
Review Article

Ocular blood flow assessment using continuous laser Doppler flowmetry

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ABSTRACT.

This article describes the technique of continuous laser Doppler flowmetry (LDF) as applied to the measurement of the flux of red blood cells in the optic nerve head, iris and subfoveal choroid. Starting with the exposition of the physical principles underlying LDF, we first describe the various devices developed to perform LDF in these vascular beds. We then discuss the clinical protocols, blood flow parameters, calibration procedures, reproducibility and limitations of the LDF technique. Various problems still need to be solved in order to bring to light the full potential of LDF in the assessment of microcirculatory haemodynamics.

Key words: choroid – circulatory physiopathology – flux of red blood cells – iris – laser Doppler flowmetry – ocular blood flow – retina

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Preliminary remarks

The intention of this and similar articles is to standardize methods in ocular blood flow assessment that are widely used, but not to the exclusion of other approaches or additional tests that individual laboratories may choose or continue to use. The main objective of these consensus reports is to standardize measurement protocols and promote consistent quality of testing and reporting within and between centres. It is intended as a guideline for clinical and scientific use for the assessment of ocular microcirculation using continuous laser Doppler flowmetry (LDF).

The consensus reported here was achieved by delegating specialists on

the topic to write a draft that was submitted for a first reading to a reading committee. After a first revision, the manuscript was sent to further experts in the field among the group. Their comments were implemented and the manuscript submitted again to the reading committee. Final input was requested from all the members of the Ocular Blood Flow Research Association prior to submission.

Introduction

The present article aims to describe LDF for online continuous recording of blood flow in the tissue of: (i) the optic nerve head (ONH); (ii) the sub-

foveal choroid; and (iii) the iris. The importance of such measurements is twofold: (i) scientific, by gaining insight into the physiology of the deep vascular beds that are under local and central nervous control; and (ii) clinical, through the possibility of assessing alterations of blood flow and its regulation early in the course of a disease, whether these alterations are associated with specific ocular diseases or resulting from systemic disturbances. Furthermore, the evaluation of the effect of treatment on the disturbed flows also represents an important area of application of LDF.

Basic technology

The advent of the laser, a device that emits optical waves of almost a single frequency, has made possible the detection with extremely high resolution of the Doppler shift that light undergoes when scattered by a moving particle. This has allowed the measurement of a broad range of particle velocities (from $\mu\text{m}/\text{second}$ to many km/second). Following the pioneering publications of Stern et al. (1977), who proposed examining skin blood flow by using coherent light scattering, and Bonner & Nossal (1981), who established the basic principle of LDF and a model thereof in microvascular tissue, Riva et al. extended this technique to the measurement of blood flow in the vascular beds of the ONH (Riva et al. 1992, 1982; Petrig & Riva

1999), subfoveal choroid (Riva et al. 1994a, 1994b) and iris (Chamot et al. 1999).

Underlying physical principle

The basis of LDF is the Doppler effect, first described in 1842 by the Austrian physicist Christian Doppler in an article entitled ‘On the colored light of double stars and some other heavenly bodies’, which described the frequency shift that a sound or light wave undergoes when emitted from an object that is moving away from or towards an observer. The Doppler effect manifests itself, for example, in the increased pitch of the siren of an approaching ambulance.

Consider a single particle such as a red blood cell (RBC) moving at velocity \vec{V} in the direction shown in Fig. 1. A laser beam of single frequency f_i is incident on this RBC at an angle α_i with \vec{V} and scattered by the RBC in various directions. In the direction of the detector, defined by α_s with \vec{V} , the frequency of this light differs from f_i by an amount

$$\Delta f = n \left| \vec{V} \right| (\cos \alpha_s - \cos \alpha_i) / \lambda,$$

where Δf is the so-called Doppler shift. Its magnitude depends upon $|\vec{V}|$, α_s , α_i , the index of refraction n of the medium containing the particle and the wavelength λ of the laser light in vacuo. Although extremely small compared to f_i ($\sim 5 \times 10^{14}$ Hz), this shift can be detected using optical mixing spectroscopy (OMS) in the autodyne mode (Benedek 1969). This OMS technique works as follows when applied to the detection of Doppler

