Observation of Choroidal Circulation Using Index of Erythrocytic Velocity

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Objective: To describe a noninvasive method to visualize choroidal circulation by means of erythrocytic velocity.

Methods: Laser speckle flowgraphy (LSFG) and indocyanine green (ICG) angiography were performed on 9 volunteers. The LSFG measures the quantitative relative velocity index of erythrocytes (normalized blur [NB] value) in retinal and choroidal vessels. We averaged NB values from 3 pulsations and made composite 1.5-mm-square NB maps during 1 pulsation. By overlapping 5 adjacent maps, we created a panoramic 3.0-mm-square NB map of the posterior pole. The vascular patterns of the panoramic map and ICG angiography were compared. To determine the influence of retinal vessels, we induced branch retinal artery occlusion in 2 monkey eyes and compared the panoramic maps before and after occlusion.

Results: The NB map showed pulsatile blood flow in choroidal and retinal vessels. Vascular pattern contrast was improved in the NB map. Choroidal vessels in ICG angiography corresponded to those in the NB map. Vascular patterns in the map changed little before and after branch retinal artery occlusion.

Conclusions: The LSFG noninvasively visualized the hemodynamics of choroidal circulation, and the vascular pattern, which is mainly choroidal in origin, was comparable with that of ICG angiography.

Clinical Relevance: The LSFG may be used to evaluate choroidal hemodynamics in various choroidal diseases.

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Retinal vascular diseases have been studied by fluorescein angiography and recently by indocyanine green (ICG) angiography. Fluorescein angiography demonstrates the dynamics of plasma flow when free fluorescein is dissolved and leaks through the choriocapillaris. Indocyanine green angiography allows observation of choroidal vessels because excitation of light and fluorescence are within the infrared wavelength. In addition, ICG angiography demonstrates the dynamics of ICG-bound plasma albumin, which does not penetrate the choriocapillaris. Recently, the important role of the choroidal circulation has been recognized in various fundus diseases, such as central serous chorioretinopathy, hypertensive choroidopathy, and polypoidal choroidal vasculopathy. Indocyanine green angiography and fluorescein angiography demonstrate the plasma flow in the choroid; however, the dynamic blood flow is observed only during the early phase of the angiograms.

An apparatus based on the laser speckle phenomenon, which we call laser speckle flowgraphy (LSFG), targets moving erythrocytes in the eye. This apparatus has been used to measure the blood flow velocity in the retina, optic nerve head, choroid, and iris with the diode laser, mainly with wavelengths of 808 or 830 nm. When applied to the ocular fundus, LSFG shows the retinal and choroidal components. The manner in which the hemodynamics of the retina and the choroid contribute to the image of LSFG has not been reported.

The prototype of the LSFG measured an area of the ocular fundus that was approximately 1.0 mm square, which was too small and too low in contrast to demonstrate the complex pattern of the choroidal vessels. In the current study, we enlarged the area of observation to 1.5 mm square and increased the contrast of the LSFG images. In addition, we compared a panoramic 3.0-mm-square image with an ICG angiogram. To evaluate the influence of the retinal circulation in LSFG, we...
induced branch retinal artery occlusion (BRAO) in monkey eyes.

METHODS

LASER SPECKLE METHOD AND THE NORMALIZED BLUR

Our LSFG is the next generation of the previously reported instrument.\textsuperscript{10,13,17} A diode laser (wavelength, 830 nm) illuminates the ocular fundus. The reflected lights from the ocular tissue produce the speckle pattern at the plane where the area sensor is focused (Figure 1). Reflected lights from moving erythrocytes induce blurring in the speckle pattern. The relative velocity of the erythrocytes is calculated from variations in the blurring, which we call the normalized blur (NB) value.\textsuperscript{11,12} The area sensor with 100 × 100 pixels detects the NB value in a 1.5-mm-square area of the fundus. The NB value is calculated 16 times in 1 second and displayed as an NB map in either a false color-scale image or a gray-scale image. The mean NB value of the NB map shows the pulsatile fluctuation synchronized with the cardiac rhythm.\textsuperscript{13} We examined a 1.5-mm-square area for about 4.5 seconds. We overlapped a series of NB maps during 3 cardiac rhythms and averaged them in 1 fictitious cardiac rhythm, which we called the composite NB map. We made the panoramic composite map 3 mm square by combining 5 adjacent composite maps.

