A comparison between laser interferometric measurement of fundus pulsation and pneumotonometric measurement of pulsatile ocular blood flow

1. Baseline considerations

LEOPOLD SCHMETTERER,
SUSANNE DALLINGER,
OLIVER FINDL,
HANS-GEORG EICHLER,
MICHAEL WOLZT

Abstract

Purpose **Several methods have been proposed for the investigation of the human choroidal circulation. The aim of the present study was to compare laser interferometric measurements of cardiac synchronous fundus pulsations with pneumotonometric measurements of intraocular pressure pulse and pulsatile ocular blood flow in humans.**
Methods **The association between fundus pulsation amplitude as assessed with laser interferometry and pulse amplitude (PA) and pulsatile ocular blood flow (POBF) as assessed with pneumotonometry was investigated in 28 healthy subjects. Additionally, we investigated the distribution of fundus pulsation amplitude (FPA) in a region of $-15°$ to $+15°$ around the macula ($n = 18$) and the influence of accommodation paralysis with cyclopentolate on FPA ($n = 10$).**
Results **There was a high association between FPA and PA ($r = 0.86$, $p < 0.001$) and FPA and POBF ($r = 0.70$, $p < 0.001$). Fundus pulsations in the macula were significantly smaller than in the optic disc, but significantly larger than those in peripheral regions of the retina. Administration of cyclopentolate did not influence FPA.**
Conclusions **On the basis of the strong correlation between laser interferometric measurements of FPA and pneumotonometric measurements of PA and POBF, we conclude that the FPA is a valid index of pulsatile choroidal perfusion in humans.**

Key words Fundus pulsation, Ocular blood flow, Pneumotonometry

A variety of common ocular diseases, including glaucoma, age-related macular degeneration and diabetic retinopathy, are associated with ocular perfusion abnormalities. Hence there is considerable interest in the development of techniques for the specific investigation of retinal, optic nerve and choroidal blood flow in man.

Information on ocular blood flow can be obtained with techniques based on Doppler shifting of light on moving erythrocytes. This phenomenon has been used to study blood flow in large retinal vessels,[1] in the optic nerve microvasculature[2] and in the submacular choriocapillaries,[3,4] and to perform retinal and optic nerve blood flow mapping.[5] Doppler shift of ultrasound waves can be used to study blood flow velocities in retrobulbar vessels.[6]

Information on retinal and choroidal blood flow can also be obtained from angiographic methods. Attempts have been made to obtain quantitative information on retinal[7,8] and choroidal[9,10] blood flow from fluorescein and indocyanine green (ICG) angiography.

The present study focused on the comparison of two methods which have been proposed for the assessment of pulsatile ocular blood flow. Pneumotonometry measures changes in intraocular pressure (IOP) during the cardiac cycle. These changes have been used for quantitative estimation of pulsatile ocular blood flow (POBF).[11,12] Laser interferometry is used to measure ocular fundus pulsation, which is the distance change between cornea and retina during the cardiac cycle.[13] The aim of the present study was to investigate the association between the two methods. An additional aim of this study was to determine the topographic distribution of fundus pulsations.

L. Schmetterer
S. Dallinger
H.-G. Eichler
M. Wolzt
Department of Clinical Pharmacology
University of Vienna
Vienna, Austria

L. Schmetterer
Institute of Medical Physics
University of Vienna
Vienna, Austria

O. Findl
Department of Ophthalmology
University of Vienna
Vienna, Austria

Leopold Schmetterer, PhD ✉
Department of Clinical Pharmacology
Währinger Guertel 18–20
A-1090 Vienna, Austria
Tel +43 1 40400/2981
Fax: +43 1 40400/2998
e-mail: Leopold.Schmetterer@univie.ac.at

Received: 24 May 1999
Accepted without revision: 13 September 1999

Methods

Subjects

The study protocols were approved by the Ethics Committee of Vienna University School of Medicine. One study was performed to investigate the association between pnemotonometric parameters and fundus pulsation amplitude (FPA; $n = 28$). One study was performed to investigate the distribution of ocular fundus pulsation in a region of $-15°$ to $+15°$ around the macula ($n = 18$) another study was performed to investigate the distribution of ocular fundus pulsation in the optic disc region ($n = 8$), and one study was performed to investigate possible influences of changes in lens thickness during accommodation on interferometrically assessed fundus pulsations ($n = 10$). Male and female non-smoking subjects, aged between 19 and 35 years, were studied. All participating subjects were informed of the nature of the study and signed a written consent. An ophthalmic examination was performed in each subject prior to the study day. Inclusion criteria were normal ophthalmic findings, ametropia of less than 3 dioptres, and an ametropia difference between the two eyes of less than 1 dioptre. In all subjects the right eye was studied.