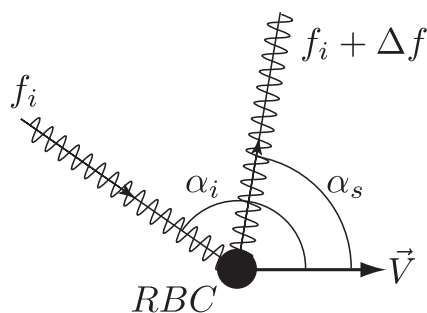


Fig. 1. The Doppler effect. The frequency of the light scattered by the particle [red blood cells (RBC)] with velocity \vec{V} in the direction defined by α_s is shifted in frequency by an amount Δf compared to that of the light of frequency f_i , which is incident on the RBC at an angle α_i .

shifts in the light scattered from a microvascular bed:

Let us consider a laser beam illuminating a number of RBCs moving through a network of capillaries at various velocities and in different directions (Fig. 2). The light scattered by the RBCs consists of a summation of waves with various Δf s. It can be generally noted that most of the light emerging from a tissue has been scattered solely by static structural components of the tissue. This non-shifted light acts as a reference signal that is mixed at the surface of the photodetector with the Doppler-shifted light scattered by the moving RBCs (Cummins & Swinney 1970). Because the detector squares the sum of the shifted and non-shifted light (square-law detector) at its surface, its output current contains only the components oscillating at the various Δf s, not the original f_i or f_s . A plot of the power of the photocurrent as a function of Δf constitutes the Doppler shift power spectrum (DSPS). Furthermore, the Doppler shift of the light components can be positive or negative, depending on the direction of the various velocities of the RBCs relative to the incident light and detector directions. Because the ‘square law’ detectors used typically in LDF devices (photomultiplier tubes or avalanche photodiodes) cannot discriminate between positive and negative Doppler shifts, the DSPS spans only positive frequencies.

Devices available to record the DSPS

Fundus camera-based system for ONH and subfoveal choroidal blood flow

As described originally by Riva et al. (1981) (Fig. 2A), an optical system adapted to a standard fundus camera delivers a laser beam from an He-Ne (632.8 nm) or diode laser (670 nm) to a discrete site at the optic disc for optic nerve blood flow (F_{onh}) or centred on the fovea for subfoveal choroidal blood flow (F_{ch}) measurements. The diameter of the illuminated spot at the fundus is about $150 \mu\text{m}$. The scattered light is collected by an optical fibre with the image of its aperture focused onto the illuminated site (also approximately $150 \mu\text{m}$ in diameter). This fibre guides the scattered light to a photodetector. The fundus is illuminated in red-free light (30° angle) for observation and positioning of the

laser beam. For some applications requiring measurements in darkness, a laser probing beam in near-infrared (IR) (between 750 and 805 nm) is used and the fundus is also illuminated in near-IR light (826 nm). In this case, observation of the fundus is achieved by means of a charged-coupled device (CCD) camera and video monitor (Riva et al. 1987; Logean et al. 2005). In addition, the position of entrance of the laser beam at the pupil can be monitored by means of a CCD finger camera (Logean et al. 2005). For precise placement of the probing laser beam at the desired site of the optic disc for F_{onh} , a point-like fixation target is presented to the patient. It consists of the aperture of a $50\text{-}\mu\text{m}$ -diameter optical fibre illuminated by a red or green diode, which can be focused in the conjugate retinal plane behind the ophthalmoscopic lens and moved in this plane to move the patient’s eye to the desired retinal location. For the investigation of the effect of increased retinal activity on F_{onh} , a system for delivering visual stimuli (flicker and contrast reversal pattern) has been adapted to the fundus camera-based instrument (Logean et al. 2005).

Confocal laser Doppler flowmeter

A newly developed compact device now allows the measurement of F_{ch} in a confocal arrangement of the laser delivery and detection systems (Fig. 2B). This instrument, which simplifies the alignment with the patient’s eye, detects the light scattered by the RBCs within an annulus ($180 \mu\text{m}$ at the fundus) centred on the illumination site of the laser at the fovea (Geiser et al. 1999). A miniaturized version of this instrument has been mounted on an helmet (Geiser et al. 2001), thus facilitating measurements during various types of dynamic physiological manoeuvres, such as cycling. In both instruments, the illuminated laser spot ($12 \mu\text{m}$ at the fovea) and the detection annulus are self-aligned. The pupil of the optical system is small enough (4 mm) to allow measurement without pupil dilatation.

