

COMPACT LASER DOPPLER CHOROIDAL FLOWMETER

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ABSTRACT

A compact instrument is described that allows the measurement of the laser Doppler flow parameters, i.e., the velocity, the volume, and flow of blood in the foveal region of the human choroidal vascular system. This new device uses the optical principle of confocality for the delivery of the laser light to the site of measurement and heterodyne detection of the Doppler frequency shifted scattered light. Power of the incident light (785 nm) at the cornea is 90 μ W. Measurements were obtained in both eyes of a group of 21 normal volunteers without pupil dilatation. We determined the intrasubject reproducibility and the minimum statistically significant detectable changes in the flow parameters for a group of 21 eyes (one in each subject). Linear correlations were also established between the flow parameters in the right and left eyes. © 1999 Society of Photo-Optical Instrumentation Engineers. [S1083-3668(99)00704-2]

Keywords laser Doppler flowmetry; heterodyne detection; blood flow; choroid; laser in medicine.

1 INTRODUCTION

Laser Doppler flowmetry (LDF) is a noninvasive technique which allows the measurement of the flux of moving red blood cells (RBCs) in the microvasculature of a tissue, such as the skin, the brain, and others.¹ The application of LDF to the human eye includes the optic nerve head,² the retinal microcirculation,³ and more recently, the choriocapillaris in the foveal region of the choroid.⁴ The measurement of choroidal blood flow (ChBF) may become important in the investigation of the pathogenesis of age-related macular degenerations.^{5,6}

Previous LDF measurements of ChBF in the human eye have been performed using laser delivery and photodetection systems that were adapted to a standard fundus camera.⁴ A laser beam in the near-infrared (811 nm) was delivered through the fundus illumination optical system of the camera. The light scattered by the RBCs in the tissue directly illuminated by the incident laser beam was detected in the retinal image plane of the fundus camera by an optical fiber and guided to an avalanche photodiode. The subject was asked to look directly at the beam, which was defocused to illuminate an area of the fundus of about 300 μ m in diameter.

The instrument was presented at the Association for Research in Vision and Ophthalmology (ARVO) Meeting, Fort Lauderdale, Florida, May 10–15, 1998, abstract (4598-B349).

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The operator observed on the monitor of a charge coupled device camera system the illuminated area of the fundus and placed the aperture of the detecting optical fiber on top of the image of the area of the choroid illuminated by the incident laser.

The experience gained from using this system in volunteers revealed a number of shortcomings that needed to be overcome to facilitate the clinical application of the method. One was the bulkiness of the instrument, requiring the patient to be tested at a fixed location. The second resulted from potential errors in the placement of the aperture of the optical fiber on top of the illuminated region and variations of this placement in consecutive measurements. Third, the lack of spatial separation between the illuminated area and the area from which the scattered light is detected made the measurements sensitive to unwanted specular reflection of the incident light at the fundus tissue, and also rendered difficult their interpretation in terms of flow, when the latter is derived using Bonner and Nossal's theory of LDF.⁷

The purpose of this paper is to describe a new and improved device to measure ChBF in the foveal region of the fundus of the human eye and to discuss results obtained in volunteers. To facilitate understanding of the principle of the ChBF measuring method, we first provide a brief description of LDF.

2 LASER DOPPLER FLOWMETRY

The LDF technique is based on the Doppler effect: laser light scattered by moving particles, such as

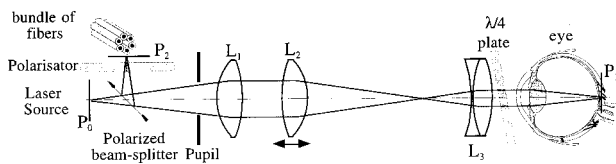


Fig. 1 The optical system which focuses the pinhole P_0 at P_1 in the plane of the retina by adjustment of lens L_2 has a magnification of one.

