LASER DOPPLER INSTRUMENTATION FOR THE MEASUREMENT OF RETINAL BLOOD FLOW: THEORY AND PRACTICE

FEKE G.T.[1]

ABSTRACT

The theory underlying the development of laser Doppler instrumentation for the measurement of retinal blood flow is framed in terms of (a) the enunciation of the Doppler principle; (b) the invention of the laser; and (c) the invention of the technique known as optical mixing spectroscopy. The features of the instrumentation, beginning with the first prototype in 1972 and culminating with the introduction of the Canon Laser Blood Flowmeter in 1998 are presented in detail. Results from seven separate studies reporting on the reproducibility of retinal blood flow measurements using the Canon instrument, as well as a review of 12 separate presentations made at the 2004 annual meeting of the Association for Research in Vision and Ophthalmology (ARVO) using the Canon instrument in studies involving retinal circulatory physiology and associated clinical research are also presented.

KEYWORDS

Laser Doppler, retinal blood flow, optical mixing spectroscopy, Canon Laser Blood Flowmeter

RÉSUMÉ

La théorie à la base du développement de l'instrumentation du laser Doppler pour la mesure du flux sanguin rétinien est structurée en termes de (a) l'énonciation du principe Doppler; (b) l'invention du laser; et (c) l'invention de la technique connue sous le nom de spectroscopie à mélange optique. Les caractéristiques de l'instrumentation, débutant avec le premier prototype en 1972 et culminant avec l'introduction du débitmètre sanguin laser Canon en 1998 sont présentées en détail. Les résultats de sept études distinctes sur la reproductibilité des mesures de flux sanguin rétinien en utilisant l'instrument Canon, ainsi qu'un compte-rendu de 12 présentations différentes réalisées lors de la réunion annuelle de 2004 de l'Association for Research in Vision and Ophthalmology (ARVO) utilisant l'instrument Canon dans des études impliquant la physiologie circulatoire rétinienne et la recherche clinique sont aussi présentés.

MOTS-CLÉS

Laser Doppler, flux sanguin rétinien, spectroscopie à mélange optique, débitmètre sanguin laser Canon

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[1] *Schepens Retina Associates Foundation for Clinical Research, Boston, MA*

INTRODUCTION

Any discussion of the theory underlying the development of laser Doppler instrumentation for the measurement of retinal blood flow must be framed in terms of the confluence of three major events. First was the enunciation of the Doppler principle. Second was the invention of the laser. Third was the invention of the technique known as optical mixing spectroscopy that made the measurements possible.

Specific to the case of blood cells moving through blood vessels is the additional theoretical question of the description of the propagation and scattering of laser light in a dense suspension of "particles" that are large compared to the wavelength of the probing radiation.

Finally, specific to the case that the blood vessels are in the retina of a living human eye, are laser safety issues and, perhaps, the greatest engineering challenge of all, the need to overcome involuntary eye movements and maintain the incident laser beam on the exact center of the target blood vessel for a sufficient length of time to acquire data over several cardiac cycles.

THE DOPPLER PRINCIPLE

When a source of sound moves toward or away from a listener, the pitch, or frequency of the sound, is higher or lower than when the source is at rest. Today, a common example is the rise and fall of the pitch of an ambulance siren as it approaches and recedes from a listener. Similarly, as one approaches and then recedes from a stationary sound source, a rise and then fall of the pitch is also heard. These phenomena, termed "Doppler" shifts, are named for Christian Johann Doppler, an Austrian physicist who first considered them in 1842. Doppler also predicted in that year that the color of a luminous body in motion would also change in a similar manner, due to the relative motion of the body and the observer.

Thus, when a luminous source emitting visible light waves approaches an observer, the waves in front of the moving source are crowded together so that the observer sees a larger number of waves in a given time that would have been seen if the source were stationary. The perceived higher frequency and shorter wavelength make the source appear more blue. Similarly, if the source is moving away from the observer, the waves are spread apart so that the observer sees a fewer number of waves in a given time that would have been seen if the source were stationary. The perceived lower frequency and longer wavelength make the source appear more red.

The magnitude of the Doppler shift depends directly on the relative velocity between the source and the observer. Thus, measurement of the Doppler shift provides a measure of the velocity of moving objects. For a Doppler shift to be measurable, however, the fractional change in wavelength or frequency must be measurable. This fractional change is given by the ratio of the velocity of the moving object to the velocity of the emitted radiation. All electromagnetic radiation travels at the speed of light, which is 3×10^8 meters per second in a vacuum. For an object that appears red (eg. 633 nm wavelength) when stationary to then actually appear blue while moving (eg. 488 nm wavelength) would require a velocity of 6.9×10^7, nearly one-fourth of the speed of light! Realistically then, measurable changes in wavelength occur only in the astronomical realm where distant galaxies recede at extremely high velocities, or in the sub-atomic realm where the emission of gamma rays from radioactive nuclei are studied using the Mössbauer effect.

