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Ultrasonic Doppler measurements of blood flow velocity of rabbit retinal vessels using a 45-MHz needle transducer

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Abstract

Background—The purpose of this study is to measure blood flow velocity of rabbit retinal vessels using a 45-MHz ultrasonic Doppler system with a needle transducer.

Methods—A high-frequency pulsed Doppler system that utilizes a 45-MHz PMN-PT needle transducer was developed to measure retinal blood flow velocity in situ. The pulsed Doppler allowed the differentiation of retinal from choroidal blood flow velocity. The needle transducer was inserted into the vitreous cavity through a 20-gauge incision port to access the retinal vessels. The first phase

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of the experiment evaluated the reproducibility of the measurements. The second phase measured velocities at four positions from the optic disc edge to the distal part of each vessel in nine eyes for the temporal and six eyes for the nasal portions. The angle between the transducer and the retinal vessel at each site was measured in enucleated rabbit eyes to estimate and compensate for measurement errors.

Results—In the first phase, the average measurement error was 5.97±1.34%. There was no significant difference comparing all eyes. In the second phase, the velocities gradually slowed from the disc edge to the distal part, and temporal velocities were faster than nasal velocities at all measurement sites.

Conclusion—This study demonstrated the feasibility of reliably measuring retinal blood flow velocity using a 45-MHz ultrasonic Doppler system with a needle transducer.

Keywords

Blood flow velocity; Rabbit; Retina; High-frequency Doppler system; Intraocular transducer

Introduction

Hemodynamics of retinal circulation remains an active area of study, since measurement of retinal and choroidal blood flow has been one of the most difficult challenges in ophthalmology. Ocular hemodynamic changes are known to be associated with ocular diseases such as retinal arterial and venous occlusion, macular degeneration and edema, glaucoma, and diabetic retinopathy [1]. Any technique that allows an accurate measurement of retinal blood flow would be an important diagnostic tool and would help clinicians corroborate diagnoses, evaluate therapeutic treatments, and provide early detection in at-risk populations [2].

Several techniques have been used to measure retinal blood flow. Most experimental methods, such as the isotope clearance method [3], the microsphere method [4,5], the heated thermocouple technique [6], and the hydrogen gas clearance method [7] were too invasive and therefore deemed inappropriate for human applications. Laser Doppler velocimetry, laser Doppler flowmetry, fluorescein angiography, indocyanine green angiography and blue field entoptic technique are used to measure retinal and choroidal blood flow. However, the suitability of methods to measure ocular circulation is limited by their sensitivity to small hemodynamic changes and by their reproducibility [1]. Measuring a selected portion of the retinal vessels during eye surgery is more difficult because of the functional limitations of these methods and patient positioning during surgery.

Ultrasonic techniques have been developed to investigate blood flow velocity in ocular arteries and veins. Color Doppler imaging has been used extensively to measure blood flow velocity in the ophthalmic artery and the posterior ciliary artery [1,8–12]. However, conventional ultrasonography cannot measure the diameter of small retinal vessels for volumetric blood flow because of poor resolution. Moreover, the sensitivity of conventional ultrasonography to slow blood flow velocity in small retinal vessels is inadequate.

Our laboratory recently developed a high-frequency Doppler system with a needle transducer that could measure in vivo retinal blood velocity in the rabbit using a minimally invasive approach [13]. This previous study showed just the possibility of measuring retinal blood flow but did not measure the blood flow velocity from retinal vessels extensively with spatial information. One challenge in using this measurement technique is differentiating between the retinal and choroidal vessels, since these vessels are located closely. Therefore, our current study monitored the time-gated Doppler signals to ensure separation of retinal vessels from choroidal vessels.

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Although the structure of rabbit retinal and choroidal vessels is not identical to the corresponding human vessels, this work should be useful since rabbits are often used in animal studies of eye diseases, especially for those diseases related to vascular occlusion [14].

Materials and methods

An angled high-frequency needle transducer

PMN-33%PT was chosen as the piezoelectric material for the active transducer element because of its high electromechanical coupling coefficient, low dielectric loss, and high relative clamped dielectric constant. These features, in combination, make it an ideal candidate for fabricating very sensitive, small-aperture, high-frequency transducers. The resulting instrument makes it easier to detect blood flow from the small retinal vessel (<200 μm in diameter) [13].

The center frequency of the transducer was about 45 MHz. The bandwidth at −6 dB was measured to be approximately 53%. Its sensitivity was sufficient for detecting weak Doppler signals from retinal vessels based on the previous study [13].