RBCs, is shifted in frequency by amounts proportional to the speeds of the particles. This scattered light is optically mixed with the light reflected by the nonmoving tissue and detected by a photodetector. The current at the exit of the detector is analyzed according to the scheme developed by Bonner and Nossal⁷ to obtain the Doppler shift power spectrum (DSPS). When the laser beam is focused at the choroidal microvasculature behind the fovea, the following choroidal blood flow parameters are derived from the DSPS:

1. the number of RBCs in the sampled blood volume, defined as

$$\text{ChBVol} = \frac{1}{A_{dc}^2} \int P(f) df, \quad (1)$$

where $P(f)df$ is the spectral power between frequency shifts f and $f + df$ and A_{dc}^2 the dc value of the photocurrent signal, which is proportional to the intensity of the detected light;

2. the mean velocity of RBCs, which corresponds to the first moment of the DSPS

$$\text{ChBVel} = \frac{\int fP(f) df}{\int P(f) df}; \quad (2)$$

3. the flux of the RBCs, defined as

$$\text{ChBF} = \text{ChBVel} \cdot \text{ChBVol}. \quad (3)$$

ChBVel is expressed in kHz and ChBVol and ChBF in arbitrary units. Since the backscattered light is strongly dependent on the optical properties of the examined tissue, and these can differ markedly between individuals, direct comparison between the flow values from different subjects is in general of limited value. However, comparison of changes in the flow parameters in a given subject is of great interest from a physiological and pathological point of view and valid under the assumption that the properties of the tissue at the site of LDF measurements have not changed between measurements.

3 THE CHOROIDAL BLOOD FLOWMETER

3.1 OPTICAL SYSTEM

The optical system for the delivery of the laser beam and the detection of the scattered light is based on a confocal arrangement (Figure 1). The

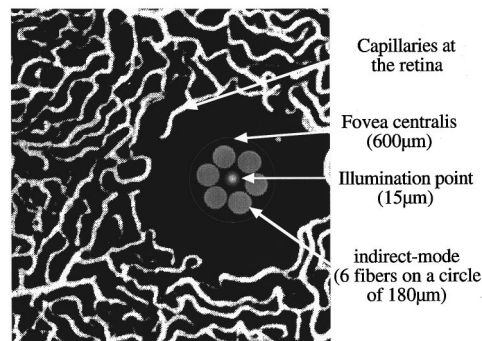


Fig. 2 Cast of the monkey macular region of the retina to illustrate the location of the laser illumination and the optical fibers detecting the scattered light in the avascular zone. The image of the input apertures of the six collecting fibers do not completely fill the fovea centralis, the region exempt of retinal capillaries. Numbers in parenthesis indicate diameters. [Adapted from K. Shimizu and U. Kazuyoshi, *Structure of the Ocular Vessels*, Igaku-Shoin, Tokyo, New York (1978).]

polarized laser source with a diameter of $100 \mu\text{m}$ at $\lambda = 785 \text{ nm}$ in P_0 is relayed with a 1:1 optical system consisting of two lenses (L_1, L_2), each of 80 mm focal length and then focused at the subject's retina by a lens L_3 (focal length=17 mm). The point source in retinal plane P_0 , the illuminated area at the foveola in retinal plane P_1 and the aperture of the optical fibers which detect the scattered light in plane P_2 are located in conjugated planes. In order to increase the signal to noise ratio, a $\lambda/4$ plate was placed between L_3 and the eye of the subject so that the light backscattered by the RBCs in the choriocapillaris returns to the instrument with a polarization perpendicular to the illumination light. This optical scheme eliminates most of the stray light arising from multiple reflections at the various elements of the system. The laser beam has a diameter of 1.3 mm and an intensity of $90 \mu\text{W}$ at the cornea. Its calculated diameter at the fovea, assuming a Gaussian beam and no tissue scattering, is $12 \mu\text{m}$.