More readily measured are fractional changes in the frequency of the detected electromagnetic radiation. Radio waves are a form of long wavelength-low frequency electromagnetic radiation. Air traffic control RADAR (RAdio Detection And Ranging) systems operate at approximately 1000 megahertz, or 10^9 Hz. Global positioning radar systems operate at approximately 1.5×10^9 Hz. Doppler Radar is now commonly used to track weather systems and for the surveillance of automobile speeds. Visible light radiation, however, oscillates at much higher frequencies. Light from a He-Ne laser (wavelength = 633 nm) oscillates at a frequency of 4.7×10^{14} Hz. Doppler frequency shifts due to blood moving in the retinal circulation are now known to range from zero to approximately 50 kHz. Thus, the resolution required to measure these Doppler frequency shifts is of the order of one part in 10^{11}.

LASERS IN THE EYE

The first successful operation of a LASER (Light Amplification by Stimulated Emission of Radiation) was reported in 1960.[23] Soon after, ophthalmologists began using lasers as "surgical" tools in the treatment of eye diseases. There has been a steady progression in the usage of laser "surgical" instruments from the earliest ruby laser photocoagulators [3] to the myriad of laser instruments currently available for the treatment of retinal detachment,[41] diabetic eye disease,[6] glaucoma,[12] and age-related retinal degeneration.[22] These laser instruments make use of two of the unique properties of laser light: its high intensity and low beam divergence.
Laser Doppler instruments, however, make use of two other unique properties of laser light: its extreme monochromaticity and its great coherence. The monochromaticity arises from the quantum nature of atomic absorption and emission. When radiation is absorbed or emitted, an atom is raised or lowered from one quantized energy level to another. The energy difference between the levels is related to the frequency, ν, of the radiation as follows:

$$h\nu = E_2 - E_1$$

where h is Planck's constant and E_2 and E_1 are the energies of the upper and lower levels. In principle, the frequency of the radiation is precisely determined. In practice, in a He-Ne laser, there is a spread of approximately 2 Hz in the bandwidth of the 633 nm radiation oscillating at 4.7×10^{14} Hz.[4] This tiny spread is due to atomic collisions.
Until the invention of the laser, electromagnetic radiation in the visible region could not be generated in the same controlled way as in the radio and microwave regions. An optical Doppler instrument for retinal blood flow measurement is thus, of necessity, a laser Doppler instrument.

OPTICAL MIXING SPECTROSCOPY: THE KEY TO THE RETINAL DOPPLER SHIFT

With the advent of the laser, the technique of optical mixing spectroscopy became widely used as a precise analytical means of providing valuable information in the fields of physics, chemistry, biology, and engineering.[2,4] The first application of this technique to the measurement of the velocity of particles suspended in a flowing medium was accomplished by Yeh and Cummins in 1964.[49]
Figure 1 A shows a schematic representation of the illuminated volume of a retinal vessel from which scattered laser light is detected.[35] It is assumed that the incident laser light illuminates the entire cross section of the vessel. According to the Doppler principle, laser light that is scattered from a moving particle is shifted in frequency by an amount

$$\Delta f = (1/2\pi)\,(\mathbf{K}_s - \mathbf{K}_i) \bullet \mathbf{V} \qquad (1)$$

where V is the velocity vector of the particle; K_i and K_s are, respectively, the wavevectors of the incident and scattered light. When $|V| << c$, $|K_s| \approx |K_i| = 2\Pi n/\lambda$, where n is the refractive index of the medium, λ is the wavelength in vacuo of the laser light, and c is the speed of light.
When the incident laser beam uniformly illuminates the entire cross section of a blood vessel, the scattered light contains a range of frequency shifts corresponding to the range of particle velocities that are present. If a suspension of particles undergoes Poiseuille flow, then there exists a continuous range of velocities exhibiting a parabolic profile as a function of the radial distance from the axis of the vessel:[20]

