

# COHERENT OPTICAL TECHNIQUES FOR DIAGNOSTICS OF RETINAL BLOOD FLOW

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## ABSTRACT

Up to now, a variety of coherent optical techniques have been proposed and extensively studied for diagnostics of the retinal blood flow. These techniques are mainly based on dynamic laser light-scattering phenomena such as the laser Doppler effect and the laser speckle fluctuation. This paper reviews, first, spectral reflectance properties of the ocular fundus tissue layers and, then, principles of the techniques with the comparison of the Doppler and the speckle methods. Some physical phenomena are also discussed in the origin of the techniques such as heterodyne and homodyne beatings, and time-varying speckles. Developing processes of each technique are briefly outlined. Peculiarities of blood flow measurements at the retina are finally examined from the methodological point of view. © 1999 Society of Photo-Optical Instrumentation Engineers. [S1083-3668(99)02001-8]

**Keywords** laser Doppler; speckle; velocimetry; flow velocity; retinal blood flow; ocular fundus.

## 1 INTRODUCTION

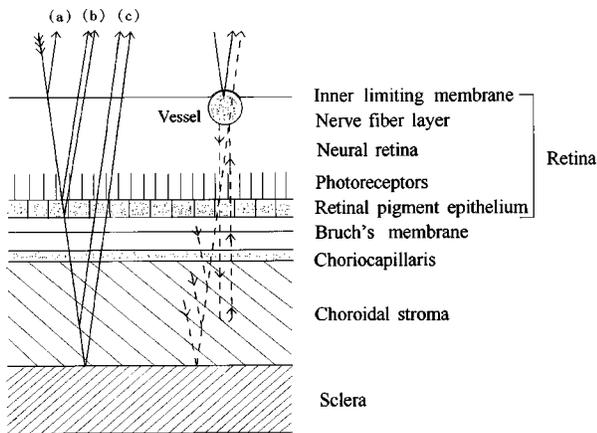
Noninvasive and quantitative evaluation of retinal hemodynamics is an important diagnostic subject while it has been one of the most difficult challenges in ophthalmology. Measurements of the retinal blood flow are expected scientifically to make valuable contributions toward physiological studies of the human eye, and also clinically to provide the possibility of an early diagnosis of various ocular diseases, such as diabetic retinopathy. A variety of techniques have been reported so far for this purpose, including the methods using microspheres,<sup>1</sup> thermocouples,<sup>2</sup> iodoantipyrine,<sup>3</sup> or hydrogen clearance.<sup>4</sup> However, these methods are clearly invasive and limited to laboratory studies. The ultrasound Doppler<sup>5</sup> and the ocular pulse<sup>6</sup> techniques were also developed to study the retinal hemodynamics, but they do not give direct measurements of the retinal blood flow. Human retinas are not physically accessible, but are visually or optically observable in a direct manner without surgery, and are only one part of a human body in such a sense. By considering this unique nature of human retinas, optical approaches are quite suitable and promising for diagnostics of the retinal blood flow. These approaches can be divided into two categories of incoherent and coherent optical techniques. The incoherent optical technique is represented by the fluorescein dye dilution and the blue field entoptic methods, while the coherent optical technique includes the laser Doppler and the laser speckle methods. Fluorescein fundus angiography

introduced by Novotny and Alvis<sup>7</sup> was historically the most well-known technique for routine clinical measurements of the retinal blood flow. This method is based on the photographic recording and analysis of fluorescein sodium passing through the retinal vasculature, and provides us with the transit time of the fluorescein dye front between two points on an arteriole, or the mean-circulation time<sup>8</sup> of fluorescein from artery to vein. Up to now, a variety of improvements and extensions were taken to obtain reliable and quantitative results, such as cineangiography<sup>9</sup> or video angiography<sup>10</sup> and fluorophotometry.<sup>11</sup> These methods could provide us with quantitative measurements in clinical studies, but still have problems. Injection of dye is still invasive and repeatable measurements are difficult, actually. The blue field entoptic method employs an entoptic phenomenon<sup>12</sup> in which white blood cells flowing in capillaries are visually perceived by a subject within his/her own eye under illumination of a strong blue light centered at a wavelength of 430 nm. This flow is compared with a pattern simulated by a computer in order to realize quantitative measurements of the macular blood flow.<sup>13,14</sup> This technique is certainly applied to routine measurements of the blood flow, but its use is limited to the macular area. Another problem may be that the method is basically a subjective test.

It is usually quite difficult to observe directly *in vivo* movements of red blood cells (RBCs) in retinal vessels under illumination of the incoherent light. Both the laser Doppler and the laser speckle techniques are based on the interference effect and

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**Fig. 1** Schematic description of the interaction between light and ocular fundus tissue layers.

light-scattering phenomena produced by an interaction of the illuminating laser light with RBCs as scattering particles. Thus, these coherent techniques are able to detect sensitively the movement of RBCs in an order of the light wavelength. The use of a laser beam provides us directional illumination, which is quite effective for positioning a measuring point or area at the retina. The methods are noninvasive or least invasive if used moderately, and repeat measurements are possible in principle within the eye safety to laser light irradiance. By taking account of these characteristics, the coherent optical techniques may be most promising for routine clinical measurements of the retinal blood flow in the future.

In this paper, we review the coherent optical techniques for measurements of the retinal blood flow with the special relation to the laser Doppler and laser speckle techniques. We first survey the spectral reflectance and penetration of light in ocular fundus tissues, and the basic phenomena of laser light scattering. Next, we introduce various methods using the Doppler and speckle techniques reported so far, and discuss their features including usefulness and problems. Methodological studies peculiar to the retinas are also outlined with some applications.

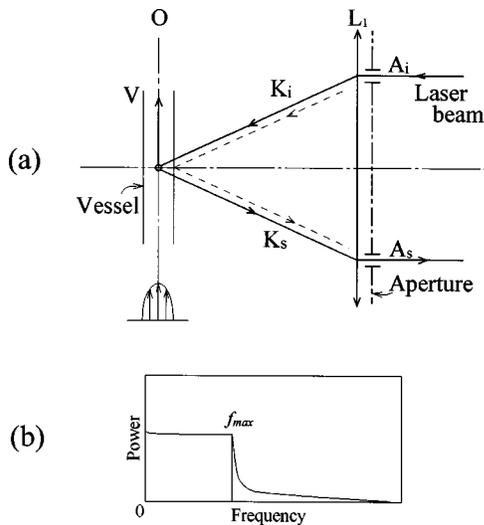
## 2 ABSORPTION, REFLECTION, AND SCATTERING OF LIGHT IN OCULAR FUNDUS

### 2.1 SPECTRAL REFLECTANCE AND ABSORPTION

Optical techniques for measuring the retinal blood flow depend strongly on the interaction between light and the fundus tissue layers such as penetration or transmission, absorption, reflection, and scattering of light. Understanding of these phenomena is important for proper interpretation and uses of the techniques. As shown in Figure 1, the ocular