Experimental design

Baseline parameters of pneumotonometry and laser interferometry were compared after a resting period of at least 20 min. Fundus pulsations were measured in the macula and the time interval between the two measurements was less than 2 min. Blood pressure and heart rate measurements were performed every 5 min to ensure stable conditions.

To measure the fundus pulsation amplitude at 35 preselected points of the eye fundus, a fixation light with adjustable angle was shown to the contralateral eye. Measurements were performed in steps of 5° between 15° nasal and 15° temporal and 10° superior and 10° inferior of the macula.

Fundus pulsation amplitudes in the optic disc region were measured on preselected points. These points were identified in each subject by use of a fundus camera, to which the laser interferometer is coupled. A schematic drawing of the selected points is given in Fig. 1. The points 1–4 were located on the neuroretinal rim, points 5–8 on the cup.

To investigate possible influences of accommodation on optical distance changes as measured with the laser interferometer we measured FPA with and without accommodation paralysis with cyclopentolate (Cyclogyl 1%, Alcon Couvreur, Puurs, Belgium). Measurements were taken 30 min after administration of cyclopentolate. To ensure stable cardiovascular conditions blood pressure and heart rate were measured before and during accommodation paralysis. FPA was measured for at least 10 cardiac cycles at baseline and during accommodation paralysis. From these 10 cardiac cycles

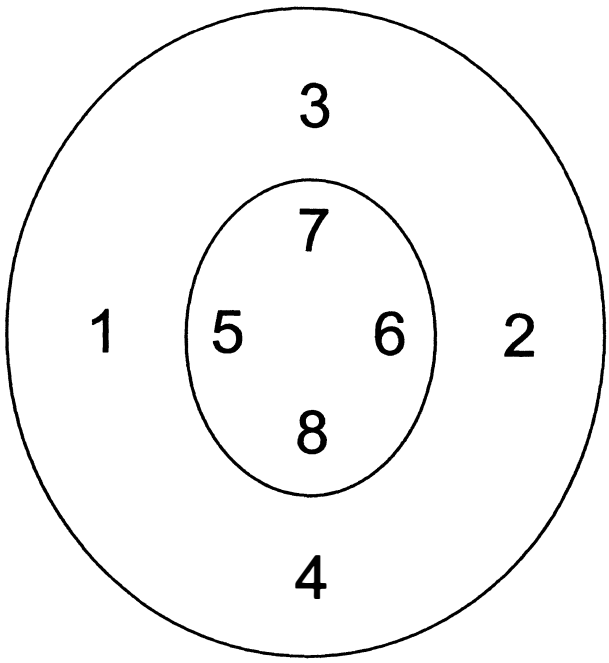

Fig. 1. *Definition of the sites of fundus pulsation measurements in the optic disc. Points 1–4 were located at the neuroretinal rim, points 5–8 were located at the cup.*

the mean FPA and the SD of the 20 readings (one systolic and one diastolic reading during each heart cycle) were calculated.

Fundus pulsation measurements

Pulse synchronous pulsations of the ocular fundus were recorded with a laser interferometric method.[13] The method uses a high-coherence laser beam with a wavelength of 780 nm for illumination of the subject's eye. The power of the laser beam is approximately 80 μW at a beam diameter of 1 mm. The light is reflected at the anterior surface of the cornea and at the fundus. The light from the front side of the cornea serves as a reference wave. This permits calculation of the relative distance changes between cornea and retina during the cardiac cycle from the interferences produced by the two reflected waves. These distance changes are in the order of several micrometres and are caused by the rhythmic filling of ocular vessels during systole and diastole. The distance between cornea and retina decreases during systole and increases during diastole. The maximum distance change between the cornea and fundus during the cardiac cycle is called fundus pulsation amplitude (FPA), yielding information on the pulsatile component of ocular blood flow.[14] The interferometer is coupled to a fundus camera (Zeiss FK-30, Oberkochen, Germany), which allows real-time inspection of the measurement point on the retina.[13]

Pneumotonometric measurement of pulsatile ocular blood flow

POBF was determined with a commercially available blood flow measurement system (OBF System 3000, OBF Labs, Malmesbury, UK).[15] The system measures changes

in IOP, which are caused by the rhythmic filling of the intraocular vessels, with a pneumatic applanation tonometer.[16,17] The maximum IOP change during the cardiac cycle is called pulse amplitude (PA). Based on a theoretical model eye, the POBF is calculated from the IOP variation with time.[11,12] This hydrodynamic model is based on the assumption that venous outflow from the eye is non-pulsatile and that ocular volume changes can be estimated from changes in IOP based on a standard ocular rigidity function. The calculation of POBF is automatically derived from the five pulses that are closest to each other in IOP beat-to-beat variation.