NORMAL HUMAN EYE

Nine eyes of 9 healthy volunteers underwent color fundus photography, ICG angiography, and LSFG after the pupil was dilated with 2 drops of 0.5% tropicamide and 0.5% phenylephrine hydrochloride (Mydrin-P; Santen Pharmaceutical Co, Osaka, Japan). The mean ± SD age of the volunteers was 37.8 ± 12.5 years (age range, 20–51 years). We compared the contrast of the image of the original NB map with that of the composite NB map. Panoramic NB maps were made from 5 adjacent composite maps around the fovea and the optic disc. The vascular patterns in the ICG angiogram were compared with those of the composite NB map and the panoramic NB map. This research was per-

![Figure 1. Schematic diagram of laser speckle flowgraphy.](image)

![Figure 2. A series of original normalized blur (NB) maps in case 1, a 39-year-old man (right eye, −1.5 diopters). The image of the NB map changed every one sixteenth of a second. B, Graph showing that the mean NB value of each original map fluctuates in synchronicity with the cardiac rhythm. Because the heart rate of the participant is 60 beats/min, 1 second (bar) corresponds to 1 pulsation, which includes 16 NB maps. The arrow indicates the 48 NB maps during 3 pulses selected for the composite NB maps in Figures 4 and 5.)](image)

![Figure 3. The normalized blur map during the diastolic phase in Figure 2 shown in false color scale (A) and gray scale (B). The scale bars indicate the normalized blur value. The contrast of the vascular image is diminished by noisy dots.](image)
formed in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants. The safety of the LSFG for retinal application was already confirmed.18

EXPERIMENTAL BRAO

Two adult Japanese monkeys, Macaca fuscata, weighing 5.5 and 6.7 kg, were used and treated in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. General anesthesia was induced by intravenous injection of pentobarbital sodium, 10 mg/kg. All experiments were performed in steady state, and the pupil was dilated with 2 drops of 0.5% tropicamide–0.5% phenylephrine hydrochloride. After experiments, the monkeys recovered from general anesthesia.

We induced BRAO in 1 eye of each monkey. The retinal artery was photocoagulated with intense argon laser (Visulas Argon; Carl Zeiss Inc, Jena, Germany) with a preset lens (SuperField NC; Volk Optical Inc, Mentor, Ohio). We applied the laser 3 to 10 times (power output, 150-250 mW; spot size, 100 µm; duration, 0.5 seconds) until the retinal artery was completely occluded. In one eye, the first branch of the central retinal artery was occluded in the optic nerve head, and in the other eye, the second branch was occluded 3 disc diameters from the disc margin.

Panoramic NB maps were examined before and after induction of BRAO. We performed fluorescein and ICG angiography immediately after all LSFG examinations.

RESULTS

NORMAL HUMAN EYE

The sequence of the NB maps showed pulsatile fluctuations in all 9 eyes (Figure 2A). The mean NB value in a 1.5-mm-square NB map that contained 100×100 pixels also demonstrated fluctuations synchronized to the cardiac rhythm (Figure 2B). The NB maps can be presented in a false color-scale image or in gray scale. Slight differences in the NB value were clearer in the false color scale than in gray scale. The original NB map had sandy, noisy dots that decreased the contrast (Figure 3). The composite map, in which 3 pulsations were averaged into 1 pulsation, had fewer noisy dots and higher contrast. Although the intensity of the composite map fluctuated with the cardiac rhythm, the vascular pattern remained unchanged (Figure 4). When a series of composite maps from one pulsation was averaged into one image, the vascular pattern had higher contrast with less noise (Figure 5).