fundus consists of the retina, choroid, and sclera. The retinal tissues are composed of the inner limiting membrane, nerve fiber layer, neural retina, photoreceptors, and retinal pigment epithelium. The fundus is usually seen to be reddish in color. This fact means that longer wavelengths of visible light are dominantly reflected back from the fundus layers. Previous studies using monochromatic fundus photography<sup>15-18</sup> or reflectometry<sup>19-22</sup> have shown some useful experimental results and findings on the spectral reflectance characteristics of the human ocular fundus. The fundus reflectance is the lowest at the shorter wavelength, increases as the wavelength becomes large, reaches a plateau which extends from nearly 520 to 580 nm, and then again increases to the highest at the longer wavelength.<sup>19</sup> There is a difference with an order of 2 between the blue light and red light. Some results show distinct reflection minima or inflections at 540 and 575 nm. These reflectance properties are strongly influenced by the absorption of light by various factors such as blood in the retinal and choroidal vessels and capillaries, melanin pigments in the retinal pigment epithelium and the choroid, the macular pigment in the fovea, and the ocular media or lens.<sup>20</sup> The absorption spectra of oxygenated hemoglobin and melanin are generally found to decrease with an increase of the wavelength in the visible range, although the oxygenated hemoglobin shows the characteristic absorption maxima in the wavelength range of about 520-580 nm. This latter behavior is responsible for the plateau-like reflectance spectrum or the distinct reflection minima mentioned above. The green and blue lights that penetrate into the choroid are strongly absorbed by blood and melanin and, thus, have only a little contribution to the reflectance. The main reflection originates from one or more retinal layers, probably the inner limiting membrane [reflection (a) in Figure 1] and the retinal pigment epithelium (b), and also the Bruch's membrane (b). For the case of low absorption by blood and melanin in the red light, most of the light penetrates into the deeper choroidal layers and is scattered or reflected within the layers and partially from the sclera [reflection (c) in Figure 1], which produces the major components of the reflectance.

Typical coherent light sources employed in optical diagnostics of the retinal blood flow are a He-Ne laser with a 632.8 nm wavelength, and some laser diodes in the near-infrared wavelength region. When a red beam from the He-Ne laser source is focused onto the retinal vessel, a substantial amount of the light is scattered by RBCs because of the low absorption. If the blue or green laser were used here, the detector could not receive a sufficient amount of the scattered light. Thus, the choice of the red light is quite reasonable. The light scattered forward may penetrate into the choroid. Or, when the size of an illuminated area is larger than the vessel diameter, a substantial amount of the light incident near the vessel also penetrates into the



**Fig. 2** (a) Basic scattering geometry in the laser Doppler technique and (b) a typical model of the power spectrum.

choroid. These portions are scattered or reflected back to the front, partly crossing the retinal vessel as shown by the broken line in Figure 1. But, these components experience two or more events of scattering and take a rather long path length, consequently, their intensity being small and usually negligible.

## 2.2 LASER LIGHT SCATTERING: DOPPLER EFFECT AND SPECKLE FLUCTUATIONS

### 2.2.1 Doppler Effect

Two typical phenomena of laser light scattering, which are well known as the laser Doppler effect and the laser speckle fluctuation, are applied to the diagnostics of the retinal blood flow. Here, we review briefly the principles of the techniques using these phenomena for measurements of the retinal blood flow. Figure 2 shows the principle of the laser Doppler method using a backscattering configuration. The incident beam is focused onto the retinal vessel and scattered by a moving RBC. The scattered light, which is Doppler shifted in frequency, is received in a certain direction. With the non-shifted light (dashed line) reflected from a vessel wall, the heterodyne detection (or, possibly, auto-dyne detection) is realized at a detector surface to produce Doppler beat signals whose beat frequency  $f_D$  is given by

$$f_D = \frac{1}{2\pi} (K_s - K_i) \cdot V, \quad (1)$$

where  $K_i$  and  $K_s$  are the wave vectors of incident and scattered light and  $V$  is the velocity vector of a moving RBC. Equation (1) means that the velocity of a moving RBC is determined by measuring the Doppler beat frequency, if the optical geometry of  $K_i$ ,  $K_s$ , and  $V$  is specified. Originally, this principle

is valid for a single scattering by a single RBC. In a typical application<sup>23</sup> of this technique at the retina, however, the cross section of a blood vessel is entirely illuminated and, thus, velocities of RBCs ranging from zero to the maximum  $v_{\max}$  within the vessel may simultaneously be extracted by this principle. Each velocity is reflected on each Doppler-beat frequency in the spectrum with the assumption of a single scattering for each RBC. With the assumption of a Poiseuille flow, this corresponds to a flat spectrum from zero to the maximum frequency  $f_{\max}$  with a characteristic cutoff pattern at  $f_{\max}$  being represented as shown in Figure 2(b). This maximum shifted frequency  $f_{\max}$  is linearly related by Eq. (1) to the maximum velocity  $v_{\max}$  at the vessel center.

### 2.2.2 Speckle Fluctuations

Today, the speckle is universally known as phenomena showing a random granular interference pattern, which is typically produced by the laser light scattered from a diffuse object. If the diffuse object moves, the speckle grains also move and change their shapes. This phenomenon is referred to as a "speckle fluctuation." The time-dependent property of speckle fluctuations can be applied to measurements of the moving object velocity, with the established theory.<sup>24,25</sup> Dynamic speckle phenomena or time-varying speckle fluctuations can also be observed with living objects including RBCs moving in retinal vessels. Figure 3 shows the principle of the laser speckle method on the basis of the image-plane detection. An incident beam illuminates a certain area including a retinal vessel and the surrounding tissues with a little extended spot. The light scattered by RBCs, moving in the retinal vessel, is collected onto the image plane (IP). An image of each RBC cannot usually be seen in this plane due to the low resolution, but a time-varying speckle pattern is observed. A typical speckle pattern is shown in Figure 3(b). The size of the speckles is determined by the point spread function of the limiting aperture of the imaging optics. The speckle pattern is produced by the coherent addition of many scattered waves  $A_j$  ( $j=1-N$ ) coming from each RBC. The resultant amplitude detected by a detecting aperture (DA) is given by

$$A(P, t) = \sum_{j=1}^N |A_j(P, t)| \exp[i\phi_j(P, t)], \quad (2)$$

where  $\phi_j(P, t)$  is a random phase resulting from the random distribution of RBCs, and  $P$  and  $t$  indicate a detecting point and a time, respectively. Equation (2) indicates that the speckle intensity fluctuates in a space-time random fashion with movements of RBCs. It is quite natural to presume that scattered waves  $A_j$  experience multiple scattering events by RBCs in the retinal vessel and tissue, and are also temporally modulated in a complicated manner by

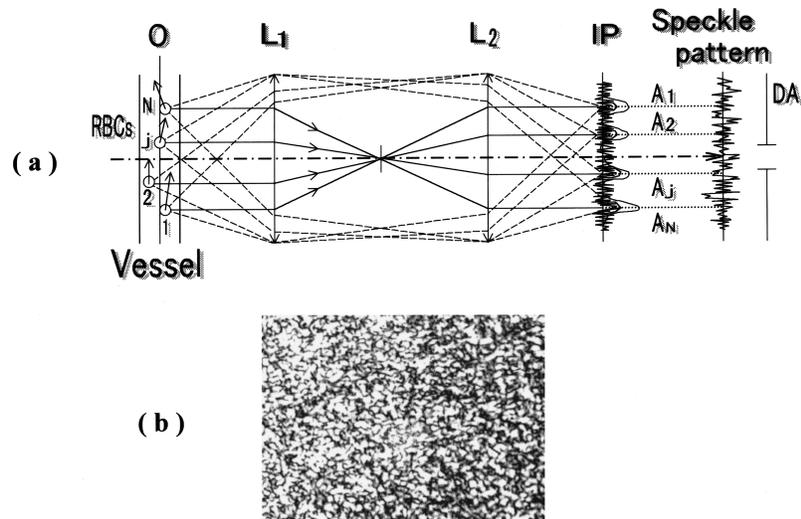


Fig. 3 (a) Principle of the laser speckle technique and (b) a typical speckle pattern.

scattering from RBCs having various velocities (including zero). The speckle fluctuations produced with these conditions are, thus, considerably different from the well-known behaviors of speckles, for example, produced by a single scattering from a diffuse plate moving with a constant velocity. To pay special attention to these speckles, they are referred to as "biospeckles"<sup>26-28</sup> by the present authors. By reason of the complexity of biospeckles, the theoretical background has not yet been established, including the proportionality between the object mean velocity and biospeckle fluctuations. However, a variety of *in vitro* experiments<sup>29-31</sup> verified that the width of broadening in the autocorrelation function or power spectrum of speckle fluctuations is linearly related to the mean flow velocity, consequently, relative measurements of the velocity being realized. Therefore, the relative velocity of the retinal blood flow can be obtained by measuring, for example, a correlation time that is defined as the delay time needed for the correlation function to decrease to the half.

