in NB_{vein}. As for the blood flow in the retinal vein, pulsatile velocity was rarely observed with laser Doppler velocimetry,[5] which is consistent with the present result.

In the present subjects, the blood velocity obtained in retinal veins with the diameter of about 50 μm averaged 11.1 mm/s. The present result is consistent with the findings of a previous study in which laser Doppler velocimetry was used, indicating that the blood velocity in human retinal veins with diameters of 64–177 μm was 8–26 mm/s.[5]

In the present study, it took 1 minute for the entire measurement and the minimum time required for a good fixation was approximately 2 seconds, covering at least two cardiac pulses. This property allows for much easier measurement and is much less burdensome for the subjects, compared to previous laser methods that are not equipped with a tracking system. In the current study, the coefficient of reproducibility of 2-minute interval measurements of NB was 2.5%. The coefficient of reproducibility is considered to be an index not only of the error of measurement but also of the physiologic fluctuations in retinal blood velocity. The current result indicates that the measured NB value is stable and suggests that there is great potential for the present method in studying the time course of change in the retinal blood velocity under various conditions.

Finally, the potential hazards to human eyes of measurements with the apparatus must be discussed. The maximum permissible exposure of the retina for viewing a diffuse reflection of a diode laser is 460 mW/cm^2 for 10-second exposures.[15] The maximum exposure of the retina with the present apparatus in the current study was about 90 mW/cm^2 and the exposure time was 10 seconds, which are well below the permissible limits.

In summary, the present results indicate that the laser speckle method can evaluate the blood velocity in a simulated human retinal vein with reasonable accuracy and reproducibility, and might be useful for studying the effects of various factors on human retinal blood flow in vivo.

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