Data analysis

Association between the laser interferometric and pneumotonometric parameters was investigated with linear correlation. For comparison of FPA in the macula (at 0,0) and FPA in the periphery the mean of the eight measurement points adjacent to the macula was calculated. Differences between FPA at the macula and FPA at the eight surrounding measurement points were assessed for significance by ±95% confidence intervals of the mean. The effect of accommodation paralysis on FPA was assessed with the Wilcoxon signed rank test. A p value < 0.05 was considered significant.

Results

The association between laser interferometric measurement of FPA and pneumotonometric measurement of PA or POBF is depicted in Fig. 2. The association was highly significant for both parameters. However, the correlation coefficient was higher for FPA versus PA than for FPA versus POBF.

The results of the regional distribution of FPA measurements are presented in Fig. 3. Fundus pulsation amplitude was higher in the macula (3.4 μm; 95%

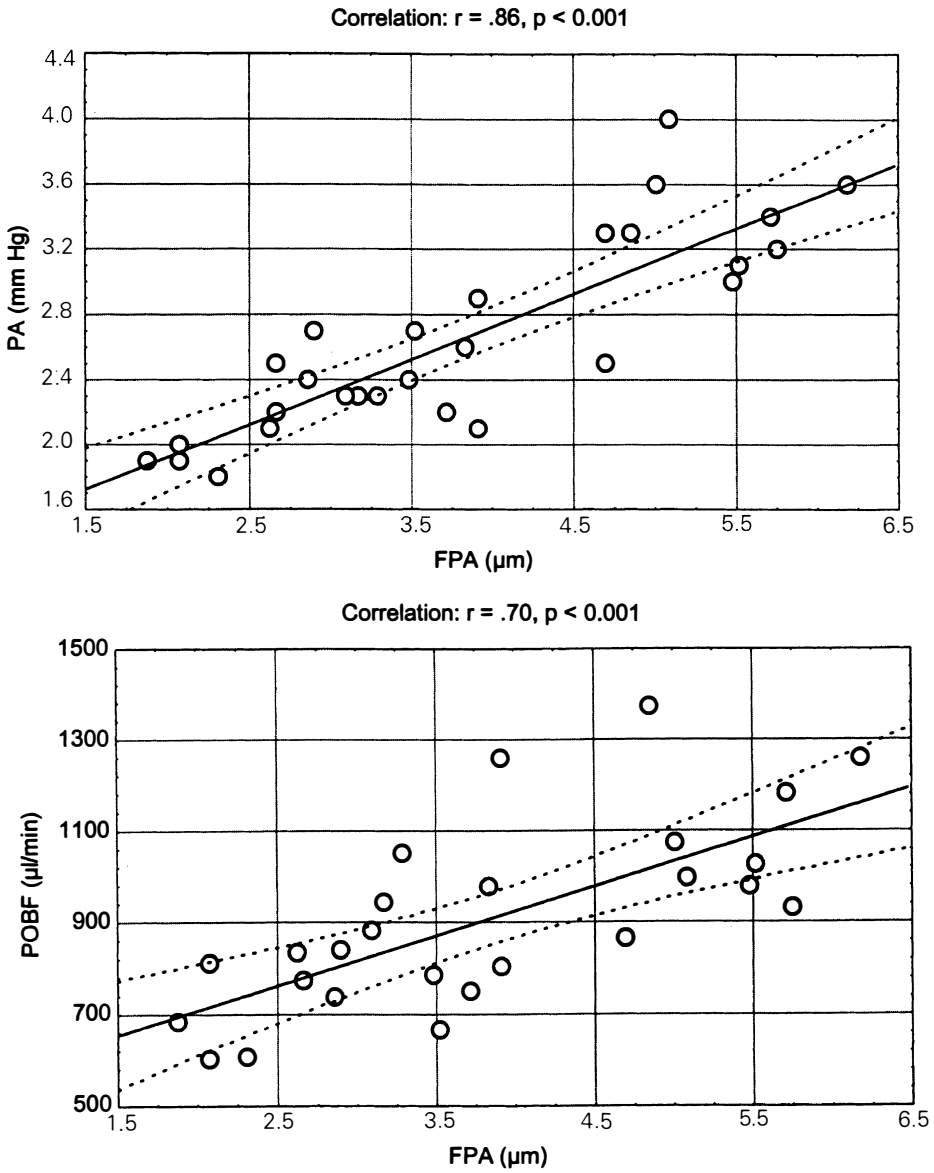

Fig. 2. *Linear correlation* (n = *28) between baseline values of fundus pulsation amplitude (FPA) and the pulse amplitude (PA, upper panel) and fundus pulsation amplitude (FPA) and pulsatile ocular blood flow (POBF, lower panel). The regression lines and the 95% confidence intervals are shown.*

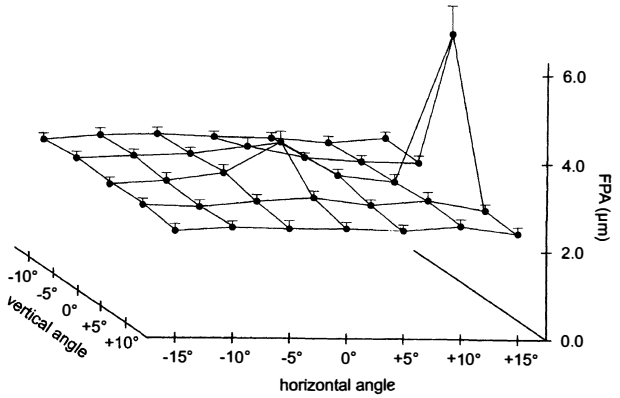

Fig. 3. *Regional distribution of fundus pulsation amplitude (FPA) measurements as obtained from 18 subjects. Fundus pulsation amplitudes are presented as the means ± SEM.*

confidence interval: 3.0–3.9 µm) than at the surrounding measurement points (2.6 µm, 95% confidence interval: 2.3–2.9 µm). In the optic disc, which corresponds to the measurement point at (+15°, 0°), the FPA was more than twice as high as at the other measurement sites.

The results of topical fundus pulsation measurements in the optic disc are presented in Table 1. There was a high variability of FPA as obtained on the neuroretinal rim and the cup. At the neuroretinal rim FPA was not measurable at two measurement sites in one subject and at one measurement site in three subjects. At the cup we did not obtain any results in one subject. Technically adequate results on each of the four measurement sites were obtained in only two subjects.

The results of FPA measurements with and without accommodation paralysis are presented in Table 2. The mean and the SD of FPA as obtained from 10 cardiac cycles after cyclopentolate were not significantly different from the mean FPA and the SD at baseline.