### 2.2.3 Inter-Relation of the Two Techniques

Both the Doppler effect and the speckle fluctuation mentioned above are obtained from laser light scattering by moving RBCs. Therefore, there should be some inter-relations between them. Briers<sup>32</sup> recently discussed this interesting topic, where he demonstrated the essential equivalence of the Doppler and speckle approaches in the measurement of line-of-sight velocities. As he claims in his paper, no research has actively conducted a comparison study of the two phenomena. To give a general solution to this topical problem is not an aim of this paper. Thus, we briefly discuss the mutual relations between the two techniques of Figures 2 and 3 in the works limited to blood flow measurements. In Figure 2, for the laser Doppler technique, the size of an

aperture that receives the scattered light is usually set to be small in order to define the direction of  $\mathbf{K}_s$  with small ambiguity. This is alternatively referred to as a coherence condition<sup>33</sup> in the Doppler technique. The detector performs a heterodyne mixing, which yields the Doppler-beat signals with frequency  $f_D$ . Contributions of randomly distributed RBCs and their velocity distribution result in Doppler broadening, although the heterodyne beat component is maintained. In the worst case, however, heterodyne components disappear due to modulation with the pedestal or homodyne components extended to high-frequency regions which become very close to those of speckle fluctuations.

In Figure 3, for the speckle technique, the scattered light is received over the whole area of a lens pupil. A range of scattering angles produces a range of Doppler-shifted frequencies. The light fields scattered in the same direction by different RBCs with the same velocity vector have the same Doppler-shifted frequencies but have random phases due to their distribution in the optical path length. Both the range of Doppler-shifted frequencies and the random phases contribute to a temporal randomization of the phase of total detected intensity signals. This may alternatively be considered as broadening in the spectrum, but not of the heterodyne beat components. The contribution of the velocity distribution enhances this randomizing effect. Thus, the temporal fluctuation of speckles can be caused not only by the spatial randomness due to the RBCs' distribution, but also by the temporal (or, possibly, frequency) randomness due to various Doppler-shifted frequencies.

In the optical configuration of Figure 3, the coherence condition is usually not satisfied because the speckle formation is prior to the heterodyne mixing. Then, the range of Doppler-shifted frequencies yields the homodyne mixing which plays a role in

the temporal variation of speckles. As Briers<sup>32</sup> describes, in this context, the speckle fluctuation and the homodyne techniques become identical, including also the photon-correlation spectroscopy. Whether or not the conventional term, "laser Doppler velocimetry," covers only the heterodyne technique or includes the homodyne technique is probably due to a difference of interpretation. It should be noticed that the homodyne mixing in the case of Figure 3 can be described only statistically, which is certainly the method employed always in the speckle theory. Sufficient randomness of the homodyne mixing is necessary for referring to the equivalence with the speckle technique. This may support the fact that the time-varying properties of biospeckles or dynamic speckles accompanied by velocity distributions or multiple scattering are insensitive to the direction of velocity, and are to the illuminating and detecting optical geometry within the use in the ocular fundus. In conclusion of the present discussion, determination of the superiority of terminology for the scattering phenomena observed here is unproductive and meaningless. What is most important is to understand fully and correctly their mutual relation.

#### 2.2.4 Multiple Scattering Effects

Some previous studies<sup>34,35</sup> suggested the high possibility of occurrence for multiple scattering of light in whole blood. If multiple scattering is dominant, the above-mentioned heterodyne technique fails in the retinal vessel. Riva and co-workers<sup>36,37</sup> experimentally investigated this question and showed that the single scattering approximation was valid for determining the maximum velocity  $v_{\max}$  using Eq. (1). This is probably supported by the consideration that since the forward scattering is dominant in blood with an average cosine of  $>0.99$ , most of the backscattered light to be detected (which is, of course, very small in amount) is given only by the contribution of a single backscattering. But, they also claimed in their paper that they hardly obtain an ideal cutoff spectrum at some sites with highly-reflective backgrounds in the ocular fundus. This fact expects local fluctuations in the reflectance of layers behind the vessel. Other researchers<sup>38,39</sup> extensively discussed the problem of the multiple scattering in the laser Doppler technique.

As speckle phenomena result from the interference of many scattered waves with random phases with respect to each other, many RBCs and other scattering centers should be contained in the illuminated area. This can lead to multiple scattering, which can cause a problem with the metrological use of speckle fluctuations. Previous studies<sup>31,40</sup> show that the reciprocal of a correlation time increases with an increasing vessel diameter or an increasing background reflectance. In those measurements, such increases of the diameter and reflectance probably enhance the order of multiple

scattering in two ways: one is within the vessel and another is by backscattering from the background. These may accelerate the randomization of a phase  $\phi_j(\mathbf{P}, t)$  and shorten the phase-consistent time or the time-correlation length, consequently, the reciprocal of a correlation time being increased. Other related studies<sup>41-44</sup> were also theoretically and experimentally performed to have an insight into these behaviors of speckles. These properties on the correlation time should be compensated in order to make the technique available for metrology. For this purpose, experimental studies<sup>30,31</sup> were conducted to calibrate the correlation time to the absolute mean velocity in the glass capillary model having a corresponding diameter.

### 3 LASER DOPPLER TECHNIQUE

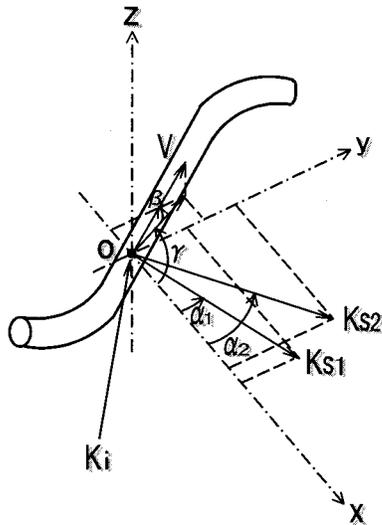
#### 3.1 HETERODYNE METHOD

Laser Doppler velocimetry was the first coherent optical technique applied to measurements of the retinal blood flow. There are some different configurations<sup>33</sup> in the laser Doppler velocimeter based on the heterodyne principle, including a reference-beam type and a differential or dual beam type. The configuration of the latter type is generally more useful than the former for practical measurements because of the freedom in detection. For application in the ocular fundus, however, a simple optical system is desirable. In the technique for the retinal vessel, a simplified reference-beam type of the laser Doppler configuration has been employed. As far as we know, Riva et al.<sup>23</sup> first demonstrated measurements of blood flows in individual retinal arteries of an anesthetized albino rabbit by using the reference-beam-type laser Doppler technique. They illuminated a vessel perpendicularly with the He-Ne laser light of a 632.8 nm wavelength and, then,  $\mathbf{K}_i \mathbf{V} = 0$  is realized in Figure 2(a). With an angle  $\theta$  between  $\mathbf{K}_i$  and  $\mathbf{K}_s$ , Eq. (1) is rewritten as

$$f_D = \frac{nV}{\lambda} \sin \theta, \quad (3)$$

where  $\lambda$  and  $n$  denote the wavelength of the laser light in vacuum and the refractive index of a flowing medium. The flow direction is assumed to be in the scattering plane, which is defined by the illuminating and detecting optical axes, or  $\mathbf{K}_i$  and  $\mathbf{K}_s$ . This simplification was a success, at least in the beginning step. After this report, Riva's group has intensively and then extensively studied this type of the laser Doppler technique, which is currently being used for various clinical studies. Since their works are well reviewed in the literature,<sup>14,45,46</sup> here we only briefly and historically outline developments of the laser Doppler technique in this type.