Iris LDF flowmeter

For measurements of blood flow through the microcirculatory network of the iris (F_{iris}) of the human eye, the laser delivery system, photodetector

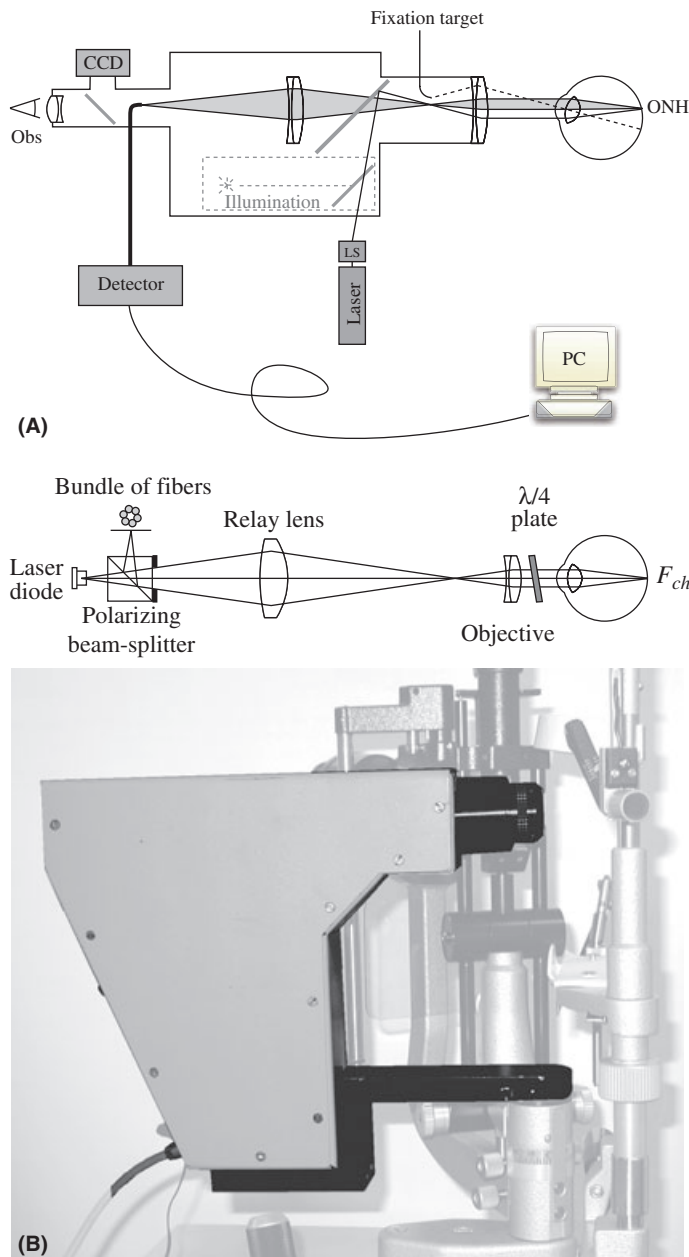


Fig. 2. (A) Optical scheme of a fundus-camera-based laser Doppler flowmetry (LDF). The probing laser beam is delivered to the fundus through a steering system (LS) along the optical path of the fundus illumination (visible or near-infrared). Observation (Obs) is achieved through an ocular when using a laser beam in the visible range of the spectrum or by means of charge-coupled device (CCD) camera and video monitor. A point-like fixation target (aperture of a thin optical fibre) is mounted in the retinal plane behind the ophthalmoscopic lens and is focused on the retina of the tested eye. Detection of the scattered light is achieved by means of an optical fibre connected to a detector. (B) Optical scheme of the compact confocal flowmeter and approximately scaled photograph of the device for F_{ch} measurements. A polarized diode laser point source is focused on the fovea. The light scattered by the region of the choriocapillaris and possibly by some larger vessels behind this layer is directed along the same optical path to reach a bundle of fibres arranged on an annulus. The latter is centred on the image of the illuminated volume. The fibre bundle guides the light to an avalanche photo-diode detector. A $\lambda/4$ plate (an optical element that transforms a linear polarized input light signal into a circular polarized output signal) is placed between the eye and the objective with the function of preventing stray light from being detected to improve the relative strength of the signal (Geiser et al. 1999, 2001).

and target fixation devices were adapted to a slit lamp (Chamot et al. 1999). The delivery system consisted

of two collinear laser beams: a near-IR probing beam (787 nm) and a red beam (670 nm). The 670 nm beam

defined the location of the IR probing beam at the iris. Total power at the cornea was $150 \mu\text{W}$, ensuring the safety of the ocular fundus for 10 seconds in case the probing beam accidentally reached the ocular fundus. The original green illumination of the slit lamp was used to constrict the pupil of the patient and allow the operator to observe the iris and maintain the beam at the desired measurement site. The scattered light was collected by an optical fibre (aperture diameter = $250 \mu\text{m}$) placed directly on the image of the illuminated spot and fed to an avalanche photodiode.