$$V(R) = V_{max}\,[1 - (R/R_o)^2] \qquad (2)$$

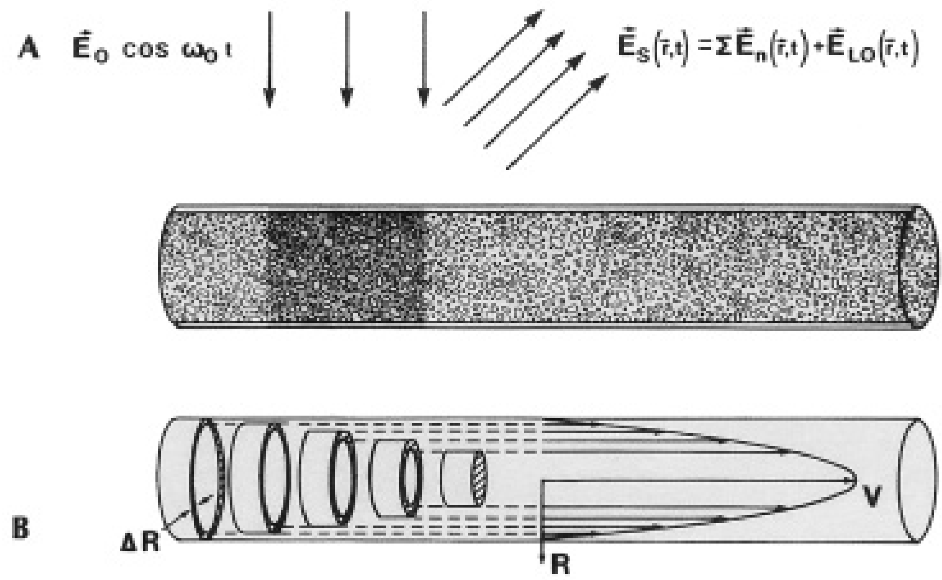

Fig. 1. (A) Schematic representation of the volume (shaded area) of a retinal vessel illuminated by an incoming laser beam (E_O) from which scattered light (E_S) is detected. E_S consists of Doppler-shifted light scattered by the flowing red blood cells as well as unshifted light (E_{LO}) scattered from the vessel wall material. (B) Representation of the parabolic velocity profile present in the retinal vessel according to Poiseuille flow, and the division of the illuminated volume into concentric cylindrical shells of thickness ΔR through which equal numbers of red blood cells flow per unit time. (From: Riva C.E., Feke G.T. - Laser Doppler velocimetry in the measurement of retinal blood flow. In: The Biomedical Laser, L. Goldman (ed.) Springer-Verlag, New York. 1981; 137).[35]

where V_{max} is the velocity at the center and R_o is the radius of the vessel. This is schematically indicated in figure 1 B. Experimental evidence [15] indicates that Poiseuille flow is present in all vessels with a diameter greater than 80 μm.

In the case of Poiseuille flow, as discussed by Riva et al.,[33] there are equal numbers of particles flowing at each increment of velocity, dV, from V = 0 to V = V_{max}. The average velocity is thus (½)V_{max}. The optical frequency spectrum of the light scattered from such a suspension is flat from f_0 to $f_0 + \Delta f_{max}$, and zero elsewhere. f_0 is the frequency of the incident laser light (4.7×10^{14} Hz) and Δf_{max} is the frequency shift corresponding to V_{max}. For maximum speeds of the order of several cm/s and for typical scattering geometries, Δf_{max} is of the order of several kilohertz. The heterodyne mode of optical mixing spectroscopy [2] is used to detect these relatively small frequency shifts.

When optical heterodyne detection is applied to the case where the incident laser light illuminates the entire cross section of a blood vessel, the light scattered at a particular angle by the flowing particles as well as the light scattered from the vessel wall is collected at the surface of a photodetector. The light scattered from the wall is unshifted in frequency and thus acts as the reference beam or local oscillator. The output of the photodetector is a current I(t) which is proportional to the absolute square of the total electric field $\mathbf{E_T}$(t) impinging on its surface.

We may write

$$\mathbf{E_T}(t) = \mathbf{E_{Lo}}(t) + \sum_{i=0}^{max} \mathbf{E_{Si}}(t) \qquad (3)$$

where $E_{Lo}(t)$ is the local oscillator field and each $E_{Si}(t)$ is the field scattered from particles moving at a particular speed V_i in the range V = 0 to V = V_{max}. The photocurrent is thus given by

$$I(t) \approx |\mathbf{E_T}(t)|^2 \approx |\mathbf{E_{Lo}}(t)|^2 + 2|\mathbf{E_{Lo}}(t) \sum_{i=0}^{max} \mathbf{E_{Si}}(t)| + |\sum_{i=0}^{max} \mathbf{E_{Si}}(t)|^2 \quad (4)$$

For pure heterodyne detection the magnitude of the local oscillator field must be much greater than the magnitudes of each of the scattered fields arising from the flowing particles. The third term in equation (4) may then be neglected. If E_{Lo} varies in time as $A_0 \cos\omega_0 t$, then the time dependence of each E_{Si} may be written as $A_i \cos(\omega_0 + \Delta\omega_i)\, t$, where $\omega_0 = 2\,\Pi\, f_0$ is the angular frequency of the incident light and $\Delta\omega_i = 2\,\Pi\,\Delta f_i$ is the shift in the angular frequency of the light scattered from particles flowing at the speed V_i. Since all the A_i are equal for Poiseuille flow, the photocurrent is given by

$$I(t) \approx A_0^2 \cos^2\omega_0 t + 2\, A_0 A \cos\omega_0 t \sum_{i=0}^{max} \cos(\omega_0 + \Delta\omega_i)\, t \quad (5)$$

Writing:

$$\cos^2\omega_0 t = (½)\,(1 + \cos 2\omega_0 t) \quad (6)$$

and

$$\cos\omega_0 t \cos(\omega_0 + \Delta\omega_i)\, t = (½) \cos\Delta\omega_i t + (½) \cos(2\omega_0 + \Delta\omega_i)\, t \quad (7)$$

and noting that optical frequencies are beyond the response range of photodetectors, we see that

$$I(t) \approx A_0^2/2 + A_0 A \sum_{i=0}^{max} \cos \Delta\omega_i\, t \quad (8)$$

The photocurrent thus contains a constant term plus terms oscillating at each difference frequency, $\Delta\omega_i$, in the range $\Delta\omega = 0$ to $\Delta\omega = \Delta\omega_{max}$.