The blood flow velocity was then measured in human retinal vessels by Tanaka et al.<sup>47</sup> using a photon-correlation technique. Their measurements produced monotonically decayed autocorrelation



**Fig. 4** Basic scattering geometry in the bidirectional-type laser Doppler technique.

functions which did not follow the principle of Eq. (1). The difference from Eq. (1) was probably caused by multiple scattering. They used the results of a glass capillary test for calibration. Pike et al.<sup>48</sup> also developed a prototype of the measuring system partly using a fundus camera, and meaningfully compared the results obtained by this system with those by fluorescein angiography in the cat eye. Reliable measurements in human retinal vessels were then reported by Feke and Riva<sup>49</sup> using the principle of Eq. (3). They successfully obtained the expected cutoff spectra [as in Figure 2(b)] in 0.1 s and also recorded a velocity variation in arterial blood flows during each cardiac cycle.

Equation (3) is simple and seems to be convenient, but it requires estimating the intraocular scattering geometry, which means each angle specifying the vectors  $\mathbf{K}_i$ ,  $\mathbf{K}_s$ , and  $\mathbf{V}$  "within ocular." It is actually difficult to determine these intraocular angles. In order to overcome the difficulty, Riva et al.<sup>50</sup> developed the bidirectional-type laser Doppler technique. Figure 4 schematically shows the basic geometry of the bidirectional type. The  $y$ - $z$  plane means an ocular fundus plane and the  $x$  axis corresponds to the eye axis. In this scheme, the light scattered by a RBC at the origin is detected in two distinct directions  $\mathbf{K}_i$  and  $\mathbf{K}_s$  separated by a known angle  $\Delta\alpha = \alpha_2 - \alpha_1$ . Each Doppler beat frequency is given by Eq. (1) and, then, their difference  $\Delta f$  is derived, with permissible approximation, as

$$\Delta f = f_{D2} - f_{D1} = \frac{1}{2\pi} (\mathbf{K}_{s2} - \mathbf{K}_{i1}) \cdot \mathbf{V} = \frac{nV\Delta\alpha \cos\beta}{\lambda}. \quad (4)$$

As the angle  $\beta$  is measurable, the flow velocity  $V$  can be determined in an absolute value from the measured beat frequency  $\Delta f$ . This principle has finally been adopted in the current laser Doppler in-

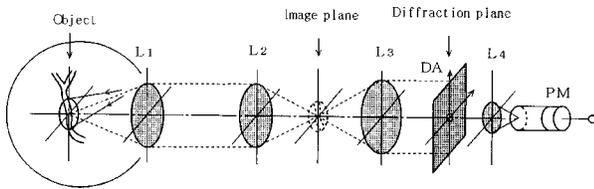
strument of the Riva group. The feasibility of the bidirectional method was experimentally verified first by recording signals consecutively with two scattering angles by rotating the receiving optics in the slit lamp. Later, a fundus camera-based apparatus equipped with two receiving optical systems was developed<sup>51</sup> for simultaneous detection of the two beat frequencies. The apparatus based on a fundus camera eliminates the need for a contact lens but allows determination of the scattering geometry, thus, the procedure of retinal blood flow measurements are simplified.

Further significant developments in the Riva group were achieved in two lines. One<sup>52</sup> is the fast and automatic signal analysis and the spot identification by means of advanced microcomputer technology. The improvement was aimed to realize clinical uses as a routine diagnostic tool. Another<sup>53,54</sup> is the use of a near-infrared laser diode as a light source and an avalanche photodiode (APD) as a detector. These choices are reasonable and advantageous because they provide increased retinal irradiance, better quantum efficiency of the APD with increased signal-to-noise ratio, compactness and lightness, no need for pupil dilatation, and no glaring effect.

A different approach<sup>55</sup> was tried by using the differential-type laser Doppler technique. Two beams divided from the laser light are focused by a fundus camera onto a retinal vessel, and the scattered light is collected with a certain range of solid angles. Photomultiplier outputs are analyzed by the photon-correlation technique. The beams, crossing at the vessel, may provide a high spatial resolution, and the light detection over the range may result in an increased intensity. *In vivo* measurements were demonstrated with human retinal vessels, but their results suffered from a low signal-to-noise ratio and were unsatisfactory. This would probably be due to the difficulty in the exact beam crossing at a measuring point on the vessel. This was clearly a delicate task at the human ocular fundus. There is no continuing study in this scheme.

### 3.2 HOMODYNE METHOD

The homodyne principle was also used by introducing the laser Doppler "flowmetry" (LDF), which was originally developed for the estimation of skin tissue microcirculations. Riva and co-workers<sup>54,56,57</sup> applied this method to measurements embedded in the tissue, yielding a monotonically decreasing frequency spectrum but not showing the heterodyne beat component peak or the cutoff characteristics.<sup>58</sup> As we discussed in Secs. 2.2.3 and 2.2.4, many RBCs and tissue scatterers in a random distribution, a variety of the illumination and detection angles, and the velocity distribution, all contribute to the randomization of optical path lengths and Doppler-shifted frequencies. Sufficiently randomized phases produce time-varying



**Fig. 5** Basic optical system in the diffraction-plane detection of the biospeckle technique.

speckle fluctuations or homodyne beat components, which result in a broader frequency spectrum or a narrower autocorrelation function, both showing a monotonically decreasing curve. Therefore, the LDF technique is much more equivalent to the speckle fluctuation technique than the laser Doppler technique.

The relative blood flow parameter was used to investigate the response to a change of the intraocular pressure in the optic nerve head. Some applications<sup>57</sup> of the homodyne method were made to investigate the oxygen reaction and the response to the intraocular pressure in choroidal tissue layers.

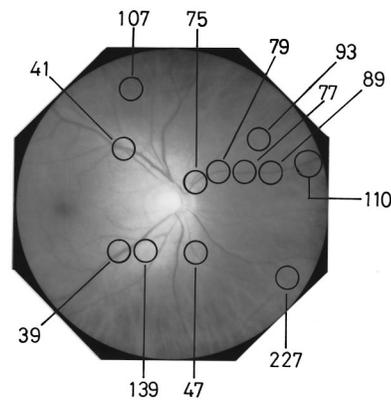
## 4 LASER SPECKLE TECHNIQUE

Time-varying laser speckles have been applied in two different approaches to an evaluation of the retinal blood flow: measurements of a relative flow velocity at a point or a flow parameter in a small area, and mapping or visualization of a relative flow velocity distribution in some extended retinal area. The former is called "biospeckle flowmetry" by the present authors while the latter includes speckle photography and is called "laser speckle flowgraphy."