Clinical protocol

Preparation of the patient

With the fundus camera LDF system, adequate dilatation of the patient's pupil is necessary. Because it cannot be excluded that α -adrenergic agonists like phenylephrine influence the ONH and choroidal circulation (Busch et al. 1991; Takayama et al. 2009), a muscarinic antagonist (i.e. tropicamide) is recommended for pupil dilatation. However, the measurement of F_{ch} with the confocal instrument or with the fundus camera near-IR system does not require pupil dilatation. Before starting the measurements, a resting period should be scheduled in order to obtain stable cardiovascular conditions, as evidenced by a stable blood pressure and heart rate. Other requirements have to be considered in designing the study protocol, such as abstinence from coffee, alcohol drinking and smoking, refraining from a heavy meal, exercise, etc, all of which may affect the LDF measurement.

Evaluation procedure

For F_{onh} , the instrument is aligned with the patient's eye, as one usually proceeds with a retinal fundus camera. Under observation of the fundus, the laser beam is directed at the disc. To record the flux of the RBCs moving in the capillary bed, the beam is aimed at disc sites mostly free of visible vessels. In particular, it is important to avoid the large vessels where the motion of RBCs is unidirectional. This aiming is achieved by moving the target at which the patient is fixating or by positioning the laser beam in the x-y directions.

Once the beam is at the chosen location, it is focused and the aperture of the optical detection fibre guiding the scattered light to the detector (photomultiplier tube or avalanche photodiode) is placed above the retinal image of the sampled volume. For F_{ch} , either with the camera-based system or with the confocal system, the patient is asked to look directly at the probing beam. To perform measurements of F_{iris} , the red laser beam mounted on a slit lamp is aimed at a small area of the tissue while the patient is fixating a point-like target (Chamot et al. 1999).

Functional stimuli applicable

The property of the LDF parameters to provide changes of flow that are proportional to the real changes of tissue blood flow makes this technique particularly suitable for investigating the regulation of F_{onh} and F_{ch} in response to various physiological stimuli. For studies of F_{onh} in normal patients, these stimuli include: decreases in mean ocular perfusion pressure (PP_m) induced by increases in intraocular pressure (IOP) (Pillunat et al. 1985; Riva et al. 1997a, 1997b, 1997c) and by increases in IOP combined with squatting (Polska et al. 2007); increases in PP_m produced by increases in systemic blood pressure with isometric exercises (Movaffaghy et al. 1998); and breathing of pure O_2 (hyperoxia) and mixtures of O_2 and CO_2 (Harris et al. 1996), including carbogen and increased neuronal activity (Riva et al. 2001; Falsini et al. 2002; Garhöfer et al. 2002; Riva et al. 2004a, 2004b, 2005).

Investigations of F_{onh} in diseases include studies of baseline haemodynamic parameters (Grunwald et al. 1998a, 1998b, 1998c, 1999; Piltz-Seymour 1999; Riva et al. 2004a, 2004b), the effect of flicker (Riva et al. 2004a, 2004b), of changes in PP_m and of drugs on these parameters in glaucoma patients (Pournaras & Riva 2001; Fuchsjäger-Mayrl et al. 2004; Pournaras et al. 2004a, 2004b; Weigert et al. 2005). Furthermore, important information has been obtained regarding the clinical outcome of patients from the measurement of F_{onh} and the prognostic significance of glaucomatous damage (Zink et al. 2003).

Measurements of F_{ch} and F_{iris} are recent. Studies in humans include the

effect on F_{ch} of increases in PP_m by means of static (Riva et al. 1997a, 1997b, 1997c) and dynamic (Lovasik et al. 2003) exercises; decreases of PP_m by scleral suction cup (Riva et al. 1997a, 1997b, 1997c); valsalva manoeuvres (Riva et al. 1994a, 1994b); breathing of various gas mixtures (pure O_2 , O_2 and CO_2) (Geiser et al. 2000; Gugleta et al. 2005); the effect of light and darkness (Longo et al. 2000; Fuchsjäger-Mayrl et al. 2001, 2003; Huemer et al. 2007) and choroidal vascular reaction to hand-grip stress (Gugleta et al. 2003). The effect of aging, age-related macular degeneration and choroidal neovascularization have been reported (Grunwald et al. 1998a, 1998b, 1998c; Pournaras et al. 2004a, 2004b, 2006). Schmetterer and colleagues in Vienna have studied the effect of numerous pharmacological agents on F_{ch} (Riva & Schmetterer 2008). The technique has been applied to investigate the effect of increased IOP on F_{iris} in humans (Chamot et al. 1999, 2000).