The power spectrum of the photocurrent is measured via Fast Fourier Transform analysis. Since the optical frequency spectrum is flat for Poiseuille flow, the resultant heterodyne Doppler shift frequency spectrum is also flat from $\Delta f = 0$ to $\Delta f = \Delta f_{max}$.

In 1972 Riva et al.[33] first demonstrated the feasibility of using optical mixing spectroscopy to measure blood flow in individual retinal vessels. They were able to measure the Doppler shift frequency spectrum of the light scattered from red blood cells (RBC's) flowing in a retinal artery of an anesthetized rabbit. The incident laser beam power was 10 mW and the measurement time was 2 min. The spectrum exhibited a cutoff frequency, which is necessary for determining the maximum RBC velocity V_{max} directly from the measurement. For Poiseuille flow, determination of V_{max} is sufficient for obtaining the volumetric flow rate F, since $F = ½\, V_{max}\, S$, where S is the cross-sectional area of the vessel at the measurement site.

THE FIRST MEASUREMENTS IN THE HUMAN EYE

In order to apply the technique to blood flow measurements in human retinal vessels, it was necessary to reduce the retinal irradiance to permissible levels and to shorten the measurement time so that the effects of eye movements could be minimized. In an attempt to meet these requirements, Tanaka et al.[46] measured the temporal autocorrelation function of photopulses arising from the light scattered by RBC's flowing in human retinal vessels. The autocorrelation function is related to the frequency spectrum through a Fourier transformation.[32] They used an incident laser beam power of 18 μW, which results in a retinal irradiance well below the maximum permissible level for continuous illumination.[43] Their measurement time of 10 s, however, was not short enough to fully eliminate the effects of eye movements.

The measured correlation functions did not exhibit oscillations corresponding to a cutoff in the spectrum. Instead, they were Lorentzian-like curves exhibiting no distinctive features that could be directly related to the maximum RBC speed. An estimate of the average speed was obtained by using an empirical calibration procedure. The half-width of a correlation function obtained from a retinal vessel was calibrated against the half-width of a correlation function obtained from blood flowing at a known rate through a glass capillary tube with an internal diameter approx-

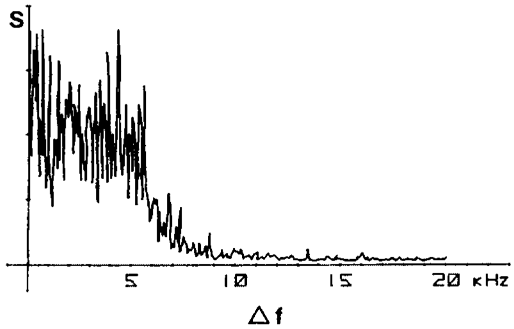

Fig. 2. A Doppler-shift frequency spectrum of laser light scattered from red blood cells flowing through a retinal artery. The spectrum exhibits large fluctuations in power up to a clearly measurable frequency shift, Δf_{max} at approximately 5.5 kHz. This shift arises from scattered light Doppler-shifted by cells flowing at the maximum speed (V_{max}) at the center of the vessel. (From: Feke G.T., Riva C.E. - Laser Doppler measurements of blood velocity in human retinal vessels. J Opt Soc Am 1978; 68: 529).[7]

imately equal to that of the vessel. It was assumed that multiple scattering of the laser light within the blood vessel was responsible for smoothing the oscillations in the correlation function which, if observed, could have been used to directly determine the maximum RBC speed.

In 1978, Feke and Riva [7] presented and interpreted measurements of Doppler shift spectra obtained from human retinal vessels. The spectra exhibited the cutoff frequencies that are necessary for the direct determination of maximum RBC speeds. They were able to measure meaningful spectra from human retinal vessels in times as short as 0.1 s at permissible retinal irradiance levels.

Current applications of the laser Doppler technique for the measurement of blood speed in human retinal vessels have evolved from the specific techniques first described by Feke and Riva.[7] They showed that Doppler-shift frequency spectra of laser light scattered from red blood cells flowing through individual retinal vessels exhibit large fluctuations in spectral power up to a clearly measurable frequency shift, Δf_{max}. This shift arises from scattered light Doppler-shifted by cells flowing at the maximum speed (V_{max}) at the center of the vessel. An example of such a spectrum is shown in figure 2.

With the optical arrangement used in the first instrument [7] however, it was difficult to determine the angle between the incident laser beam and the velocity vector as well as the angle between the detected scattered light and the velocity vector, a requirement for computing the actual blood speed from the measured frequency shift (See equation 1, which involves a vector dot product). Therefore, initial applications of the technique were limited to experimental studies in which the light scattering geometry was held constant while the retinal circulation underwent a perturbation, and to clinical studies involving serial measurements from the same vascular sites. Relative changes in V_{max} were determined from the measured changes in Δf_{max}.