### 4.1 BIOSPECKLE FLOWMETRY

#### 4.1.1 Blood Flow Parameter in a Small Area

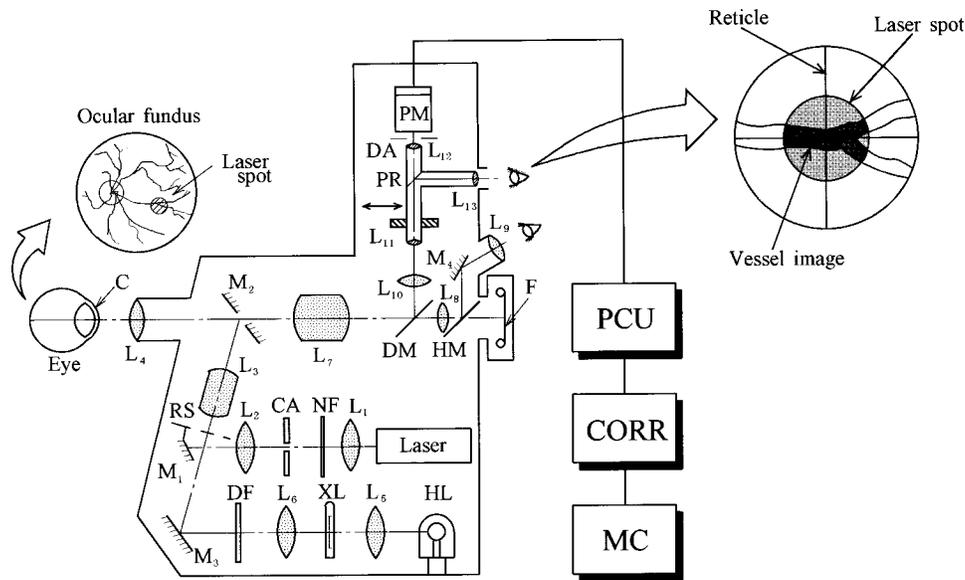
Time-varying biospeckle fluctuations were used by Fujii et al.<sup>59</sup> to monitor the overall information of blood microcirculations in some illuminated area of the skin tissue. This approach was, then, modified and applied to an evaluation of blood flows at the ocular fundus of an albino rabbit<sup>60</sup> and human subjects.<sup>61,62</sup> Figure 5 shows the basic optical system of this type of the biospeckle flowmeter. The laser light illuminates a certain retinal area with an extended spot having a diameter of 1–3 mm, and is scattered by moving RBCs in blood vessels and capillaries and by ocular fundus tissues. As described in Sec. 2.2.2 with Figure 3, a time-varying speckle pattern is observed in the image plane. By using a lens  $L_3$ , the light through the image plane is further collimated to the Fraunhofer diffraction plane where one can also observe a dynamic speckle pattern and obtain speckle fluctuation signals through a detecting aperture DA followed by a detector. Owing to the extended illuminating spot and the



**Fig. 6** Typical correlation times ( $\mu\text{s}$ ) measured at various points in the human ocular fundus.

detection in the diffraction plane, the detector receives a superposition of many light fields scattered from the whole spot area. Since each scattered light field holds information about the moving velocity, the speckle fluctuations produced by the coherent addition of all the fields reflect the velocities of corresponding moving RBCs. Therefore, this optical system enables us to measure the overall activity of various blood flows contained in the spot area, rather than a flow velocity at a certain point on a retinal vessel.

A measuring apparatus is composed of a standard fundus camera, a He-Ne laser, and a photomultiplier. Output fluctuation signals are evaluated by the mean frequency  $\langle f \rangle$  (Ref. 28) in the spectrum analysis or by the correlation time in the photon-correlation spectroscopy. Due to the principle, the results are provided as a blood flow level or degree in a relative quantity. The experiments for an anesthetized albino rabbit demonstrated an expected increase of the mean frequency corresponding to an increase of the blood flow with an application of a gas mixture of  $\text{CO}_2$  and  $\text{O}_2$ . The autocorrelation functions were measured by a photon correlator at various positions in the ocular fundus of normal human volunteers. Figure 6 demonstrates values of the evaluated correlation time at some probe area in the fundus. The smaller values in the correlation time are obtained from the areas containing one or more major retinal vessels. The high velocities and a large number of RBCs probably contribute to the high-frequency fluctuations or rapidly decaying correlation functions. The longer correlation times are obtained in the area showing no visible major vessel. Such low fluctuations in these areas probably result from retinal microcirculations and partly from the choroidal blood flows. The overall analysis in a small area is complementary to the point analysis of a local velocity on a vessel, and is also insensitive to the flow direction. The aiming of a laser beam can easily be performed at a position of interest. In these points, we consider that a biospeckle flowmeter of this type has a potential use-



**Fig. 7** Schematic diagram of the biospeckle measuring apparatus using a fundus camera.

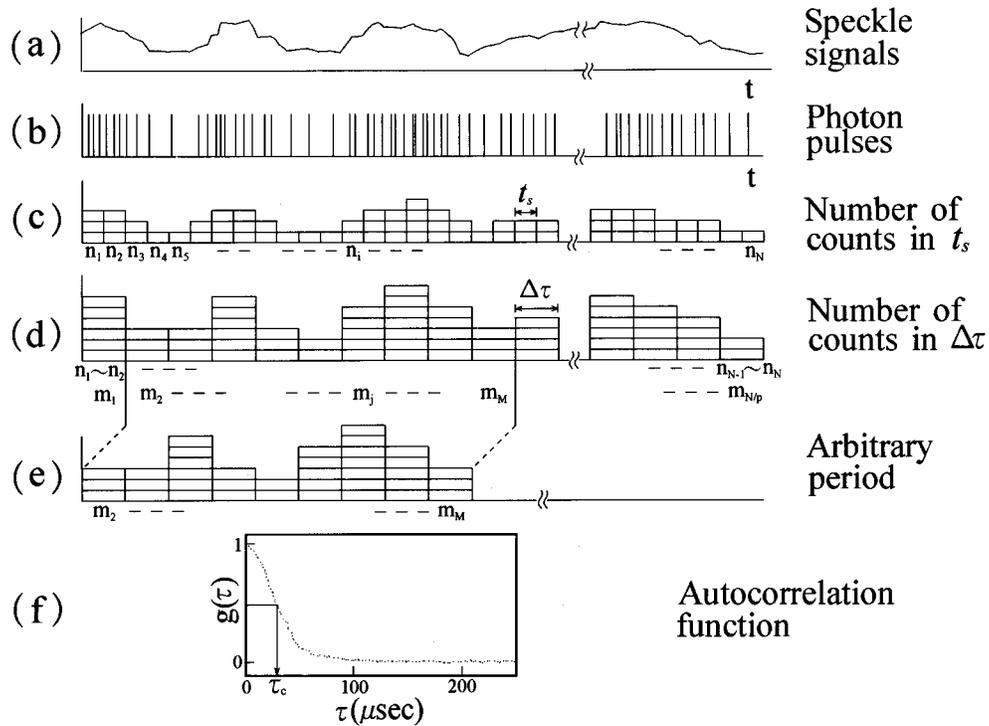
fulness, especially for the retinal capillary network. However, the major problem is a low signal-to-noise ratio in the output signals. A primary noise source is a predominant level of the stationary speckle intensity, because the spot usually contains a small portion of blood vessels and a large portion of static tissues. For an application to the choroidal blood flow, a wavelength of the laser source should be appropriately chosen, possibly in the near-infrared region from the discussion of Sec. 2.1. The blood flow information obtained by the laser Doppler flowmetry is likely to be equivalent to that obtained by this technique, apart from terminology.