The incident light is scattered by the RBCs in the choriocapillaris layer and by the nonmoving surrounding tissue structures and some of this scattered light exits the pupil. After reflection at the polarized beam splitter, this light is collected by a bundle of six optical and guided to an avalanche photodiode. Each fiber has a core diameter of $110 \mu\text{m}$. The cladding was removed to pack the fibers close to each other. The fibers are arranged on a circle with a diameter of $180 \mu\text{m}$ which is imaged around the incident beam at the fovea (Figure 2). This detection mode represents the indirect mode of the confocal arrangement (also called Schlieren).

Subjects were asked to look directly at the laser beam and to focus it by moving lens L_2 (Figure 1) using an appropriate mechanical device. The Doppler signal at the output of the detector was also fed into a loudspeaker, the output of which could be heard by the subject and operator. Figure 3 is a pic-



Fig. 3 Photo of the compact confocal choroidal flowmeter.

ture of the device. The instrument alignment was checked by maximizing this audio signal.

3.2 SIGNAL ANALYSIS

The signal analysis has been previously described^{8,9} and only a short summary will be given here. The photocurrent is sampled 21 times per second. The sampled values are Fourier transformed in a range of frequencies from 0 to 10 kHz to obtain the DSPS (Figure 4). The measured flow parameters and the dc level are calculated in real time using software developed for a NeXT computer, displayed on the computer monitor and stored in a file. The involuntary movements of the eye induce spikes in the flow parameters, and particularly in ChBVol which have amplitudes much larger than those due to the movement of the RBCs. These spikes were removed manually by the operator before calculating the average of the flow parameters over a given recording

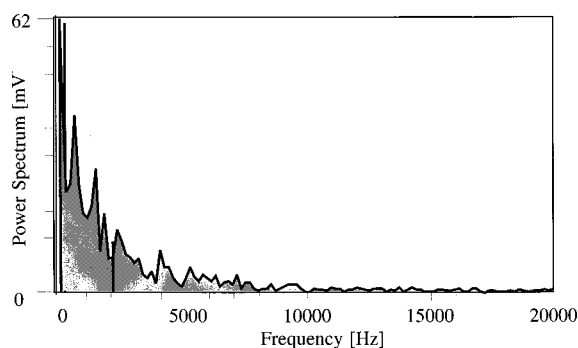


Fig. 4 Sample power spectrum of light scattered by RBCs in the choroid obtained from a volunteer. Determination of the flow parameters is based on the portion of the spectrum between 193 Hz and 10 kHz. The small vertical bar around 2000 Hz represents the velocity, determined from the model of Bonner and Nossal (Ref. 7).

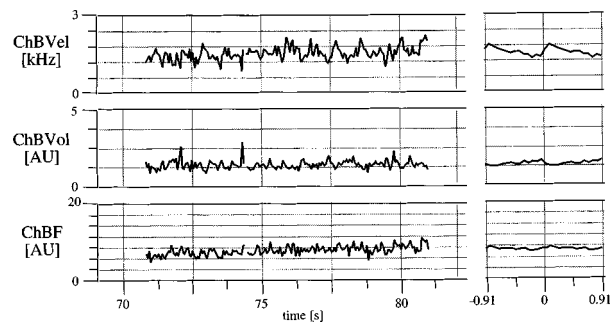


Fig. 5 Sample measurement of the flow parameters obtained from a normal volunteer. On the right are average changes during two heart cycles obtained from the 10 s recording (see text for averaging procedure). Phase zero corresponds to the ascending part of the pulse pressure wave (inflexion point).

time, as described elsewhere.¹⁰ Each flow parameter is measured every 46 ms for 10 s (Figure 5). Data points are then averaged in phase with the heart pulse, which is continuously recorded by an infrared earlobe transducer. All data points which have the same phase delay after the start of the pulse are averaged, and this procedure is repeated for all phase delays, producing an average wave form representative of the change of the flow parameter during the heart cycle (Figure 5). The pulsatility P of each parameter is then determined. For example, the pulsatility of ChBF is $P_{\text{ChBF}} = (1 - \text{ChBF}_{\text{diast}} / \text{ChBF}_{\text{sys}})$, where $\text{ChBF}_{\text{diast}}$ and ChBF_{sys} are the values of ChBF at end diastole and peak systole, respectively. The pulsilities P_{ChBVel} and P_{ChBVol} are defined in a similar way.