VALIDITY OF THE MODEL

The ability to observe clearly defined maximum frequency shifts, Δf_{max}, in the measured Doppler spectra was surprising since equations 1-8 are based on a model which assumes single scattering of the incident laser light by the RBCs. Since blood is a dense suspension, it would be expected that multiple scattering of the incident light by many RBCs would be a dominant factor, so that light reaching a detector would have undergone a number of Doppler shifts. If that were the case, there would be no clear Δf_{max} that could be related to a single maximum RBC speed at the center of the blood vessel.

This issue was addressed in two studies reported by Riva et al.[37-38] In the first study,[37] the angle between the velocity vector and the plane of the detected scattered light was varied from 0 to 180 degrees as Δf_{max} was measured first from a dilute suspension of polystyrene spheres in water flowing through a 200 μm diameter glass capillary tube placed in the retinal plane of a model eye, and then from RBCs flowing through a 120 μm diameter retinal vein of a healthy subject. The angular dependence of Δf_{max} was identical in both cases, and followed the predictions inherent in the assumption of single scattering. As an explanation, the authors suggested that due to the fact that the light scattered from individual RBCs is overwhelmingly in the forward direction (the mean cosine of the scattering angle at wavelengths in the red is 0.99), the most important contribution to the detected scattered light arises from events where only single backscattering is involved.

Experimental proof for this explanation was presented in the second study.[38] The authors studied the characteristics of Doppler shift spectra obtained from retinal vessels in the cat under two conditions: (1) from vessels located in front of the highly reflective tapetum and (2) from vessels located in front of the highly absorbing pigmented region. The spectra obtained under the two conditions were markedly different from each other. When vessels were in front of the tapetum, the spectral shapes were exponential-like and arose from scattered light that was almost completely depolarized. There were no observable cutoff frequencies, and the spectral widths did not vary with scattering angle. In this case, the main component of the detected scattered light is that which has been multiply scattered on its way through the vessel, reflected by the tapetum, and then multiply scattered again on its way back through the vessel. However, when vessels were in front of the pigmented region, the spectral shapes were much like that shown in figure 2, essentially flat up to a clearly defined cutoff frequency which varied with the scattering angle. In addition, these spectra arose from scattered light whose polarization properties were almost identical to that of the incident beam. Thus it appears that when retinal vessels are located in front of an absorbing pigmented background, the detected scattered light consists primarily of singly backscattered light from the various depths of the blood column as well as a strong component of light scattered by the vessel wall. As pointed out by Riva et al.,[38] a detailed theoretical treatment of laser Doppler measurements in blood [44] predicts that the single backscattering process should allow true Doppler shift measurements of blood velocity to a depth of at least 166 μm.

THE BIDIRECTIONAL LASER DOPPLER TECHNIQUE

In order to facilitate measurements of V_{max} in actual speed units, a new approach, the so-called bidirectional laser Doppler technique,[34] was developed. The instrumentation could now be used for site-to-site comparisons in the same eye, for comparisons between the two eyes in the same patient, and for comparisons between patients. In the bidirectional technique, one incident beam is used to illuminate a site along a retinal vessel. The light scattered by the blood cells at the illuminated site is detected simultaneously in two distinct directions separated by a known, fixed angle. Two Doppler-shift frequency spectra are measured, each with a particular value of Δf_{max}. The difference between the two values is used to calculate the value of V_{max} in units of speed, for example, in cm/sec.

The first instrument to successfully implement the bidirectional laser Doppler technique was described by Riva et al.[36] A standard Topcon retinal camera was modified with a laser delivery system and a bidirectional laser light collection system. The Δf_{max} values present in pairs of Doppler-shift frequency spectra from retinal vessels were determined by visual inspection of the spectra, and the centerline speed was calculated.

The second successful bidirectional laser Doppler system was described by Feke et al.[8] In this system, a Nikon slit-lamp microscope was modified with laser input optics and a pair of photomultiplier tube detectors for collection of the scattered light. A data analysis procedure was designed for use on retinal arterial measurements where the blood speed is pulsatile and synchronous with the cardiac cycle. In this case, Δf_{max} undergoes regular systolic/diastolic variations. A signal processor that produces a continuous output proportional to the instantaneous value of Δf_{max} was used to provide a means of identifying portions of the recorded signal obtained when the incident laser beam was centered on the target retinal vessel. In addition, the processor output provided a reliable means for determining the exact time windows for blood speed measurements at peak systole and minimum diastole during the cardiac cycle, measurements used to calculate the time-averaged value of V_{max} during the cardiac cycle. In this system, Δf_{max} values were also determined by visual inspection of the spectra.

While these instruments proved to be effective in the hands of their developers, they were not amenable to routine clinical use. The determination of Δf_{max} values by visual inspection could be reliably done only by experienced observers. More importantly, an accurate laser Doppler measurement of the centerline blood speed in a retinal vessel depends critically on the centering of the incident beam on the target vessel. Patient eye movements would cause the incident beam to land partially or completely off of the vessel center. In this case, since the blood cell speed is highest at the centerline and decreases as the periphery of the vessel is approached, a blood speed measurement away from the centerline would yield a value lower than the true centerline speed. Such an error would most often occur in measurements on the largest retinal veins.