#### 4.1.2 Local Blood Flow Velocity

The use of a detecting aperture in an image plane means the definition of a measuring point as well as the detection of speckle fluctuations. As described in Sec. 2.2.3, this type of biospeckle flowmetry enables us to measure the relative blood flow velocity in a retinal vessel. Figure 7 shows a schematic diagram of the measuring apparatus<sup>27</sup> consisting of a standard fundus camera (Kowa RC-X) with a He-Ne laser source of a 632.8 nm wavelength and a photomultiplier (PM). A laser beam of 40–80  $\mu\text{W}$  in power illuminates an area of nearly 700  $\mu\text{m}$  in diameter at the fundus including a retinal vessel of interest. The light scattered from the area is detected by the PM, and the output signals are sent into the signal processing system using a photon correlator. A subject eye fundus is roughly positioned by a fixation target and, then, the detecting aperture is adjusted to a desired measuring point by moving a reticle of the eyepiece. The mean size of the speckle grains in the detecting plane depends on the receiving optical system, including the magnification, and was observed to be nearly 200  $\mu\text{m}$  in

diameter. A typical size of the probing cross-sectional area is defined to be nearly 20  $\mu\text{m}$  in diameter by a detecting aperture of 400  $\mu\text{m}$  in diameter and imaging magnification of 22.8 $\times$ . The probe volume depth is not definitely provided, because it is a matter of light penetration in the vessel with the background tissue layers, and is not determined by the focal depth. By referring to the discussion in Sec. 2.1, the measured results may reflect primarily the blood flow in the whole depth of the vessel and secondarily the choroidal blood flow.

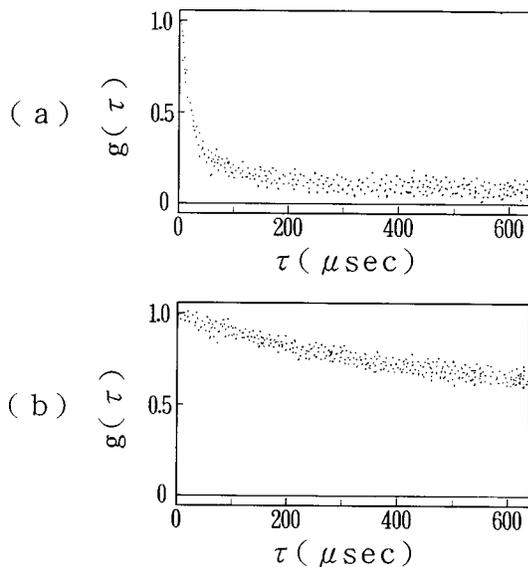
Figure 8 schematically describes the principle of flexible correlation analysis<sup>63,64</sup> aimed particularly at retinal blood flow measurements. Speckle fluctuation signals (a) are detected as a train of photoelectron pulses (b), and counted every sampling time  $t_s$ , which is variable. The number of pulse counts (c) is sequentially stored into the memory and, thus, these raw data can be used repeatedly. The stored data  $n_i$  are read out for analysis and are integrated, if necessary, in every time segment defined by  $\Delta\tau = pt_s$  ( $p = \text{an integer, usually } 1\text{--}5$ ). This operation can be used for changing a unit of the delay time or a full-scale range. The integrated pulse counts (d) are, then, used to calculate the second-order autocorrelation function (f) in 256 channels on the basis of the digital correlation technique.<sup>65</sup> The correlation function is finally smoothed out to evaluate the correlation time. To realize time-division correlation analysis, it is also possible to calculate the correlation function only from a certain limited part of the sequential data (e). Since the sequential raw data are stored in the memory, one can repeat the analysis without limiting the calculations of the correlation function with different analyzing conditions such as a  $p$  value or time division.



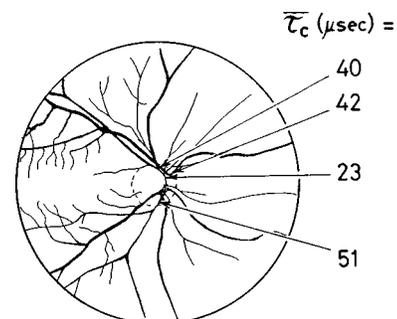
**Fig. 8** Schematic principle of the flexible photon-correlation analysis.

Figure 9 shows typical correlation functions obtained on (a) a major retinal vessel and (b) the surrounding tissue area of the human ocular fundus. The result (a) presents a rapidly decaying correlation due to a certain normal blood-flow velocity in the vessel, while the function (b) demonstrates a slowly decreasing correlation due to a low degree of capillary blood circulations and secondary blood circulations in the choroidal layers. The correlation

function changes its width, reflecting the flow velocity. To obtain the well-converged correlation function needed for precise measurements, the diameter of the detecting aperture should be twice as large as that of the speckle grains.<sup>30</sup> It may provide the best convergence in the correlation while it causes an acceptable small integration effect. Examples of the evaluated correlation time are shown in Figure 10 for four different measuring points in the human optic nerve head, which is known as the area having high reflectance. The effect of multiple scattering is strong in that area and the heterodyne Doppler technique usually fails to obtain the characteristic cutoff spectrum. Since the biospeckle technique evaluates such an effect as highly randomized or modulated intensity fluctuations, the blood flow in this area can be measured in the same way



**Fig. 9** Typical correlation functions obtained from (a) a major retinal vessel and (b) the surrounding tissue area.



**Fig. 10** Typical correlation times ( $\mu s$ ) measured at four different points in the human optic nerve head.

as in the other retinal areas. The measured correlation time is calibrated to an absolute mean velocity in the corresponding glass tube model by using the equation<sup>30,31</sup> obtained experimentally:

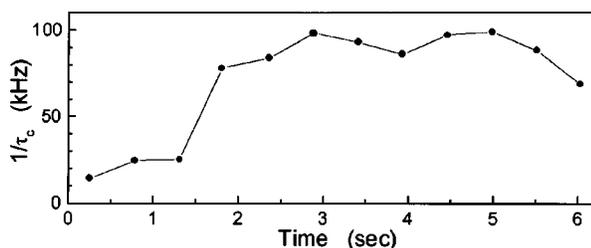
$$\nu_0 = \frac{1}{10^a R^b} \frac{1}{\tau_c}, \quad (5)$$

where  $a$  and  $b$  are functions of the diameter  $d$  and experimentally evaluated to be  $9.5 \times d^{0.0439}$  and  $0.00437 \times d^{-0.425}$ , respectively, and  $R$  is the background reflectance. The diameter is obtained by the measurements (see Sec. 5.3) while the reflectance is given by values in the literature, including a case of the optic nerve head.

Figure 11 shows an example of the time-division correlation analysis. After recording photon counts for about 6 s, they were divided into 12 subperiods and each set was analyzed to obtain the correlation function. A reciprocal of the correlation time is small in the beginning and then increases after 1.5 s. This variation agreed well with the visual observation of vessel movements from the surrounding tissue into the vessel during the measurement. Reproducibility was experimentally estimated by the coefficient of variations, which was obtained to be 3% for *in vitro* measurements and less than 20% for *in vivo* measurements. The linearity errors in velocity measurements were investigated by glass capillary tests, and were found to be less than 7.8%. The spatial resolution is determined by the size of the probe cross-sectional area and is currently 20–50  $\mu\text{m}$  in diameter. The temporal resolution and the measuring time depend strongly on the detected intensity of the signals and the ability of a signal-processing system, and are at present 65.5 ms and a range of 65.5 ms–6 s, respectively.