The needle transducer was designed with a 45° beveled tip for easy access to the retinal vessels and its outer diameter is around 1 mm. [15]. A representative finished PMN-PT high sensitivity needle transducer is shown in Fig. 1.

High-frequency Doppler system

Because of the small backscatter coefficient of blood, the signal-to-noise ratio (SNR) of the Doppler system plays a significant role in blood flow velocity measurement. Therefore, a customized high-frequency pulsed-wave Doppler system was developed to achieve a high SNR. The digitized Doppler signals were converted into a directional spectrogram in real time using Labview-based software (National Instruments Corp., Austin, TX, USA). Further off-line analysis was conducted using MATLAB-based software (The MathWorks Inc., Natick, MA, USA) [15]. To differentiate retinal blood flow velocity from choroidal blood flow velocity, an electronic time gating was applied to 200 ns from the first echo signals from the tissue (Fig. 2). The Doppler sampling gate was 100 ns. All system and software were developed at the University of Southern California.

Rabbit experiments with surgical procedures

All animal procedures were carried out in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and with the Institutional Animal Care and Use Committee of University of Southern California.

Pigmented rabbits, each weighing from 2 to 3 kg, were used for all experiments. The left eyes of five rabbits were used for the first phase of the study, the repeatability test. The left eyes of nine rabbits were used in the second phase to measure blood flow velocity of retinal vessels. The right eyes of all rabbits were used as controls. All eyes were examined and confirmed intact and normal before beginning the study.

Initial sedation was induced with intramuscular injection of ketamine hydrochloride (25 mg/kg) and xylazine hydrochloride (6 mg/kg). The pupils were dilated with a topical application of phenylephrine hydrochloride 2.5 % and tropicamide 0.5 % eye drops. After the experiment was completed, the rabbits were euthanized with an intracardiac injection of sodium pentobarbital (120 mg/kg).

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The procedures were performed under anesthesia using an operating microscope; a speculum was used to keep the eyelids open. A vitrectomy lens was placed on the cornea with a lens handle ring to visualize the fundus and the vitreous cavity. A scleral incision was made in the inferonasal portion of the left eye 1.5 mm behind the limbus, using a 20-gauge blade.

The intraocular Doppler ultrasound transducer was inserted through this scleral incision and the tip was directed to the measurement site (Fig. 3). Blood flow velocities in retinal veins and arteries were then measured.

Measurement sites and methods

From the optic disc edge to the distal part of the retinal vessels, four positions on one retinal vessel were selected. The position on the optic disc edge was designated position 0. Position 1 was 1 mm distal; position 2 was 2 mm distal; and position 3 was 3 mm, respectively, from the disc edge (Fig. 4).

During the first phase, the measurement variation caused by the human positioning of the transducers was evaluated. Ten independent measurements were repeated with the same transducer at selected sites in five eyes.

After the first phase, blood flow velocities in the retinal veins and arteries were measured by the same surgeon at all four positions (position 0 through position 3). Nine eyes were used to measure temporal vessels and six to measure nasal vessels.

Each position of the probe was controlled through the operating microscope. The distance from the vessel was fixed using real-time Doppler signal shape and the measurement site was measured with the diameter of the transducer as a 1-mm marker.

Doppler angle estimation

Estimation of blood flow velocity by high-frequency ultrasound has been studied [11]. Flow velocity can be estimated by the Doppler equation [12]:

$$v=\frac{\Delta f}{2f_0\cos\theta}c \tag{1}$$

Δf is the measured Doppler shift frequency, f_0 is the center frequency of the transducer, c is the sound velocity in a medium; and θ is the angle between the ultrasound beam and the flow [15]. In our ultrasound Doppler measurements, f_0 was 45 MHz and c was 1,500 m/s. θ is often estimated from the corresponding B-mode images; however, B-mode images were not available in our system [15]. To overcome this limitation, the Doppler angle was estimated in the following manner. A masked experiment was performed to measure the Doppler angle between the front surface of the transducer and retinal vessels at both sides of position 3 and position 0 in ten enucleated rabbit eyes (Fig. 5)

Results

The average Doppler angle measured by a masked observer was 79.89°±2.26° at the nasal aspect, 79.50°±3.63° at the optic disc, and 80.13°±4.67° at the temporal aspect. Since the mean and standard deviation of the estimated Doppler angle was 79.84°±3.46°, the Doppler angle θ was estimated at 80° for this experimental study.

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During the first phase of the study, the average measurement error of ten Doppler measurements at a selected site of retinal vessel was 5.97±1.34%. There was no significant difference for all eyes.