Discussion

The relative fraction of the pulsatile component of blood flow in the eye has not been determined. Estimates vary between 80%[18] and 50%.[19] Nevertheless the determination of POBF has attracted much interest and recent investigations argue that information on flow pulsatility can be obtained from Doppler sonographic measurement of blood flow velocities in retrobulbar arteries.[20] In the present study we compared the results obtained with laser interferometric measurement of fundus pulsation and pneumotonometric measurement of ocular pressure pulse. We observed a high association between FPA and PA, which is in agreement with our previous data.[21] Whereas PA is the maximum IOP change during the cardiac cycle FPA is the maximum distance change between cornea and retina during the cardiac cycle. Deviations from a perfect correlation between the two parameters obtained in the present study can be caused by at least four different reasons:

(1) The two parameters (PA and FPA) are related by the so-called ocular pressure–volume relationship. Changes in ocular volume, as caused by the pulsatile inflow of arterial blood during systole, cause concomitant changes in ocular pressure. This relationship was defined by Friedenwald[22] as:

$$E = (\log IOP_1 - \log IOP_2)/(V_1 - V_2) \quad (1)$$

where E is the ocular rigidity, $IOP_1 - IOP_2$ (= ΔIOP) is a change in intraocular pressure, and $V_1 - V_2$ (= ΔV) is the corresponding change in intraocular volume. The semilogarithmic form of this equation implies that the pressure change induced by a specific volume change is dependent on baseline IOP. When the pressure changes are small, which is the case if they are induced by the increase in blood volume during systole, the equation may be simplified to

$$E = \Delta IOP/\Delta V \quad (2)$$

It should be noted that in this equation the pressure–volume relationship is no longer dependent on baseline IOP. Applying equation (2) to the present situation, PA denotes ΔIOP. FPA, on the other hand is a point measure of ΔV (assuming an infinitely small laser spot on the fundus). It is therefore obvious that the relation between PA and FPA is dependent on the ocular rigidity. However, ocular rigidity shows a considerable degree of variation among subjects[23,24] and therefore identical changes in blood volume do not necessarily induce identical changes in IOP. Moreover, the ocular pressure–volume relationship is dependent on the arterial pressure.[25]