Specific parameters

Vascular beds and the measured haemodynamic parameters

In the measurements of F_{onh} , F_{ch} and F_{iris} using the fundus camera and slit-lamp-based systems, the LDF parameters are calculated based on the model of Bonner & Nossal (1990). This model assumes that the light impinging on the RBCs is completely randomized and that the network of microvessels in the tissue is random on a length scale defined by the mean distance between scattering events by the RBCs. Consequently, when calculating the Doppler shifts in scattered photons, one assumes that the RBCs' velocities are also distributed randomly in direction. The LDF parameters are derived from the DSPS in real time (21 times/second) using an algorithm operating on a NeXT computer (Petrig & Riva 1996) and more recently on a PC (Petrig et al. 2000). This algorithm includes a fast Fourier transformation of the detector signal. The measured parameters are: Vel , the mean speed of the RBCs in the sampling volume (proportional to the mean Δf); and Vol , the number of moving RBCs in this volume (propor-

tional to the area under the DSPS curve). These LDF parameters are determined using the following equations:

$$Vel = \frac{\int_{30Hz}^{\Delta f_{high}} \Delta f P(\Delta f) d\Delta f}{\int_{30Hz}^{\Delta f_{high}} P(\Delta f) d\Delta f},$$

$$Vol = \frac{1}{A_{dc}^2} \int_{30Hz}^{\Delta f_{high}} P(\Delta f) d\Delta f,$$

A_{dc} is the amplitude of the direct photocurrent (DC, non-Doppler-shifted light). Doppler shifts below 30 Hz are filtered out to avoid artifacts caused by slow motion of the tissue and eye motion. For F_{onh} and F_{iris} measurements, Δf_{high} is set at 3000 Hz because the Doppler shifts from this tissue are below this value. For F_{ch} , a value of 20 000 Hz is most often appropriate. The RBC flux in the ONH (F_{onh}) is calculated as $F_{onh} = k \times Vel_{onh} \times Vol_{onh}$. A similar definition applies for F_{ch} and F_{iris} . Vel_{onh} is expressed in Hz and Vol_{onh} and F_{onh} are in arbitrary units; k is a proportionality constant. The same applies for the LDF parameters measured from the choroid and iris.

The software for determining these LDF parameters also allows rejection of the blinks or other spurious signals caused by eye motion, which cause gaps in the continuous display of the data. However, all data between blinks are analysed and can be displayed with various time constants. In addition, this software allows averaging of the signal according to the phase within the heart cycle. This permits precise measurement of systolic and diastolic values of each parameter. As an illustrating example, Fig. 3A shows continuous recordings of Vol_{onh} , Vel_{onh} and F_{onh} obtained from a temporal site of the ONH of a volunteer under resting conditions. At the right of the figure, the time course of these parameters was obtained by averaging the recordings during 20 seconds (shaded area) according to the phase of the heart cycle.

In addition to these parameters, the DC term ($= A_{dc}^2$) is also recorded (not shown in Fig. 3A). For repeated measurements from the same site of the fundus, it is important to maintain