In the second phase, as shown in Table 1, the blood flow velocity was fastest in the artery at the edge of the optic disc and slowest in the most distal part of vein. From the central to the distal part of retinal vessels, the velocities were slower and the arterial spectrum changed from a peaky to a blunt shape (Fig. 6).

Discussion

During the second phase of the experiments, blood flow velocity increased as the measurement site approached the optic disc. Arterial peak velocity was found to be approximately twice as fast as venous velocity at all measurement sites. The change in velocity is dependent on multiple factors, such as vessel diameter, vessel resistance to crossroad and branch, vessel tone, turbulence, and blood pressure. The decrease in velocity towards the distal part of the vessel (closer to the capillary) has been reported in previous studies [16–18].

Several previous studies [19,20] discuss the difference between temporal and nasal ocular blood flow in humans. Human capillary blood velocity from temporal sites was reported to be, on average, 15% faster than that from nasal sites, corresponding to the equally greater distribution of ganglion cell axons within the same area [20]. Although structures of the retina and the retinal and choroidal vessels of rabbits are not identical to those of humans [15], the temporal velocities were faster than nasal velocities at all of our measurement sites and were, on average, 13.6% faster than the nasal velocities in our results. More systematic measurements are needed to better understand retinal blood flow physiology.

Others have attempted to measure retinal blood flow velocities in rabbits. While one study [21] reports that the velocity at the rabbit central retinal artery measured with a pulsed Doppler was around 6.5 cm/s, similar to our result at the optic disc edge; other studies, using a scanning laser ophthalmoscope [18] and fluorescein leukocyte angiography [16,17] report that the maximum velocity in a large rabbit retinal vessel was less than 1 cm/s. However, these latter techniques could only measure average velocity over the vessel diameter and over a cardiac cycle; and their measurement sites were more peripheral than ours, which would result in a much slower velocity compared to arterial peak velocity and the center of the vessel.

Ultrasound has been used as a non-invasive diagnostic method for many years. The ultrasonic transducer's relatively small diameter and needle shape, which is similar to that of microsurgical instruments used for 20-gauge vitrectomy, would allow for easy manipulation and would be acceptable for the vitreous surgeon [15]. The surgeon can use the transducer to measure blood flow velocity at any site of the retinal vessels in spite of any axial length while monitoring through the operating microscope.

The ultrasound Doppler angle and the distance from the target are important factors that contribute to measurement error [22]. Angle estimation in this study may not be optimal, but it should be considered reasonable without B-mode imaging. The distance from the target could be compensated for by electronic time gating. Moreover, this study demonstrated a small standard deviation in angle estimation and a low measurement error with human positioning during the first phase, demonstrating that this measurement for retinal blood flow velocities of rabbits is repeatable and reliable. In the next generation of our Doppler system, a B-mode image with Doppler spectrogram in Duplex imaging [15] will be developed for more accurate measurement of the Doppler angle. Moreover, the size of the intraocular needle transducer will be decreased to allow use with smaller-gauge (23-gauge/25-gauge) vitrectomy systems.

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This minimally invasive high-frequency Doppler measurement technique may not be practical for routine measurement of retinal blood flow velocity, but it may be useful for monitoring retinal blood flow in real time before, during, and after surgery.