(2) As mentioned above, FPA is a point measure of volume changes during the cardiac cycle. To calculate E using formula (2) one would need to know the overall

Table 1. *Fundus pulsation amplitude in the optic disc (µm)*

	Sites at the neuroretinal rim				Sites at the cup			
Subject no.	1	2	3	4	5	6	7	8
1	7.4	–	6.8	7.7	9.1	8.3	9.9	
2	5.7	6.0	5.1	6.7	8.8	9.3	–	9.8
3	7.8	5.6	7.5	5.8	6.3	9.8	7.8	6.1
4	8.9	9.4	7.6	9.2	9.3	7.9	8.2	10.3
5	7.0	6.4	–	7.8	9.1	–	–	–
6	–	–	6.9	8.4	–	–	–	–
7	8.0	5.5	7.3	–	6.8	–	–	7.4
8	8.7	9.3	11.5	9.6	–	9.5	13.4	12.0

Measurement sites are defined in Fig. 1. At some measurement sites we did not obtain technically adequate interferograms for the evaluation of FPA.

Table 2. *Fundus pulsation amplitude (μm) as calculated from 10 cardiac cycles*

Subject no.	Mean (bl)	SD (bl)	Mean (ap)	SD (ap)
1	2.02	0.21	1.98	0.27
2	3.54	0.37	3.60	0.32
3	4.80	0.36	5.17	0.29
4	2.43	0.32	2.39	0.33
5	5.74	0.34	5.76	0.36
6	3.54	0.38	3.66	0.41
7	2.90	0.45	2.76	0.30
8	5.58	0.39	5.42	0.41
9	3.48	0.39	5.42	0.41
10	4.25	0.25	4.35	0.31
All subjects	3.83	0.34	3.86	0.32

Fundus pulsations were assessed during baseline (bl) and during accommodation paralysis (ap). Data are presented as the mean ± SD.

ocular volume change (ΔV). This total volume change cannot be estimated from single-point FPA measurement, because FPA in the macula may also depend on the local angioarchitecture and local irregularities in scleral rigidity.

(3) The IOP pulse is caused by the pulsatile retinal and choroidal arterial blood flow. By contrast, the FPA in the macula is only dependent on the choroidal circulation, because the retina lacks vascularisation in this region. It was, however, first observed by Bynke and Schele[26] that the ocular pressure pulse is mainly caused by the choroidal circulation. This is due to the fact that the choroid has a much greater blood volume than the retina. Whereas the mean POBF in the present study was approximately 900 μl/min, laser Doppler velocimetry studies indicate that the total retinal blood flow in healthy subjects is less than 40 μl/min.[27] Hence the contribution of retinal blood flow to the ocular pressure pulse should be negligible.

(4) PA and FPA were not taken from the same pulse periods in the subjects under study, because it is technically impossible to assess PA and FPA simultaneously.

The association between POBF and FPA was weaker than the association between PA and FPA. Calculation of POBF from the time course of the ocular pressure pulse is based on a theoretical model derived by Silver.[11,12] It is obvious that a calculation of POBF in microlitres per minute requires a correction for pulse rate. The model is further based on the assumption of a pulsatile arterial inflow and a steady venous outflow. With this limitation the pulsatile net flow curve in the eye can be calculated as the first derivative of the change in intraocular volume, which is derived from equation (2). In other words, the POBF depends not only on PA but also on pulse rate and the shape of the IOP changes with time, which in fact may be responsible for the weaker correlation between FPA and POBF. The factors mentioned in this paragraph obviously also limit the correlation between PA and POBF. Applying this result to pharmacodynamic studies it is obvious that FPA can be taken as relative measure of pulsatile choroidal blood flow if there is no pronounced change in pulse rate and no significant change in the time course of IOP and ocular volume changes. We have used this fact to study the influence of hyperoxia and hypercapnia,[28,29] pentoxifylline,[30] nitric oxide synthase inhibitors,[31,32] exogenous endothelin-1,[33–34] sumatriptan[35] and topical antiglaucoma drugs[36] on blood flow. In cases where changes in FPA in response to systemically administered drugs only occur at doses which induce significant changes in pulse rate and blood pressure, such as with exogenous angiotensin II,[20] or phenylephrine and sodium nitroprusside,[14] the interpretation of the data is more difficult.