4 EXPERIMENTAL PART

Measurements were performed in both eyes of 21 healthy, nonsmoking volunteers having no ocular disease. Age ranged from 8 to 60 years (mean 30.7 ± 14.9 sd) and refractive error from -4 to 4 diopters. The procedures were approved by the Medical Faculty Ethical Committee of the University of Lausanne and conducted in accordance with the tenets of the Declaration of Helsinki. The following studies were performed.

4.1 LINEARITY

The linearity of the instrument response was assessed by using dilute (1:2) milk flowing in a glass capillary with a diameter of $200 \mu\text{m}$, which was placed in the focal plane of a model eye. Such a solution gives rise to DSPS similar to those obtained from the choroid. The mean speed of the milk was controlled by changing the height of the reservoir containing the solution.

4.2 INSTRUMENT COMPARISON

In order to compare this new device with the one previously described,⁴ which is based on a fundus camera, the changes in ChBF in response to a step

increase in intraocular pressure (IOP) were measured in two subjects with both instruments. For this, a suction cup was applied on the conjunctiva, near the limbus. A suction of 50 mmHg was applied to hold the cup and after checking the instrument alignment, ChBF was recorded for 30 s. Then the suction was increased to 200 mmHg and ChBF recorded for another 10 s. The experiment was done with the compact system first and the next day with the fundus camera based system. The procedure was repeated on a different day in both subjects and, instead of recording ChBF, the IOP was measured with a Langham pneumatometer.

4.3 BASELINE MEASUREMENT OF THE FLOW PARAMETERS

In one eye of each subject, whose eyes were not dilated, two measurements of the flow parameters, each of 10 s duration, were obtained within 1 min. Then the other eye was measured in the same way and the whole procedure was repeated 1/2 h later.

4.4 INTRASUBJECT REPRODUCIBILITY

This reproducibility was determined as the average over 21 subjects of the coefficient of variation of four 10 s measurements done in the same eye.

4.5 DETECTION SENSITIVITY (S) OF THE FLOW PARAMETERS

S represents the minimum statistically significant change in a flow parameter that can be detected with the method, based on measurements in one eye of *N* subjects. It is defined as

$$S = \frac{SD}{\bar{x}} \cdot \frac{k}{\sqrt{N}}, \quad (4)$$

where SD is the standard deviation of the difference between the first and the second measurement for all subjects, \bar{x} the mean value of all measurements, and *k* the two-tail value of the *t* distribution at 0.05 significance level for *N*−1. *S* was calculated based first on two measurements of 10 s duration which were performed within 1 min (*S*_{1 min}) and second, on two measurements done at a 30 min interval of time (*S*_{30 min}). *S* was calculated for the left eye (OS) and the right eye (OD).

4.6 CORRELATION BETWEEN THE FLOW PARAMETERS OBTAINED IN THE RIGHT AND LEFT EYES

For each flow parameter, the values from OD were correlated with those from OS and a linear Pearson regression was determined.

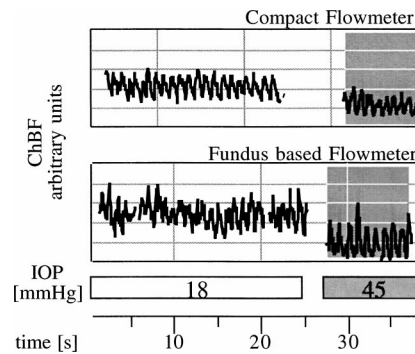


Fig. 6 ChBF change induced by an increase in IOP from 18 to 45 mmHg obtained in one subject with the compact flowmeter and with the fundus camera based system. The average decrease amounted to 43% with the new device and 41% with the fundus camera based system [Ref. 4].