#### 4.2 SPECKLE PHOTOGRAPHY

Fercher and Briers<sup>66–68</sup> first reported a method for visualizing two-dimensional (2D) retinal blood-flow distributions using single-exposure speckle photography. When a photograph of the time-varying biospeckle pattern is taken from the retina with a certain exposure time, a time-integrated speckle pattern is recorded. Speckle grains in the area containing a high velocity of the flow fluctuate



**Fig. 11** Temporal variation of a reciprocal of the correlation time when the measuring point was moving into the retinal vessel from the tissue during measurement.

with high frequency, while those in the area with a low flow velocity or without movement move slowly or remain stationary. A photograph of such a speckle pattern demonstrates low-contrast or blurred areas for active flows and high-contrast areas for inactive or no flow. Thus, the contrast map corresponds to a map of the flow activity. Generally, the speckle contrast  $C$  is defined as

$$C = \frac{\sigma_s}{\langle I \rangle}, \quad (6)$$

where  $\sigma_s$  and  $\langle I \rangle$  are the standard deviation and the mean value of the speckle intensity fluctuations. In this method, the speckle contrast recorded in the photographs is a function of the flow velocity and the exposure time. Some theoretical treatments were given to show the change of the speckle contrast from near zero to near unity depending on an increase in the ratio of the speckle correlation time to the exposure time.

In order to enhance the recorded speckle contrast and to make it clearly observable, an analog spatial filtering technique was used in the beginning. This process results in a conversion of the contrast to the intensity variation. This postprocessing technique was later alternated with a technique<sup>69,70</sup> using digital image processing, by which the contrast variations are converted to pseudocolor variations with relatively small error. Experimental results of the retinal blood flow map demonstrate their potential usefulness for diagnostics of the retinal blood-flow state, particularly in instantaneous 2D distributions. Future improvement may be made through more quantitative evaluations that enable us to analyze the difference in velocities. The blood flows in the optic nerve head and in the choroidal layers may also be explored by speckle photography. The technique ingeniously utilizes the two-dimensionality of the speckle pattern and, at this point, it is of considerable use for future applications in comparison with point measurements by the laser Doppler or biospeckle technique. The photographic process is certainly disadvantageous for practical uses, but Briers and his co-workers<sup>71,72</sup> have recently developed a fully digitized imaging/processing technique called "laser speckle contrast analysis (LASCA)," instead of using photography. This approach may be one of the promising steps in the development of this technique.

#### 4.3 LASER SPECKLE FLOWGRAPHY

Fujii<sup>73</sup> applied his earlier works,<sup>74</sup> which visualize a microcirculation map in a skin tissue for evaluation of the retinal blood flow. A near-infrared laser diode of 830 nm wavelength is used to illuminate some retinal areas by using a fundus camera. The scattered light is collected to form a speckled image of the illuminated area where an area sensor is placed. The sensor scans a fluctuating speckle pattern by its pixels in order and captures one image.

This is successively repeated with a high rate of scannings and stored directly into a large memory. Consider the speckle intensity at a certain pixel point in the measured area. If RBCs pass with a high velocity through this point, the speckles fluctuate rapidly and the recorded intensities show a substantial difference between one scan and the successive scan in the pixel. Low-velocity flow results in a small or almost no difference. On the basis of this principle, a useful measure, an average derivative (AD) was defined to express the changing rate of the speckles as

$$AD_k = \frac{\sum_n^{N-1} |I_k(t_{n+1}) - I_k(t_n)|}{(N-1)\langle I_k \rangle}, \quad (7)$$

where  $I_k(t_n)$  denotes the intensity detected at the  $k$ th pixel of the  $n$ th scan, and  $\langle I_k \rangle$  is its average intensity for  $N$  total scans. The AD values of the total pixels are converted with a color code to a 2D color map. From some experiments, this parameter was verified to be useful for a relatively smaller range of object velocities, but it was saturated or decreased with higher velocities.

This problem was later improved<sup>75</sup> by introducing another measure of a blur rate (BR) defined as

$$BR_{k,j} = \frac{\langle I_{k,j} \rangle}{(1/N) \sum_{n=1}^N |I_{k,j}(t_n) - \langle I_{k,j} \rangle|}, \quad (8)$$

where  $I_{k,j}(t_n)$  is the value of  $I_k$  in the  $j$ th measurement. The parameter BR, which is equivalent to a reciprocal of the speckle contrast, was found by *in vitro* experiments to be almost proportional to the object velocity. By using the parameter BR, the technique was compared with the microsphere technique and was applied to investigate the effect of the ocular perfusion pressure in the retina, choroid, and optic nerve head.<sup>76,77</sup> Reproducibility of this technique was reported<sup>78</sup> to be about 10% for real-time measurements of the human optic nerve head. The problem may be the limited area of view, but it is not considered to be an essential drawback.

## 5 METHODOLOGIES PECULIAR TO RETINA

### 5.1 POINT MEASUREMENTS AND AREAL VISUALIZATION

In diagnostics of the retinal blood flow, point measurements are effective for major retinal arteries and veins with relatively large diameters, roughly  $> 50 \mu\text{m}$ . In this case, the measuring quantity is an absolute maximum flow velocity at the center of a vessel in the laser Doppler technique, or a relative mean-flow velocity in the biospeckle technique. Thus, quantitative information can be directly provided and is quite convenient for interpretation. Since the probe cross-sectional area is usually limited within the vessel, the blood flow in the vessel is more dominantly reflected on measurements than that in the retinal capillary and in the choroidal lay-

ers. When the velocity is combined with the vessel diameter, this gives a flow volume rate, which is quite an important measure in ophthalmic diagnostics. The point measurements suffer from eye movements more seriously than the areal visualization and, of course, are meaningless in capillary networks.

Although the areal visualization can be used both in major vessels and capillary networks, it works prominently, especially for capillary networks. From the physiological and clinical points of view, the state of retinal microcirculations is another and probably a more important measure for diagnostics than the velocity in major vessels. Both techniques using speckle photography and laser speckle flowgraphy are able to potentially provide useful information. It is useful to see the simultaneous flow states at various positions. The colored mapping is also visually advantageous, but its coded values are inferior to the direct flow velocity in quantitative analysis. This visualization may carry information about choroidal blood circulation but it is sometimes difficult to discriminate information of the retina from that of the choroid. The choice of the laser light wavelength may remove this ambiguity. One of the best ways is to combine point measurements with areal visualization, since they are complementary to each other.

### 5.2 REAL-TIME OPERATION

Optical measurements in the eye should be generally conducted in a very short time. There are at least three reasons for this requirement: to detect the blood flow pulsation or to eliminate its effect, to reduce the influences of eye movements, and to ensure eye safety to laser light exposure. In order to achieve real-time measurements, time-division processing is usually employed for the power spectrum analysis, photon-correlation analysis, and 2D image analysis. The laser Doppler technique has already been used to record in its earlier stage<sup>49,50</sup> the temporal variations of Doppler-shifted power spectra and their cutoff frequencies corresponding to the relative systolic/diastolic variations. Due to a short integration time, however, the power spectra are very noisy with statistical fluctuations and the cutoff frequency is rather ambiguous. Improvement was then made by using advanced microcomputer techniques,<sup>52</sup> by which one data set can be obtained in 80 ms. Various measurements<sup>52,79-81</sup> were performed to investigate the effect of photocoagulations on pulsation, oxygen, reactivity, the effect of increased intraocular pressure (IOP), chronic alterations in perfusion pressure, and response to light and dark. The variation of the blood flow with pulsation is easily compensated also by synchronization.