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References

1. Schmetterer L, Dallinger S, Findl O, Strenn K, Graselli U, Eichler HG, Wolzt M. Noninvasive investigations of the normal ocular circulation in humans. Invest Ophthalmol Vis Sci 1998;39:1210–1220. [PubMed: 9620081]
2. Chen HC, Gupta A, Wiek J, Kohner EM. Retinal blood flow in nonischemic central retinal vein occlusion. Ophthalmology 1998;105:772–775. [PubMed: 9593374]
3. Friedman E, Kopald HH, Smith TR. Retinal and choroidal blood flow determined with krypton-85 anesthetized animals. Invest Ophthalmol 1964;3:539–547. [PubMed: 14229941]
4. O'Day DM, Fish MB, Aronson SB, Coon A, Pollycove M. Ocular blood flow measurement by nuclide labeled microspheres. Arch Ophthalmol 1971;86:205–209. [PubMed: 4999025]
5. Geijer C, Bill A. Effects of raised intraocular pressure on retinal, prelaminar, laminar, and retrolaminar optic nerve blood flow in monkeys. Invest Ophthalmol Vis Sci 1979;18:1030–1042. [PubMed: 90027]
6. Armaly MF, Araki M. Optic nerve circulation and ocular pressure. Invest Ophthalmol 1975;14:724–731. [PubMed: 810450]
7. Stefansson E, Wagner HG, Seida M. Retinal blood flow and its autoregulation measured by intraocular hydrogen clearance. Exp Eye Res 1988;47:669–678. [PubMed: 3197768]
8. Baxter GM, Williamson TH. Color Doppler flow imaging in central retinal vein occlusion: a new diagnostic technique? Radiology 1993;187:847–850. [PubMed: 8497643]
9. Baxter GM, Williamson TH. The value of serial Doppler imaging in central retinal vein occlusion: correlation with visual recovery. Clin Radiol 1996;51:411–414. [PubMed: 8654005]
10. Ozbek Z, Saatci AO, Durak I, Kaynak S, Ergin MH, Oner B, Cingil G. Colour Doppler assessment of blood flow in eyes with central retinal vein occlusion. Ophthalmologica 2002;216:231–234. [PubMed: 12207122]
11. Suzuki A, Okamoto N, Ohnishi M, Tsubakimori S, Fukuda M. Investigation of blood flow velocity by color Doppler imaging in nonischemic central retinal vein occlusion with collateral veins. Jpn J Ophthalmol 2000;44:685–687. [PubMed: 11094189]
12. Williamson TH, Baxter GM. Central retinal vein occlusion, an investigation by color Doppler imaging. Blood velocity characteristics and prediction of iris neovascularization. Ophthalmology 1994;101:1362–1372. [PubMed: 7520147]
13. Zhou Q, Xu X, Gottlieb EJ, Sun L, Cannata JM, Ameri H, Humayun MS, Han P, Shung KK. PMN-PT single crystal, high-frequency ultrasonic needle transducers for pulsed-wave Doppler application. IEEE Trans Ultrason Ferroelectr Freq Control 2007;54:668–675. [PubMed: 17375836]
14. Ameri H, Ratanapakorn T, Rao NA, Chader GJ, Humayun MS. Natural course of experimental retinal vein occlusion in rabbit; arterial occlusion following venous photothrombosis. Graefes Arch Clin Exp Ophthalmol 2008;246:1429–1439. [PubMed: 18642023]
15. Xu, X. PhD dissertation. University of Southern California; 2007. High-frequency ultrasound imaging system with Doppler features for biomedical applications using 30~35-MHz linear arrays and analog beamformer; p. 159
16. Yang Y, Moon S, Lee S, Kim J. Measurement of retinal blood flow with fluorescein leucocyte angiography using a scanning laser ophthalmoscope in rabbits. Br J Ophthalmol 1996;80:475–479. [PubMed: 8695572]

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17. Yang Y, Kim S, Kim J. Visualization of retinal and choroidal blood flow with fluorescein leukocyte angiography in rabbits. Graefes Arch Clin Exp Ophthalmol 1997;235:27–31. [PubMed: 9034839]

18. Chou NY, Winchester LW. Retinal blood velocity measurements using laser speckle imaging. Conf Proc IEEE Eng Med Biol Soc 2005;2:1716–1719. [PubMed: 17282544]

19. Hafez AS, Bizzarro RL, Lesk MR. Evaluation of optic nerve head and peripapillary retinal blood flow in glaucoma patients, ocular hypertensives, and normal subjects. Am J Ophthalmol 2003;136:1022–1031. [PubMed: 14644212]

20. Rizzo JF 3rd, Feke GT, Goger DG, Ogasawara H, Weiter JJ. Optic nerve head blood speed as a function of age in normal human subjects. Invest Ophthalmol Vis Sci 1991;32:3263–3272. [PubMed: 1748556]

21. Yamada R, Hayashi Y, Ueno S, Yamada S. Blood flow velocity in the central retinal artery of exotoxin-induced uveitis in rabbits. Nippon Ganka Gakkai Zasshi 1999;103:591–596. [PubMed: 10466331]

22. Kagemann L, Harris A, Chung HS, Evans D, Buck S, Martin B. Heidelberg retinal flowmetry: factors affecting blood flow measurement. Br J Ophthalmol 1998;82:131–136. [PubMed: 9613377]