SOLUTIONS TO THE PROBLEM OF EYE MOVEMENTS

One solution to the problem caused by eye movements would be to employ computer-assisted rejection criteria in an attempt to identify and discard portions of the signal obtained when the incident beam was not well centered. A more direct approach would be to incorporate a feedback and control system into a laser Doppler instrument that would keep the incident beam locked onto the target vessel during eye movements.

The former approach was adopted by Petrig and Riva in the first computerized retinal laser Doppler instrument.[30] A parameter related to the shape of the Doppler-shift spectrum was used to determine whether the spectrum should be accepted as having been measured at the vessel centerline or rejected as having been measured off of the centerline. In the analysis process, the value of Δf_{max} was also determined by the computer. Subsequently, Petrig and Riva replaced the standard visible HeNe laser (λ = 633 nm) used in the instrument with a pair of near-infrared diode lasers(λ = 750-830 nm).[31] They also replaced the standard photomultiplier tube-based light detection system with an avalanche photodiode-based system. These changes produced an increased signal-to-noise ratio in the measured Doppler spectra due to the elevated allowable incident beam intensity in the infrared as well as an improved quantum efficiency in the light detection system. Perhaps the most important outcome of these modifications was the newfound ability to use light itself as a stimulus for inducing retinal hemodynamic changes without any contamination from the incident beam used for the Doppler measurements. This opened the door for a series of experimental studies of the phenomenon of neurovascular coupling in the retina.

The latter approach, involving the use of eye tracking techniques to maintain the centering of the incident laser beam on the target retinal vessel during a laser Doppler measurement, was adopted by Milbocker et al.,[25] Milbocker and Feke,[26] and Mendel et al.[24] in a series of prototype instruments. This approach led to the development of the first retinal laser Doppler instrument that was suitable for routine use in a clinical setting.

THE FIRST RETINAL LASER DOPPLER INSTRUMENT FOR ROUTINE USE IN A CLINICAL SETTING

The Canon Laser Blood Flowmeter model CLBF 100 (Canon, Tokyo, Japan) is the first retinal laser Doppler instrument appropriate for routine use in a clinical setting.[9] The unique feature of this instrument is that the measuring laser beam is locked onto the target blood vessel during eye movements via an eye-tracking feedback and control system. By combining this tracking system with laser Doppler capability, a stabilized retinal laser Doppler instrument has been created. Doppler-shifted laser light scattered from a retinal vessel is analyzed to determine centerline blood velocity. The blood column diameter is simultaneously measured, and the blood flow rate at the measurement site is automatically calculated.

In this fundus camera-like instrument, a 30 degree image of the retina is provided by a halogen light source (570-635 nm) with power of 1.2 mW. The image can be viewed directly, or, since it is also focused onto the face of a CCD camera, it can be viewed on a TV monitor. The instrument is equipped with two lasers, Eye tracking is achieved by projecting a stripe shape from a green (543 nm) HeNe laser through a beam steering galvanometer system onto the retinal vessel of interest. At the retina, the dimensions of this rectangular stripe are 1500 μm $\times$ 150 μm. The stripe is rotatable, so that the operator can adjust it to be perpendicular to the axis of the vessel. The image of the vessel and the superimposed stripe are incident on a linear CCD array sensor which detects any lateral motion of the vessel, and controls the steering system to maintain the center of the green tracking stripe on the center of the vessel during eye movements. The position of the tracking stripe is updated every 4 ms, and centration is achieved to a precision of $\pm$ 5 μm under typical conditions of eye motion. The intensity of the green beam at the cornea is 4 μW for standard illumination and 6 μW for high illumination. Only the component of motion perpendicular to the vessel axis is accounted for in this one-dimensional tracking system. The component of motion parallel to the vessel axis does not disturb the measurement since at any moment what is being measured is the velocity of the RBCs relative to the blood vessel wall. However, this requires that the segments of vessels targeted for measurements must be relatively straight over a length equivalent to several vessel diameters.

Analysis of the vessel image also provides the means for measuring the diameter of the blood column. The diameter is determined automatically by computer analysis of the signal produced by the image of the vessel on the CCD sensor using the half height of the transmittance profile to define the blood column edge. Diameter measurements are corrected for the axial length of the eye (operator input) and refractive error of the eye, which is measured by the CLBF itself, according to the assumptions of the Littmann method.[21] Diameter measurements are obtained every 4 ms during the first 60 ms and during the last 60 ms of each 2-s laser Doppler velocity measurement window.