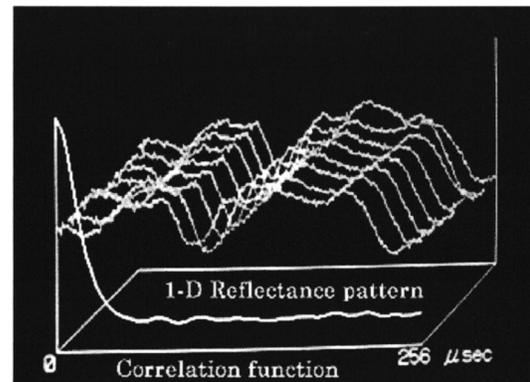
In biospeckle flowmetry, the real-time results are provided by time-division photon-correlation analysis<sup>64,82</sup> executed on the total raw data stored in

a memory beforehand. In a specially designed photon correlator that is dedicated to the biospeckle technique, one correlation function and its correlation time are obtained in 65 ms each. *In vivo* experiments<sup>26,27</sup> were carried out to show the variation of the blood flow in the retinal artery corresponding to the variation of the electrocardiogram. Laser speckle flowgraphy treats 2D data in a computer and requires the help of advanced electronics. To shorten the processing time, a specially designed hardware logic board was developed by which the real-time visualization was realized. A series of 2D maps obtained at a rate of 16 frames/s clearly visualized the pulsation in the retinal artery.<sup>75</sup> The apparatus was also used to measure the pulsating blood flows in the optic nerve head and in the choroid.<sup>78</sup> In any of the above techniques, realization of the real-time operation depends strongly on the technology of the electronics and secondarily on the detected intensity. Therefore, further improvement or development can be expected in the future.

### 5.3 VESSEL DIAMETER AND FLOW VOLUME RATE

The flow volume rate in the major retinal vessels is another important measure for the diagnostics of the retinal blood flow and is probably more informative than the flow velocity. This is because the flow volume rate is expected to reflect directly the oxygen supply to the retinal capillary network. In the optical techniques described in this paper, this value is usually obtained by the mean-flow velocity multiplied by the cross section of a vessel, which is calculated from the vessel diameter under the assumption of a circular cross section. Thus, the diagnostics of the retinal blood flow need measurements of the retinal vessel diameter at the same time and at the same position with the velocity measurements. In the laser Doppler technique, monochromatic fundus photography<sup>18</sup> is used to measure the vessel diameter. The light of the 570 nm wavelength was used for this purpose since it is able to produce a good contrast. A variety of measurements were carried out to study the physiological properties of the blood flow,<sup>83</sup> oxygen reaction,<sup>80</sup> response to the change of intraocular pressure,<sup>84</sup> and total volumetric flow rates.<sup>85</sup> However, the photographic process is indirect, time consuming, and impractical for routine uses. Another problem is that, in these studies, the time-averaged blood velocity<sup>86</sup> during the cardiac cycle was used to calculate the mean-blood-flow rate. Temporal variation of the flow volume rate cannot be obtained in this process.

The present authors developed a simple method<sup>87,88</sup> for measuring the retinal vessel diameter in the framework of the biospeckle flowmetry. One-dimensional (1D) fundus reflectance patterns are detected across the retinal vessel by using a lin-



**Fig. 12** Samples of the scanned intensity profile across the retinal vessel and the measured autocorrelation function.

ear image sensor on the basis of spectral reflectance properties. The light of the 570 nm wavelength is again used here, and the system was built into the biospeckle measuring apparatus. Then, the total apparatus enables us to measure the correlation time, vessel diameter, calibrated velocity, and flow volume rate, continuously eight times in one unit of measurement at one point. Figure 12 shows a typical example of eight scanned 1D reflectance patterns and one of the autocorrelation functions. Each pattern demonstrates the hollow nearly at the center, which corresponds to the vessel. This method can also be used to detect eye movements by monitoring the vessel position on the pixels. Related experiments using the speckle technique were carried out to investigate the blood-flow volume rate in normal human retinas,<sup>89</sup> the total volumetric flow rate,<sup>30</sup> and the effect of photocoagulation therapy.<sup>31</sup>

### 5.4 ELIMINATING EYE MOVEMENT EFFECTS

Optical diagnostic measurements in the ocular fundus suffer very often from eye-movement artifacts. There are several kinds of voluntary and involuntary eye movements. We realized empirically that the biospeckle method is troubled with small involuntary movements of the flick and drift types,<sup>90</sup> and with postural reflex movements. In the case of point measurements, such eye movements easily cause a measuring point to deviate from an initial position on the retinal vessel. The recent laser Doppler apparatus employed "active" instruments, which stabilize the measurements against eye movements in 1D<sup>91</sup> or 2D.<sup>92</sup> 1D eye tracking is made by using a linear charge-coupled device image detector that produces driving signals to control a galvanomirror. 2D eye tracking is realized by introducing a commercially available dual-Purkinje-image eye tracker, which feeds control signals to galvanomirrors. The system guarantees a high speed of response and high angular resolution, but is a large-scale instrument and costly.

The biospeckle method employs a "passive" approach<sup>63,64</sup> in which erroneous data caused by

eye movements are effectively removed through signal processing. Detection of eye movements is made by estimating a specific variance,<sup>82</sup> which is defined by

$$\frac{\sigma^2}{\langle m \rangle}, \quad (9)$$

where  $\langle m \rangle$  and  $\sigma^2$  denote the average counts and the variance of photoelectron pulses during a certain time segment, respectively. The specific variance takes a small value for the vessel and a large value for the tissue, thus becoming a useful index to specify the displacement of the measuring point. The laser speckle flowgraphic method shortens the image processing time and reduces the effect of eye movements. If eye movements occur during the measurement, one can recognize them by using an estimator,<sup>76</sup> obtaining the difference between the signal intensities for one scan and another successive scan. Apart from the blood-flow measurements, the laser Doppler technique was used to measure the eye movements by light scattering on the cornea.<sup>93</sup>

## 5.5 EYE SAFETY TO LASER EXPOSURE

In order to ensure noninvasive or least-invasive measurements, eye safety conditions must be satisfied. A reliable reference that researchers cite very often is the American National Standards Institute (ANSI) 136.1 safety standards.<sup>94</sup> Safe use of lasers also requires knowledge of eye physiology<sup>95</sup> in addition to the reference. The maximum retinal irradiance depends on the wavelength of the laser light and the duration of the light exposure. Typical retinal irradiance of laser light is approximately 50 mW/cm<sup>2</sup> in the Doppler method, 20 mW/cm<sup>2</sup> in the biospeckle method, and 2 mW/cm<sup>2</sup> in the visualization methods. Comparison of these values with the maximum permissible exposure (MPE) of the ANSI standards indicates that the values are well below the maximum permissible level for continuous irradiance. The speckle techniques employ an extended spot. In those techniques, the report of Delori et al.,<sup>96</sup> which provides values useful for the extended spot, may be used. An interesting property is found in the wavelength range of red to near infrared. The maximum permissible level of retinal irradiance increases nearly exponentially with the wavelength for a given exposure time.<sup>94</sup> This fact supports the effectiveness of using a near-infrared laser source for studies<sup>54</sup> on the ocular fundus blood flow.

## 6 CONCLUSION

This paper introduced coherent optical techniques for retinal hemodynamics investigations, and discussed their physical fundamentals and the methodologies peculiar to the ocular fundus. The subject of measuring the retinal blood flow is still difficult

and troublesome. However, a variety of techniques developed during the past 25 years surely and steadily contribute to grappling with this problem. The laser Doppler technique has currently provided an advanced instrument that seems to be almost fit for clinical uses, but it should be, for example, combined with measurements of the vessel diameter. In the speckle techniques, some numerical simulation studies may be required for establishing a strong background, which explains the relation between the speckle fluctuations and the blood-flow conditions. Today's advanced optoelectronics enable us to use various laser sources with different wavelengths, high-sensitive avalanche photodiodes, and image sensors, instead of the He-Ne laser sources and photomultipliers used in the past. These modern technologies, combined with a fast computer, will mean good prospects for future techniques.

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