5 RESULTS

5.1 LINEARITY

A linear fit of the ChBVel versus the height of the milk column gives a mean frequency shift (velocity) of 0.27 kHz when the milk is at rest. This low frequency contribution corresponds probably to the Brownian movement of the milk particles. Correlation coefficient of the linear regression is 0.983 (*p* < 0.01).

5.2 INSTRUMENT COMPARISON

The increase in suction cup pressure from 50 to 200 mmHg induced increases in IOP from 18 to 45 mmHg in one of the subjects and 21 to 43 mmHg in the other. In the first subject, these increases induced decreases in ChBF of 43% and 41%, with the compact and fundus camera based instruments, respectively (Figure 6). In the other subject, these decreases amounted to 20% and 26%, respectively.

5.3 BASELINE VALUES OF THE FLOW PARAMETERS

The baseline values (mean ±SD) of the parameters were 1.8±0.5 kHz (range 1.0–2.7) for ChBVel, 0.5 ±0.2 a.u. (range 0.2–0.9) for ChBVol, and 19 ±8 a.u. (range 7–48) for ChBF. The pulsilities *P*_{ChBVel}, *P*_{ChBVol} and *P*_{ChBF} were, respectively, 0.26 ±0.07 (range 0.09–0.53), 0.19±0.07 (range 0.07–0.40), and 0.27±0.09 (range 0.09–0.53).

5.4 INTRASUBJECT REPRODUCIBILITY

For ChBVel, ChBVol and ChBF these reproducibilities were 8.0%, 15.4%, and 17.2%, respectively, for OD and 6.7%, 15.1%, and 18.2%, respectively for OS. The average difference between both eyes was 2 ±21%, 10 ±42%, 15 ±56% for, respectively, ChBVel, ChBVol and ChBF. Based on these data, we calculated that the minimum percentage difference in the flow parameters between both eyes that would represent a statistically significant difference

Table 1 Sensitivity (S , in %) of the flow parameters. $S_{1 \text{ min}}$ is based on two measurements of 10 s each done within 1 min and $S_{30 \text{ min}}$ for two measurements done at an interval of 30 min. They are calculated for $p=0.05$ with $N=21$.

	Left eye		Right eye	
	$S_{1 \text{ min}}$	$S_{30 \text{ min}}$	$S_{1 \text{ min}}$	$S_{30 \text{ min}}$
ChBVel	3.2	4.8	3.9	5.0
ChBVol	7.1	10.2	7.3	8.2
ChBF	7.4	13.6	7.4	9.1

from normal at the 95% confidence level (2 SD) is 48% for ChBVel, compared to 59% for ChBVol and 84% for ChBF.

5.5 DETECTION SENSITIVITY OF THE HEMODYNAMIC PARAMETERS

Table 1 shows the sensitivities, $S_{1 \text{ min}}$ and $S_{30 \text{ min}}$, for the flow parameters based on two 10 s measurements performed within 1 and 30 min, respectively.

5.6 CORRELATION BETWEEN THE FLOW PARAMETERS OBTAINED IN OD AND OS

Statistically significant linear correlations between eyes were found for ChBVel ($r=0.54$, $p<0.05$, Figure 7) and ChBVol ($r=0.44$, $p<0.05$), but not for ChBF ($r=0.41$). Linear correlations for the average pulsatility were statistically significant ($p<0.05$) for P_{ChBVel} ($r=0.47$), P_{ChBVol} ($r=0.41$) and P_{ChBF} ($r=0.61$).