A panoramic composite 3-mm-square map including the fovea and its surroundings demonstrated that the choroidal vessels corresponded to those of ICG angiography. The vascular pattern in the composite map was more comparable with that of ICG angiography in gray scale than in false color scale (Figure 6A and 6B). The intensity of the panoramic map was low during the diastolic phase and high during the systolic phase but showed little change in vascular pattern related to the cardiac rhythm (Figure 7). The ICG angiography showed a different vascular pattern in the early and late phases (Figure 6D and F). One second after the beginning of the choroidal flush, the choroidal arteries were mainly perfused. The choroidal vascular pattern in early-phase ICG angiography was similar to that of the composite map (Figure 6B). In the late phase of ICG angiography 40 seconds after the choroidal flush, the choroidal veins became evident, but the arteries were hard to detect (Figure 6F). The panoramic composite NB map contained the choroidal vascular pattern during the arterial and venous phases in ICG angiography. The ICG angiography did not show the avascular fovea, because the retinal capillary was not
demonstrated. The composite map also failed to show the avascular fovea.

The panoramic composite map in the area of the optic disc showed vascular patterns similar to the retinal arteries and veins in ICG angiography (Figure 6E and F). The peripapillary choroidal vessels in the composite map were the sum of the vascular images during the early and late phases of ICG angiography.

EXPERIMENTAL BRAO

Fluorescein angiography showed complete occlusion of the temporal retinal artery (monkey 1) and a branch of the temporal artery (monkey 2) at the photocoagulation site. Retrograde dye filling was seen in these non-perfused arteries from the surrounding capillaries (Figure 8A and B). Indocyanine green angiography showed intact choroidal vessels in the area of BRAO (Figure 8C), except for a zonal area of delayed dye filling peripheral to the laser spot in monkey 2.

Because of the short time after the induction of BRAO, the retina was still transparent at the time of LSFG. The panoramic composite NB maps were made before and after induction of BRAO in the areas of ICG angiography within a dotted black line. The panoramic composite map before induction of BRAO demonstrated the retinal arteries, veins, and choroidal vessels. After photocoagulation, the image of the occluded retinal artery disappeared (Figure 9). The vascular pattern of the choroid changed little after induction of BRAO. The mean

Figure 6. Case 1. Panoramic composite normalized blur maps of the fovea and the optic disc. A, Color scale. B, Gray scale; the choroidal vascular image is clearer than in color scale. C, Color fundus photograph. D and E, Early-phase indocyanine green angiograms. F, Late-phase indocyanine green angiogram. Arrows and arrowheads in all parts indicate choroidal arteries and veins, respectively. They correspond to the choroidal vascular images in A and B. The time in each angiogram indicates the time from the choroidal flush.
NB value in the rectangle (Figure 9) within the area of BRAO decreased from 5.8 to 5.3 (91%) in monkey 1 and from 6.1 to 5.7 (93%) in monkey 2.

**COMMENT**

The use of LSFG demonstrated the hemodynamics of the choroidal vessels synchronized with the cardiac rhythm (Figure 2). When the original NB maps of 3 pulsations were superimposed and averaged into 1 pulsation, the composite map had higher contrast with less noise (Figure 4). We produced a panoramic composite 3-mm-square map by combining the adjacent 1.5-mm-square composite maps. The vascular pattern of the panoramic map was comparable with that of ICG angiography. The panoramic composite map showed the choroidal arteries and veins seen in ICG angiography.