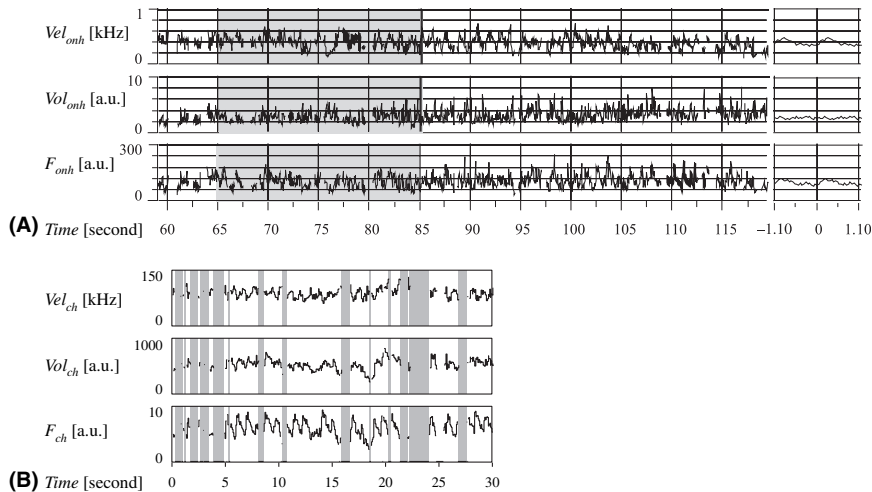


Fig. 3. (A) Recordings of the flow parameters (Vel_{ohh} , Vol_{ohh} and F_{ohh}) using the fundus-camera-based system and the algorithm developed for the NeXT computer (Petrig & Riva 1999) and (B) the confocal laser Doppler flowmetry (LDF) and Labview-based analysis (Geiser et al. 1999). At the right of the recordings in (A) we have displayed the variations during the heart cycle of the LDF parameters (shown twice). They were obtained by averaging the recordings during 20 seconds (shaded area) according to the phase of the heart cycle. The grey bars in the recordings of the F_{ch} parameters in (B) represent the portions of the signals that occurred during eye or head motion and blinks. These portions are automatically removed before computation of the flow parameters.

this term constant (arbitrarily within 10% of the mean) to ensure that the variations of the LDF parameters are not caused by variations in the intensity of the probing beam at the fundus or variations in the site of measurements. Constancy of the DC can be improved by maintaining the same entrance location of the probing beam at the pupil of the tested patient, as described in section Devices available to record the DSPS.

With the confocal flowmeter, the signal is digitized at 240 kHz and analysed 11 times per second using a program written in Labview running either with Windows or Linux. This program uses the same algorithm that was originally developed for the NeXT (Petrig & Riva 1996) and PC (Petrig et al. 2000) systems. In general, a record of 30 seconds is stored in memory for further analysis and determination of the LDF parameters (Fig. 3B). Typically, as described earlier, only measurements where the DC value does not vary by more than $\pm 10\%$ of the most probable DC level are kept for further analysis. Furthermore, the software automatically removes signal noise caused by blinks and microsaccades. For research purposes, the fundus-camera-based LDF is available from Oculix, Sarl (Arbaz, Switzerland) and the F_{ohh} confocal system can be obtained from the

Haute Ecole Spécialisée de Suisse Occidentale (Sion, Switzerland).

Why the LDF parameters are expressed in relative units

It is important to understand the reasons why LDF provides only relative measurements (Riva & Petrig 2003). Indeed, laser radiation upon a tissue undergoes scattering and absorption. Both processes influence the penetration pattern of the laser light. Penetration may differ from one region of a tissue to another, depending on the optical properties of the tissue. Thus, spatial or temporal variations in tissue structure and vascularization – as is the case, for example, in the ONH in glaucoma – will affect the LDF measurements. Furthermore, direct comparison between the LDF values from different tissues may not be valid because of variations in optical properties resulting from differences in tissue structure and composition. In particular, comparing F_{ohh} data from healthy individuals and patients with glaucoma or F_{ch} data from healthy individuals and patients with age-related macular degeneration (AMD) may be problematic under certain conditions. Furthermore, measurements obtained with different lasers may not be comparable if the optical characteristics of the lasers (wavelength, beam divergence, penetration

into the tissue) are different. The measured flow is usually referred to as ‘blood flow’. However, what is actually measured is the flux of the RBCs (Shepherd 1990; Petrig & Riva 1999). Blood flow changes are directly proportional to changes in RBC flux only if the haematocrit remains constant during the changes in flux.

Linearity of the LDF parameters

Linearity between the changes in the LDF measured blood flow and the changes in the real tissue flux of the RBCs has been documented for various tissues, such as the skin, skeletal muscle, cerebral cortex, nerves and others (Shepherd 1990). The assumption of linearity of the flow as determined by LDF has been shown to be valid for F_{ohh} and F_{ch} by demonstrating the effect of a rapid decrease in PP_m (i.e. within a few seconds) on these flows in animals (Riva et al. 1992, 1994a, 1994b). This property of linearity is most important because it justifies using the LDF technique to determine changes in flow in response to various physiological stimuli or pharmacological interventions, even though the measurements are in relative units.

Calibration

Preparation of the device

Under calibration, we mean here that an LDF instrument provides the same laser intensity at its output over time, because the LDF parameters are affected by this intensity. Keeping this intensity constant makes it possible to compare the LDF parameters obtained over periods of time. Although this procedure has not been followed so far, it would be advisable to check periodically the values of the LDF parameters using a test object, such as a diffuser disc rotating at a known speed. The laser power used for F_{ohh} and F_{ch} measurements is kept below 100 μW ; it is 150 μW for F_{iris} . These values are well below the maximum permissible level of illumination as defined by ANSI Z136.1 guidelines.