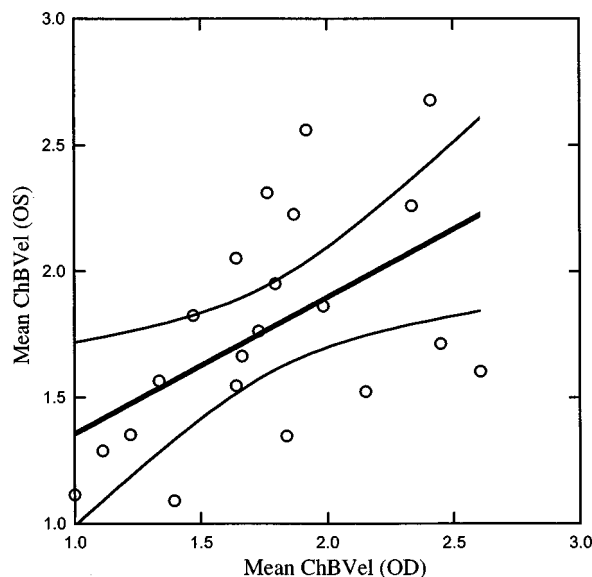


Fig. 7 Linear correlation of ChBVel between OS and OD ($r=0.54$, $p<0.05$).

6 DISCUSSION

In this paper, we have described a new laser Doppler flowmeter for the measurement of choroidal blood flow in the region behind the fovea. Compared to the device described previously,⁴ the present instrument is more compact, can be easily moved and provides ChBF with similar changes (see results, instrument comparison). By using the confocal principle for the laser illumination and light scattering detection optics, the detection fibers are always centered on the illuminated area. Thus, alignment of the detection becomes unnecessary. The only manipulation required for the operator is the centering of the input pupil of the device in the subject's pupil and this is done by adjusting the device to maximize the dc of the Doppler signal.

With the indirect detection of the scattered light, the specular reflection of the incident light at the inner limiting membrane is avoided. This scheme is therefore more in accordance with the geometry used in conventional LDF, as described by Bonner and Nossal,⁷ which assumes a separation between the area illuminated by the incident laser and the area from which the scattered light is detected. In spite of these improvements, however, the spread of the baseline flow parameters values (factor 3 between the smallest and the largest values for the ChBVel, and factor 7 for ChBF) is similar to that previously found with the fundus camera based system.⁴ This suggests that this spread is mostly due to interindividual characteristics, rather than to factors related to the scattering and detection schemes. To be considered among these factors are the fundus pigmentation, the refractive state of the eye, and possibly the age of the subject. Further work on a larger population of normal subjects is needed to determine their specific influence on the flow parameters.

Interestingly, although ChBVel shows a clear systolic/diastolic variation (Figure 5), ChBF hardly varies during the heart cycle. This is due to the fact that the change in ChBVol is 180° out of phase with ChBVel. This suggests that the increase in blood velocity is associated with a decrease in blood volume. This decrease in volume could be due to the known variation of the IOP during the heart cycle. Further studies should be undertaken to establish the relationship between the changes in the flow parameters during the heart cycle, the IOP and the systemic blood pressure pulse. Pulsatility measurements have the potential to provide important information on vascular alterations, as it appears to be the case for the retinal circulation during the progression of diabetic retinopathy.¹¹

The intrasubject reproducibility is three times better for ChBVel than for ChBF. This would suggest that ChBVel is a better parameter to assess changes in the ocular blood supply of the choroid in a particular subject. Furthermore, for the group, ChBVel also has a higher sensitivity. It is also the most sen-

sitive parameter to reveal an abnormal difference between both eyes.

In conclusion we have demonstrated a compact confocal instrument for measuring choroidal blood flow in the foveal region of the human fundus. This instrument has several potential clinical applications, such as the investigation of the effect of pharmacological agents on the choroidal circulation, the pathogenesis of age-related macular degeneration and the effect of various treatment modalities on this pathological condition.

Acknowledgments

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