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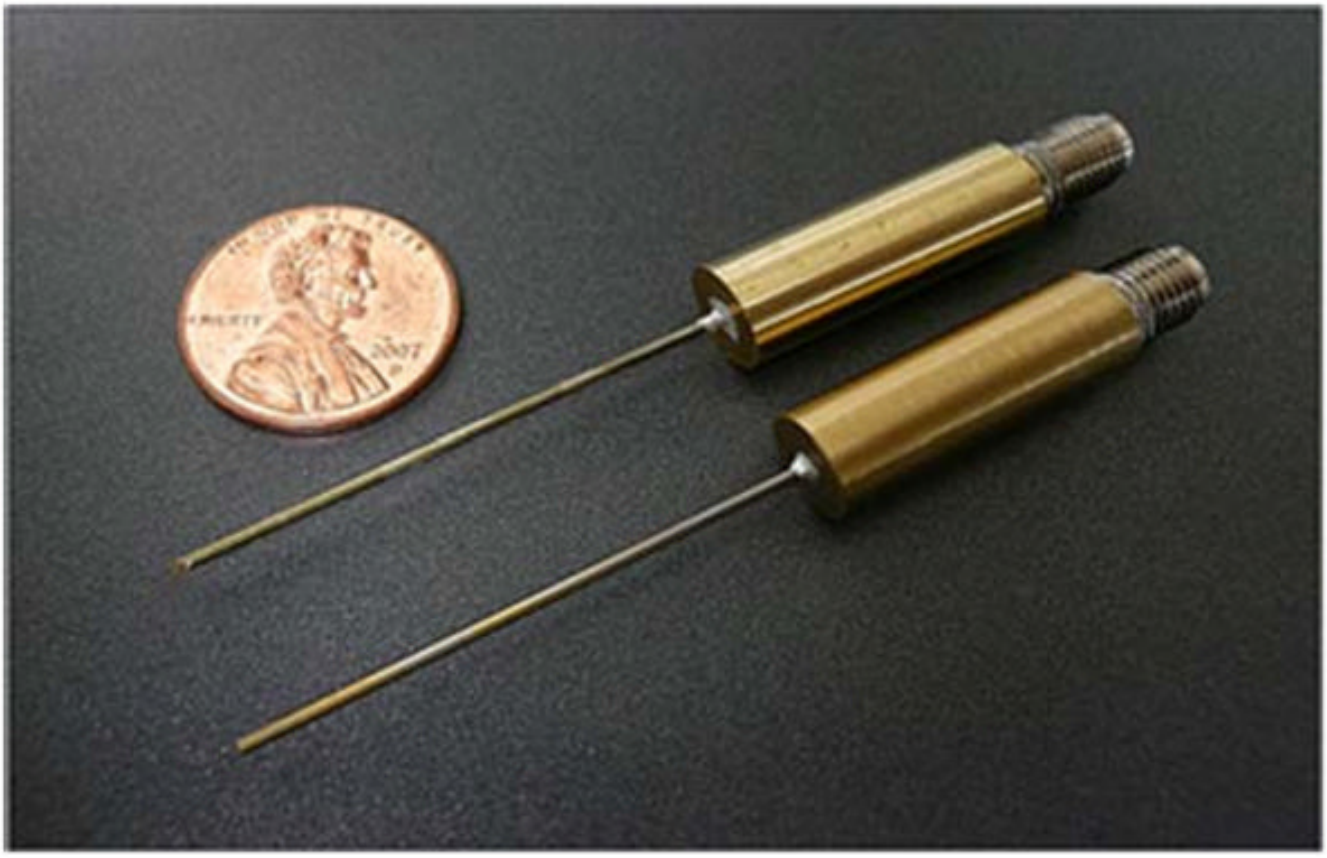