Reproducibility

Because the LDF parameters are expressed in relative units, the potential of LDF is realized mainly in the assessment of the responses of these

parameters to changes in blood flow induced by physiological stimuli or by pathologies that do not alter the optical properties of the tissue. These stimuli have been mentioned earlier. In this type of application, the sensitivity of each LDF parameter – i.e. the minimum statistically significant change that can be determined in response to a given stimulus for a given population of patients – depends mainly upon the precision with which patients can fixate a target, the type of stimulus, the site of measurements and other factors. In addition, in the studies of the regulation of blood flow requiring a prolonged measurement time, or when performing a series of measurements from the same site of the ONH (Riva et al. 2001; Falsini et al. 2002) or subfoveal choroid (Franklin et al. 1961; Fraunfelder & Meyer 1985; Geiser et al. 2000), it is important to maintain the DC signal quasi-constant across all measurements, because the blood flow values depend in part on the intensity of light incident on the tissue. Because the DC signal is strongly dependent on the scattering properties and beam incidence angle, keeping the same DC in successive measurements from the same eye location in a given patient's eye is a good indicator for the maintenance of constant beam incidence and tissue scattering.

The reproducibility of LDF for F_{onh} has been assessed in humans (Joos et al. 1997). Increased technical experience of the LDF device's operator decreases the variability of the measurements. By averaging five measurements for each session, an intra-session variation of 18% for Vel_{onh} and 24% for F_{onh} and an inter-session variation of 12% for Vel_{onh} and 32% for F_{onh} were reported. Inter-examiner variance was small. The measured variability includes components from both technical/measurement error and physiological variation. Sample size estimates were computed for experimentally induced changes in flow in single and multiple sessions: to detect a 15% difference in F_{onh} with 80% power by means of a paired *t*-test seven patients would be needed to evaluate changes within one session, whereas 43 patients would be needed to detect a change between two sessions. Therefore, LDF was found to be useful in evaluating F_{onh} in humans, particularly when

acute perturbation experiments within a single session were performed. LDF was judged to be suitable for assessing differences between patient populations (Grunwald et al. 1999). A similar conclusion was drawn for healthy individuals from a study that also demonstrated no diurnal variation in F_{onh} (Luksch et al. 2008). Comparing measurements in patients with glaucoma and controls, and using power calculations based on a non-paired test with a pooled variance estimate, the statistical power to detect a 20% decrease in the glaucoma patients was 80% or greater for a sample size of 24 glaucoma patients and 14 controls. Variability in terms of coefficient of variation (CV) of the F_{onh} LDF parameters measured at three measurement sites in normal controls ($n = 13$) and glaucoma patients with and without systemic hypertension provided the following values: CV (Vel_{onh}) = 16%, 17% and 12%, CV (Vol_{onh}) = 20%, 15% and 22% and CV (F_{onh}) = 21%, 20% and 13%, respectively (Grunwald et al. 1999).

The reproducibility of F_{onh} responses to flicker was determined based on consecutive trials performed during sessions of less than 19 min duration (Riva et al. 2001). The laser beam was aimed at the temporal rim of the ONH in the right eye. Using two different stimuli, a pure red and a pure green illuminance flicker, CV (F_{onh} response) was 26% ($n = 5$ trials) and 12% ($n = 4$), respectively.

For F_{ch} , the CV (Vel_{ch} , Vol_{ch} and F_{ch}) were reported to be 14%, 25% and 18%, respectively, in patients with AMD and 8%, 18% and 13%, respectively, in normal controls (Grunwald et al. 1998a, 1998b, 1998c). No statistically significant differences were observed between these CVs for patients with AMD and normal controls (Grunwald et al. 1998a, 1998b, 1998c). Another study in eyes with AMD of increasing severity found a CV (F_{ch}) of $10.3\% \pm 7.2\%$ ($n = 3$ trials) (Grunwald et al. 2005).

Investigation of the reliability and sensitivity of confocal F_{ch} led to the finding that tissue-scattering properties and recording settings alter the characteristics of the detected light and, consequently, the outcome of the analysis (Gugleta et al. 2002). Measurements in 10 individuals provided a sensitivity of the LDF parameters of 7.5–17.5% for

Vel_{ch} , 14.5–37.8% for Vol_{ch} and 19.2–31.6% for F_{ch} . A study in 14 patients indicated values of 6.3% for Vel_{ch} , 7.4% for Vol_{ch} and 10.4% for F_{ch} (Vinh-Moreau-Gaudry et al. 2009). In another study, intra-patient reproducibility was found to be better than 8% for Vel_{ch} and Vol_{ch} and better than 18% for F_{ch} (Geiser et al. 2000). The sensitivity of the measurements was 3.3% for Vel_{ch} , 7.1% for Vol_{ch} and 7.4% for F_{ch} (21 patients). No diurnal variation in F_{ch} was found based on a study in 16 patients and five measurement cycles between 08.00 and 20.00 hr, which provided a CV (F_{ch}) of $11.5 \pm 3.3\%$ (Polska et al. 2004).