The similarity in the vascular patterns between LSFG and ICG angiography may be the result of the similar wavelength of measurement light. The LSFG uses the diode laser at 830 nm for the measurement beam. In ICG angiography, the exciter light is approximately 780 nm, and the emission of ICG dye is around 830 nm. The composite NB map as well as ICG angiography did not demonstrate the retinal circulation at the level of the capillaries. The ICG angiography showed a veil-like hyperfluorescence that reflects the circulation of the choriocapillaris. We could not selectively differentiate the image of the choriocapillaris from the composite NB map, which involves the blood flow of the choriocapillaris and the underlying choroidal arteries and veins.

Indocyanine green angiography shows the sequence of dye filling in the choroidal arteries, capillaries, and veins. The angiogram changes from the early to the late phase. These changes were transient and not repeatable. However, LSFG represents the velocity of the erythrocytes. Thus, the composite NB map includes the choroidal arteries and the veins. The dynamic changes in the choroidal vessels are repeatable and synchronized to the cardiac rhythm.

In experimental BRAO, the choroidal vascular pattern was unchanged after induction of BRAO, which sug-
gests that the vascular pattern seen in the panoramic composite NB map is mainly choroidal in origin. The NB value is the sum of the choroidal and retinal circulation. It decreased to an average of 92% in the area of BRAO. Theoretically, it is hard to determine the exact retinal component in the composite map. First, slight retrograde circulation remained in the area of BRAO. Second, loss of retinal circulation may exaggerate the choroidal circulation component, because more of the measurement beam may penetrate the choroid in the area of BRAO.

The ratio of the retinal to the choroidal circulation has been obtained only by in vitro experiments. With the use of the labeled microspheres method,19 the blood flow rates in the monkey retina and the choroid were, respectively, 0.28 mg·min⁻¹·mm⁻² and 6.49 mg·min⁻¹·mm⁻² in the foveal region and 0.08 mg·min⁻¹·mm⁻² and 2.38 mg·min⁻¹·mm⁻² in the midperiphery. In other words, the blood flow in the choroid greatly exceeded the retinal flow by approximately 96% in each area. Although LSFG reflects the erythrocytic velocity, the 92% choroidal component in the composite map was similar to the 96% obtained from the microspheres method.

We reported images of the choroidal vessels by using the prototype of the LSFG.17 The technique targeted a 1.0-mm-square area of the fundus. Because it had no monitor system, which detects the precise location of the NB map, it was difficult to produce an accurate panoramic map. Furthermore, the image in the prototype system was the original NB map rather than a composite map, it had less contrast, and it was noisier.

Fercher and Brière20,21 presented photographs of the velocity distribution of the erythrocytes in the human retina by means of laser speckle photography. Because those investigators used a helium-neon laser with 630 nm as a probe, the beam did not penetrate the choroid, and they could not demonstrate the hemodynamics of the choroid.

Noninvasive laser Doppler flowmetry targets the movement of blood cells and provides a relative index of choroidal blood flow.22 Laser Doppler flowmetry uses a diode laser of 811 nm or 670 nm23 as a probing laser and a fixation light. During examination, patients gaze at the fixation light focused on the avascular fovea. Because the diameter of the probing laser is 200 µm within the avascular fovea, laser Doppler flowmetry reflects the choroidal blood flow (ie, choriocapillary flow). Thus, the laser Doppler flowmetry is not applied to the flowgraphy in the extrafoveal area in humans.

Scanning laser Doppler flowmetry provides the blood flow, the volume, and the velocity of the retinal vessels at the level of the retinal capillaries.24 The instrument selectively demonstrates retinal circulation maps by means of a measurement beam with a 670-nm wavelength and a confocal system.

To investigate the ocular circulation, the laser speckle method has been used as the flowmeter that detects the mean NB value. The flowmeter is not designed to show the vascular pattern of a wide area. We proposed that flowgraphy be used as a tool to investigate the choroidal circulation. Laser speckle flowgraphy noninvasively visualized the hemodynamics of the choroidal circulation, the vascular pattern of which is comparable with that of ICG.
angiography. Laser speckle flowgraphy may be used to evaluate the choroidal hemodynamics in various choroidal conditions.

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