An advantage of fundus pulsation measurements is the high topographic resolution of the method. This is of importance, as in clinical practice ischaemic or inflammatory choroidal lesions are typically localised, which may be attributed to the lobular organisations of the choroidal vessels and the existence of watershed zones.[37,38] However, from our measurements of fundus pulsations we were not able to identify segmental blood flow patterns. Hence it seems that despite this angioarchitecture the ocular pulsatile blood flow is equally distributed in the region under measurement (Fig. 3), with the exception of the macula and the optic disc. In the macula FPA is significantly higher than in peripheral regions of the ocular fundus. The reason for this observation remains to be elucidated, but could be related to the fact that the ratio of arteries to veins is particularly high in the submacular choroid,[37] or to a local irregularity in scleral rigidity. Whether the comparatively high FPA in this clinically important region is also linked to a higher choroidal blood flow in this region, which has previously been observed in monkeys,[39] is also unclear.

Whereas it is obvious that FPAs in the macula are only dependent on the choroidal blood flow the situation is more complex in other parts of the fundus. However, the fundus layer from which the main portion of light at 780 nm is reflected is most likely Bruch's membrane.[40] Therefore FPA in the peripheral retina should also be exclusively influenced by choroidal blood flow.

In the optic disc the fundus pulsation amplitude is much higher than on the other measurement sites. As shown in Table 1 there is a high local within-subject variability of the results obtained at the neuroretinal rim and the cup. It could be that the elastic properties of the eye in the optic disc are not comparable to other measurement sites, but this issue remains to be investigated. Directly comparing FPA in the optic disc with fundus pulsations in other regions is therefore not possible. It is interesting that we could not measure fundus pulsation at all measurement points under study. In these cases we did not observe interference between the light reflected from the anterior surface of the cornea and the fundus. The layers from which the light is reflected in the optic disc have not been definitely identified.[40] It may be that, due to the topography of the optic disc, the main portion of the light is not reflected at an angle of 180° to the incident beam, which is required for the observation of the interference fringes.

The studies during cycloplegia with cyclopentolate were performed to investigate a possible effect of accommodative changes in lens thickness during fixation at infinity on fundus pulsation amplitude. Cyclopentolate, which has been shown effectively to suppress accommodative fluctuations,[41] had an influence neither on mean FPA nor on the standard deviation of FPA as calculated from 10 cardiac cycles. This negligible impact is in keeping with the following theoretical considerations. Accommodative fluctuations focusing the far point under baseline conditions lead to a change in lens thickness in the order of 50 μm.[42] Focusing the near point increases lens thickness by approximately 200 μm. The FPA as measured with laser interferometry is an optical distance. Conversion into geometrical distance requires the knowledge of the group refractive index (n_g) of the cornea (n_g = 1.3856), the anterior chamber (n_g = 1.3459), the lens (n_g = 1.4070) and the vitreous (n_g = 1.3445).[42] Using the paraxial schematic eye of Le Grand and El Hage[43] with a corneal thickness of 0.55 mm, an anterior chamber depth of 3.05 mm, a lens thickness of 4 mm and a length of the vitreous of 16.6 mm a mean refractive index of this schematic eye can be calculated as n_g (eye) = 1.3456. The geometrical fundus pulsation amplitude (FPAG) can then be calculated by

$$\text{FPAG} = \text{FPA}/n_g(\text{eye}) \qquad (3)$$

in which FPA is the optical fundus pulsation amplitude, which is actually measured. A change in lens thickness caused by accommodative fluctuations may therefore lead to a change in the interferometrically determined FPA, and may result in an artefactual distance change between cornea and Bruch's membrane. Assuming an increase in lens thickness of 50 μm and corresponding decrease in anterior chamber depth of 50 μm, n_g(eye) changes to 1.3494. Equivalently a change of lens thickness of 200 μm leads to n_g(eye) of 1.3564. Hence the provoked change in mean refractive index of the schematic eye is less than 1%. Consequently, using equation (3), the influence of full accommodation on FPA is less than 1% and therefore negligible.