The beam from a red 675 nm diode laser is used for the actual laser Doppler velocity measurements. The shape of the beam at the retina is an 80 μm $\times$ 50 μm ellipse. The ellipse is always located at the center of the green tracking stripe, so that it too is centered at the center of the target vessel. The long axis of the ellipse is always oriented along the axis of the target vessel. The intensity of the red beam at the cornea is 200 μW for standard illumination and 300 μW for high illumination. Laser safety calculations were performed assuming simultaneous illumination from the fundus observation source and the two laser sources. It was determined that with standard illumination levels, 18 separate measurements at each particular site along a vessel can be made within a 24 hour period without exceeding mandated Maximum Permissible Exposure levels.[1] At high illumination levels, 12 such measurements can be made. An example of the view seen by the operator of the instrument during a measurement is shown in figure 3. It is a video print taken during a measurement on the superior temporal retinal artery in the right eye of a 51-year-old female with normal pressure glaucoma. The tracking stripe, which had been rotated

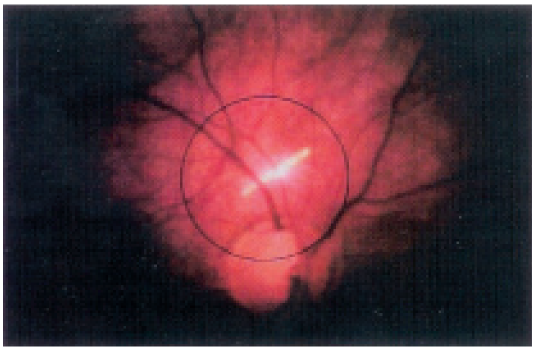

Fig. 3. A video print taken during a laser Doppler measurement with the CLBF 100 instrument on the superior temporal retinal artery in the right eye of a 51-year-old female with normal pressure glaucoma. Shown is the 543 nm tracking stripe which has been rotated to be perpendicular to the vessel axis at the site of measurement. The glow at the intersection of the tracking stripe with the artery is the 675 nm Doppler-shifted scattered light. (From: Yoshida A., Feke G.T., Mori F., Nagaoka T., Fujio N., Ogasawara H., Konno S., McMeel J.W. - Reproducibility and clinical application of a newly developed stabilized retinal laser Doppler instrument. Am J Ophthalmol 2003; 135: 357)[50]

to be perpendicular to the artery at the site of measurement, is visible. The bright glow at the intersection of the tracking stripe and the artery is the Doppler-shifted scattered light.

The Doppler-shifted light scattered from the flowing blood cells in the target vessel is detected simultaneously in two directions separated by a fixed angle according to the bidirectional technique. The signals from two photomultiplier tube detectors undergo computer-controlled spectrum analysis. Pairs of spectra are obtained every 20 ms over period of 2 seconds (100 total measurements), yielding quasi-continuous sequential measurements of the centerline blood velocity. Figure 4 shows examples of two such arterial measurements in the right eye of a 71-year-old female with a branch retinal vein occlusion in the superior temporal quadrant. Shown are measurements from the superior temporal retinal artery supplying the affected region (top), and the inferior temporal retinal artery supplying the unaffected region (bottom).

In practice, two separate 2-second measurements are made at each measurement site. What is varied in these two measurements is the angle of the incident red beam on the target vessel. This maneuver is used to resolve an ambiguity that might arise when applying the bidirectional technique. In general, optical Doppler frequency shifts may be either positive or negative depending upon the orientations of the incident and scattered beams with respect to the flow direction. The bidirectional technique requires the subtraction of one value of Δf_{max} from the other in a bidirectional pair of simultaneously measured spectra. This yields the correct result only if both optical Doppler shifts are positive, or if both are negative. However, what is measured by optical mixing spectroscopy is the absolute value of Δf_{max}. The additional bidirectional measurement with a different incident beam angle provides the means for avoiding the potential ambiguity.

In general, the blood flow rate (F) at a particular site in a blood vessel is given by $F = V_{AV} \times S$, where V_{AV} is the average velocity of the blood passing through the cross-sectional area (S) of the vessel. For Poiseuille flow, $V_{AV} = \frac{1}{2} V_{max}$, where V_{max} is the measured blood velocity at the center of the blood vessel. Since V_{max} varies over time as shown in figure 4, V_{AV} is calculated using the time average of $V_{max}(t)$ over the duration of one cardiac cycle. Therefore,

$$V_{AV} = \tfrac{1}{2}\,(1/T)\int_0^T V_{max}(t)\,dt \qquad (9)$$

where T is the time duration of the cardiac cycle. S is calculated assuming that the cross-section of the vessel at the measurement site is circular. Thus, $S = \Pi D^2/4$, where D is the average of the 60 measured blood column diameters obtained during a complete measurement cycle.