Main limitations

A central question in the application of LDF to the ONH is the depth of the sampling volume. This depth determines the relative contribution to the Doppler signal of the superficial layers, those supplied by the central retinal artery, and the deeper layers supplied by the short posterior ciliary arteries. These two vascular beds may have different blood flow regulation properties. Furthermore, the deep layers of the ONH appear to be particularly susceptible to ischaemic disorders, including glaucoma and non-arteritic ischaemic optic neuropathy (Hayreh 1969).

Investigations on a model system based on excised ONH tissue of various thickness suggest that, when the light-collecting aperture coincides with the tissue volume illuminated by the probing laser, layers of the ONH tissue of as deep as 300 μm contribute to the signal (Koelle et al. 1993). A study on monkey eyes concluded that LDF is predominantly sensitive to blood flow changes in the superficial layers of the ONH and less to those layers of the prelaminar and deeper regions of the ONH, and that their relative proportions are still unknown (Petrig et al. 1999). At present, the weaker signal from the deep layers cannot be separated from the dominant signal from the superficial layers to study the circulation in the deep layers exclusively.

A similar limitation is present in the case of F_{ch} . However, the data suggest that the Doppler signal originates predominantly from the RBCs in the choriocapillaris (Riva et al. 1994a, 1994b). In the case of confocal LDF, where the scattered light is detected

by an annulus centred on the focused probing beam spot, preliminary work also suggests a more significant contribution from the tissues beyond the choriocapillaris.

Reporting

As mentioned earlier, the data obtained with LDF are the haemodynamic parameters *Vel*, *Vol* and *F* for the different vascular beds discussed here. Furthermore, a number of studies have investigated the response of these parameters, i.e. their per cent changes from their baseline values to various physiological stimuli. Under these conditions, it is important to keep the DC value of the signal as constant as possible between the measurements of the baseline and the responses.

The NeXT- and PC-based software provides a graph of the change in each haemodynamic parameter during the cardiac cycle from which the pulsatility (*P*) can be calculated. This parameter is defined as $P(X) = (X_{\text{syst}} - X_{\text{diast}}) / (X_{\text{syst}})$, where *X* stands for any of the LDF parameters from the ONH, choroid or iris (Petrig & Riva 1999; Petrig et al. 2000). When the diastolic value is zero (systolic > 0), $P(X) = 1$. When systolic and diastolic values are equal (> 0), $P(X) = 0$. Thus $P(X)$ is always between 0 and 1 for any LDF parameter.

Unsolved open questions

Based on the content of the material developed up to this point, we believe that a better understanding of the following questions could markedly improve LDF and provide a better understanding of the data obtained with this technique. The list of questions is by no means exhaustive. Thus, it would be important to know:

(1) The exact sampled volume at the ONH and subfoveal choroid. In particular, one would like to know the contribution of the different layers of the optic nerve to the signal and the contribution of the choriocapillaris to the choroidal LDF parameters versus that of the larger choroidal vessels.

(2) The optimum probing beam wavelength to use with regard to defining the penetration of the laser beam and the sample volume.

(3) The effect of the entrance of the probing laser beam at the pupil, in particular when measuring F_{onh} , as the angle at which the probing beam impinges on the ONH rim tissue varies when the entrance pupil is moved within the pupil. Clearly, the sampled volume is dependent on the angle α_i at which the beam hits the optic disc, because of the structure and curvature of this tissue. This is especially true in glaucoma patients with a large cup-to-disc ratio.

(4) The effect of beam focus, ocular fundus pigmentation and ocular refraction on the LDF parameters.

(5) The effect of media opacities on the LDF parameters.

(6) The influence of pupil dilatation on the haemodynamic parameters.

(7) The best physiological stimuli to use clinically in terms of the optimal separation between the flow values obtained in pathological conditions and the normal values.

(8) How pathologies influence the scattering properties of the tissue, and consequently the DC value of the Doppler signal, so that changes in the LDF signal are not interpreted as haemodynamic changes when in fact these are caused by morphological changes leading to optical changes.

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