In conclusion, we have shown that there is strong correlation between FPA and PA. The correlation between FPA and POBF is weaker, which may be caused by inter-individual differences in pulse rate and the time course of IOP changes. Fundus pulsations in the macula are significantly smaller than in the optic disc, but significantly larger than those in peripheral regions of the retina. Whereas FPA in the peripheral parts of the retina shows little variation, there is a high variability of results obtained at the neuroretinal rim and the cup. Changes in accommodation state have little impact on the interferometrically determined FPA.

The authors are indebted to Chris Crowhurst and David Massey from OBF Labs (UK) Ltd for the loan of the OBF system.

References

1. Riva CE, Ross B, Benedek GB. Laser Doppler measurements of blood flow in capillary tubes and retinal arteries. Invest Ophthalmol Vis Sci 1972;11:936–44.
2. Riva CE, Harino S, Petrig BL, Shonat RD. Laser Doppler flowmetry in the optic nerve. Exp Eye Res 1992;55:499–506.
3. Riva CE, Cranstoun SD, Grunwald JE, Petrig BL. Choroidal blood flow in the foveal region of the human ocular fundus. Invest Ophthalmol Vis Sci 1994;35:4273–81.
4. Riva CE, Petrig BL. Choroidal blood flow by laser Doppler flowmetry. Opt Eng 1995;34:746–52.
5. Michelson G, Langhans MJ, Groh MJM. Clinical investigation of the combination of a scanning laser ophthalmoscope and laser Doppler flowmeter. Ger J Ophthalmol 1995;4:342–9.
6. Lieb WE, Cohen SM, Merton DA, Shields JA, Mitchell DG, Goldberg BB. Color Doppler imaging of the eye and the orbit. Arch Ophthalmol 1991;109:527–31.
7. Preussner PR, Richard G, Darrelmann O, Weber J, Kreissig J. Quantitative measurement of retinal blood flow in human beings by application of digital image-processing methods to television fluorescein angiograms. Graefes Arch Clin Exp Ophthalmol 1983;221:110–5.
8. Wolf S, Jung F, Kiesewetter H, Körber N, Reim M. Video fluorescein angiography: method and clinical application. Graefes Arch Clin Exp Ophthalmol 1989;227:145–51.
9. Duijm HFA, Rulo AHF, Astin M, Maepea O, van den Berg TJTP, Greve EL. Study of choroidal blood flow by comparison of SLO fluorescein angiography and microspheres. Exp Eye Res 1996;63:693–704.
10. Flower RW. Extraction of choriocapillaris hemodynamic data from ICG fluorescence angiograms. Invest Ophthalmol Vis Sci 1993;34:2720–9.
11. Silver DM, Farrell RA, Langham ME, O'Brien V, Schilder P. Estimation of pulsatile ocular blood flow from intraocular pressure. Acta Ophthalmol 1989;67(Suppl 191):25–9.
12. Silver DM, Farrell RA. Validity of pulsatile ocular blood flow measurements. Surv Ophthalmol 1994;28(Suppl):S72–80.
13. Schmetterer L, Lexer F, Unfried C, Sattmann H, Fercher AF. Topical measurement of fundus pulsations. Opt Eng 1995;34:711–6.
14. Schmetterer L, Wolzt M, Salomon A, Rheinberger A, Unfried C, Zanaschka G, *et al*. The effect of isoproterenol, phenylephrine and sodium nitroprusside on fundus pulsations in healthy volunteers. Br J Ophthalmol 1996;80:217–23.
15. Butt Z, O'Brien C. Reproducibility of pulsatile ocular blood flow measurements. J Glaucoma 1995;4:214–8.
16. Walker RE, Litovitz TL. An experimental and theoretical study of pneumatic tonometer. Exp Eye Res 1972;13:14–23.
17. Walker RE, Langham ME. Pneumatic applanation tonometer studies: analysis of pulsatile response. Exp Eye Res 1975;20:245–53.
18. Langham ME, Farrell RA, O'Brien V, Silver DM, Schilder P. Blood flow in the human eye. Acta Ophthalmol 1989;67(Suppl 91):9–13.
19. Krakau CET. A model for pulsatile and steady ocular blood flow. Graefes Arch Clin Exp Ophthalmol 1995;233:112–8.
20. Krejcy K, Wolzt M, Kreuzer C, Breiteneder H, Schütz W, Eichler HG, *et al*. Characterisation of angiotensin II effects on cerebral and ocular circulation by noninvasive methods. Br J Clin Pharmacol 1997;43:501–8.
21. Schmetterer L, Dallinger S, Findl O, Strenn K, Graselli U, Eichler HG, *et al*. Noninvasive investigations of the normal ocular circulation. Invest Ophthalmol Vis Sci 1998;39:1210–20.
22. Friedenwald JS. Contribution to the theory and practice of tonometry. Am J Ophthalmol 1937;20:985–1024.
23. Edmund C. Corneal elasticity and ocular rigidity in normal and keratoconic eyes. Acta Ophthalmol 1988;66:134–40.