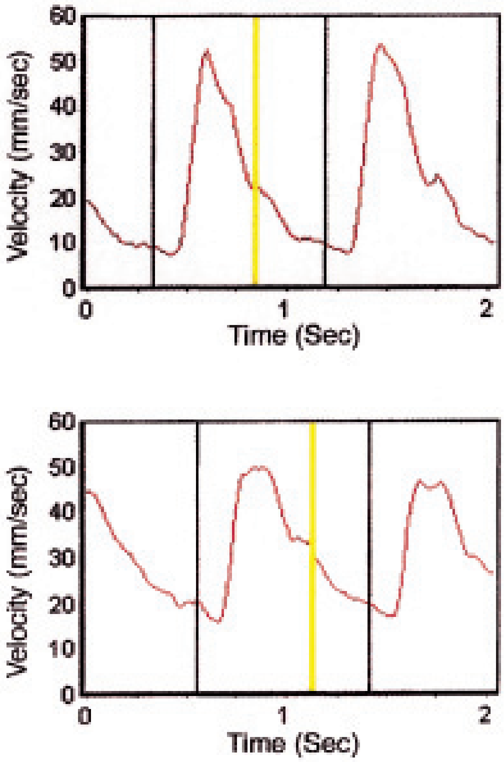

Fig. 4. The variation during cardiac cycles of the centerline blood velocity measured in the superior temporal retinal artery (top) and the inferior temporal retinal artery (bottom) of the right eye of a patient with branch retinal vein occlusion in the superior temporal quadrant of the right eye. The measurements are quasi-continuous (50 per second) as seen by the discontinuous nature of the traces, and are derived from pairs of Doppler-shift frequency spectra of scattered laser light detected according to the bi-directional principle. The waveform in the upper trace is more pulsatile than in the lower trace, indicating a greater resistance to flow distal to the measurement site as is the case in branch retinal vein occlusion. The yellow line is a moveable cursor that reads out the blood velocity at each point along the trace. (From: Yoshida A., Feke G.T., Mori F., Nagaoka T., Fujio N., Ogasawara H., Konno S., McMeel J.W. - Reproducibility and clinical application of a newly developed stabilized retinal laser Doppler instrument. Am J Ophthalmol 2003; 135: 358).[50]

REPRODUCIBILITY OF MEASUREMENTS WITH THE CLBF 100

Assessments of the reproducibility of measurements of D, V_{AV}, and F using the CLBF 100 have been reported in seven separate studies.[10,13,18-19,27,42,50] For D, coefficients of variation for repeated measurements (COV) ranged from 1.9 % to 4.9 %; for V_{AV}, COV ranged from 4.7 % to 19.9 %; and for F, COV ranged from 6.7 % to 19.3 %. The COV results from these studies are shown in table 1.

The reproducibility results and the fact that blood flow is measured in actual units of μl/min indicate that the instrument can be used for reliable comparison of blood flow characteristics at different retinal vascular sites in the same eye, at comparable sites in both eyes, and for comparison between patients and healthy control subjects.

CURRENT RESEARCH USING THE CLBF 100

A sense of the usefulness of the CLBF 100 in current research aimed at elucidating aspects of retinal circulatory physiology and pathophysiology can be gained in a review of the 12 separate

Tab. 1. *Coefficients of Variation for Repeated Measurements Reported for the Canon CLBF 100.*

Study	Vessel Diameter	Blood Velocity	Blood Flow Rate
Kida et al[18]	2.8%	11.0%	8.3%
Kida et al[19]	2.1%	12.0%	10.8%
Nagaoka et al[27]	4.1%	13.9%	16.9%
Garcia et al[10]	1.9%	4.7%	6.7%
Yoshida et al[50]	4.5%	12.5%	13.2%
Guan et al[13]	2.0%	19.9%	19.3%
Shimada et al[42]	4.9%	10.5%	11.8%

presentations made at the 2004 annual meeting of the Association for Research in Vision and Ophthalmology (ARVO) in which results provided by the instrument played a central role. In four presentations the study subjects were patients with either type 1 or type 2 diabetes. In two of these,[17,28] the changes in blood column diameter, blood velocity, and blood flow rate were measured as a function of the severity of diabetic retinopathy. In the third,[14] changes in the pulsatility of the retinal arterial flow were found to be related to changes in the risk of developing clinically significant diabetic macular edema. In the fourth,[11] the retinal vascular response to systemic hyperoxia was compared between patients with diabetes and non-diabetic controls.

Three presentations were related to glaucoma. In two of these,[16,45] the retinal circulatory changes produced by topical IOP lowering medications were assessed. In the third,[29] the hypothesis that retinal vascular dysregulation occurs in patients with glaucoma was investigated, and evidence was presented suggesting that the autoregulatory function of the retinal circulation in these patients can be preserved by appropriate topical medication having neuroprotective properties.

In three presentations, healthy volunteers were subjects in studies examining the chronic effects of aging and blood pressure on the retinal circulation,[5] the acute effects of elevated heart rate arising from dynamic exercise and the acute effects of elevated blood pressure arising from isometric exercise on the retinal circulation,[39] and the retinal vascular response to systemic hypercapnia.[47] A fourth study on healthy volunteers determined the variability of CLBF measurements acquired by different operators on the same subjects.[48]

In the 12th presentation,[40] the authors reported that all three indices of the retinal arterial blood flow pulsatility were elevated in patients with age-related macular degeneration (AMD) compared to an age-matched control group, and furthermore, that these indices increased monotonically with the severity of the AMD. This study of retinal hemodynamics in AMD suggests that there may be an association between AMD and loss of vascular compliance. Whether this is a systemic phenomenon or whether it is localized to the intraocular vasculature remains to be determined.

What these presentations indicate is the opening of a broad range of studies involving circulatory physiology and associated clinical research now made possible by the development of an easy-to-use retinal laser Doppler instrument.

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Corresponding address:

Gilbert T. Feke, PhD
Schepens Retina Associates Foundation
One Autumn Street, 6th Floor
Boston, MA 02215
feke@schepensretina.org