24. Eisenlohr J, Langham ME, Maumenee AE. Manometric studies of the pressure/volume relationships in living and enucleated eyes of individual subjects. Br J Ophthalmol 1962;46:536–48.
25. Kiel JW. The effect of arterial pressure on the ocular pressure–volume relationship in the rabbit. Exp Eye Res 1995;60:267–78.
26. Bynke HG, Schele B. On the origin of the ocular pulse. Ophthalmologica 1967;153:29–36.
27. Riva CE, Grunwald JE, Sinclair SH, Petrig BL. Blood velocity and volumetric flow rate in human retinal vessels. Invest Ophthalmol Vis Sci 1985;26:1124–32.
28. Schmetterer L, Wolzt M, Lexer F, Alschinger C, Gouya G, Zanaschka G, *et al*. The effect of hyperoxia and hypercapnia on fundus pulsations in the macula and the optic disc region. Exp Eye Res 1995;61:685–90.
29. Schmetterer L, Lexer F, Graselli U, Findl O, Eichler HG, Wolzt M. The effect of different mixtures of O_2 and CO_2 on ocular fundus pulsations. Exp Eye Res 1996;63:351–5.
30. Schmetterer L, Kemmler D, Breiteneder H, Alschinger C, Koppensteiner R, Lexer F, *et al*. A randomized, placebo-controlled, double blind crossover study of the effect of pentoxifylline on ocular fundus pulsations. Am J Ophthalmol 1995;121:169–76.
31. Schmetterer L, Findl O, Fasching P, Ferber W, Strenn K, Breiteneder H, *et al*. Nitric oxide and ocular blood flow in patients with IDDM. Diabetes 1997;46:653–8.
32. Schmetterer L, Krejcy K, Kastner J, Wolzt M, Gouya G, Findl O, *et al*. The effect of systemic nitric oxide-synthase inhibition on ocular fundus pulsations in man. Exp Eye Res 1997;64:305–12.
33. Schmetterer L, Findl O, Strenn K, Jilma B, Graselli U, Eichler HG, *et al*. Effects of Endothelin-1 (ET-1) on ocular hemodynamics. Curr Eye Res 1997;16:687–92.
34. Strenn K, Matulla B, Wolzt M, Findl O, Bekes MC, Lamsfuss U, *et al*. Reversal of ET-1 induced ocular hemodynamic effects by low dose nifedipine in man. Clin Pharmacol Ther 1997;63:54–63.
35. Schmetterer L, Woltz M, Krejcy K, Graselli U, Findl O, Eichler HG, *et al*. Cerebral and ocular hemodynamic effects of sumatriptan in nitroglycerin headache model. Clin Pharmacol Ther 1996;60:199–205.
36. Schmetterer L, Strenn K, Findl O, Breiteneder H, Graselli U, Agneter E, *et al*. Effects of antiglaucoma drugs on ocular hemodynamics in healthy volunteers. Clin Pharmacol Ther 1997;61:583–95.
37. Fryczkowski AW, Sherman MD, Walker J. Observations on the lobular organisation of the human choriocapillaris. Int Ophthalmol 1990;15:109–20.
38. Hayreh SS. *In vivo* choroidal circulation and its watershed zones. Eye 1990;4:273–89.
39. Alm A, Bill A. Ocular and optic nerve blood flow at normal and increased intraocular pressures in monkeys: a study with labelled microspheres including flow determinations in brain and some other tissues. Exp Eye Res 1996;15:15–29.
40. Drexler W, Hitzenberger CK, Sattmann H, Fercher AF. Measurement of the thickness of fundus layers by partial coherence tomography. Opt Eng 1995;34:701–10.
41. Drexler W, Baumgartner A, Findl O, Hitzenberger CK, Fercher AF. Biometric investigation of changes in the anterior eye segment during accommodation. Vision Res 1997;37:2789–800.
42. Hitzenberger CK. Optical measurement of the axial eye length by laser Doppler interferometry. Invest Ophthalmol Vis Sci 1991;32:616–24.
43. Le Grand Y, El Hage SG. Physiological optics. Berlin: Springer, 1980.