Fig. 1.
A photograph of PMN-PT needle transducers

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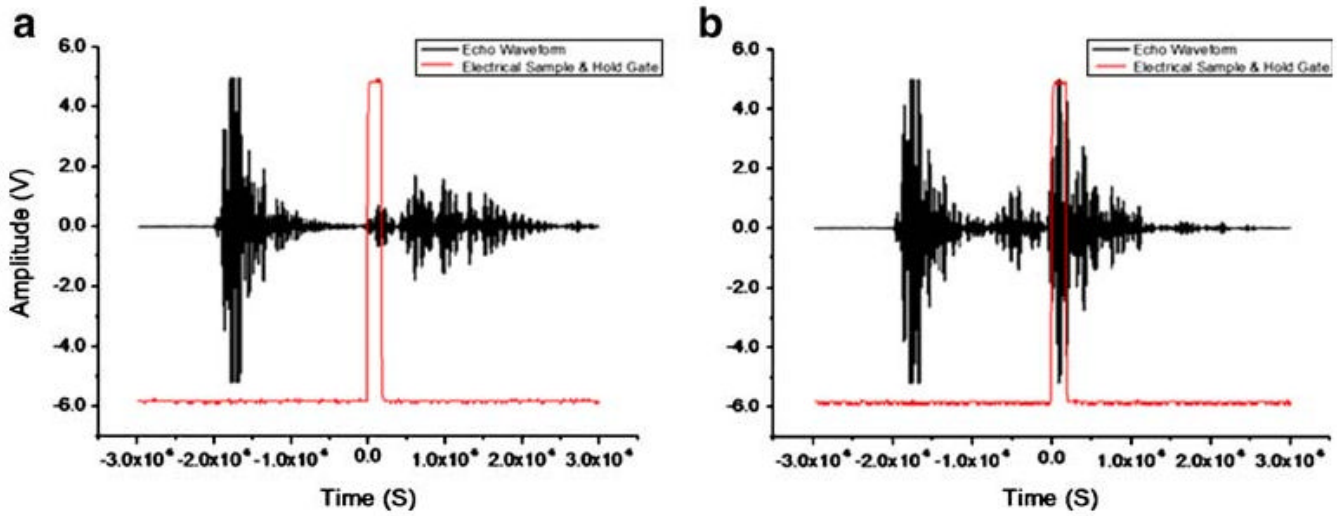

Fig. 2.

Typical radio frequency signal with time gate windows of the Doppler measurements for retinal vessels (**a**) and choroid vessels (**b**)

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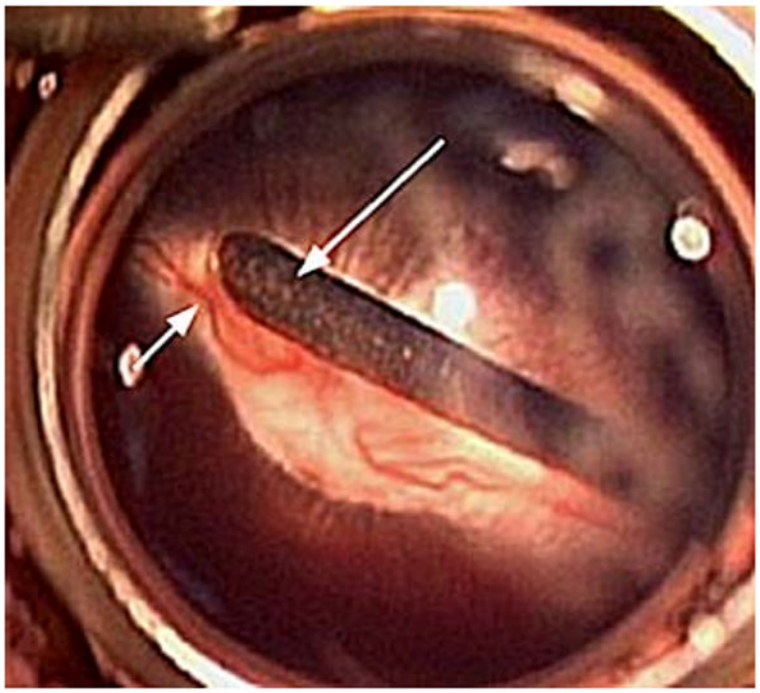

Fig. 3.
A representative image, showing the intraocular ultrasound Doppler transducer (*long arrow*) placed over the temporal retinal vessels (*short arrow*) at the edge of the optic disc

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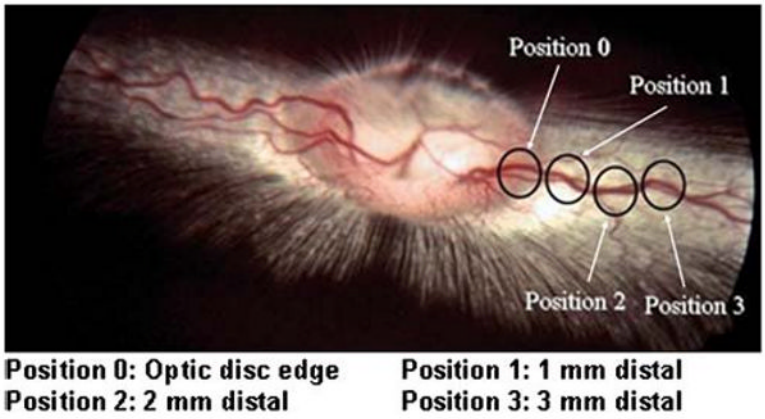

Fig. 4.
The measurement sites were designated according to the distance from optic disc edge

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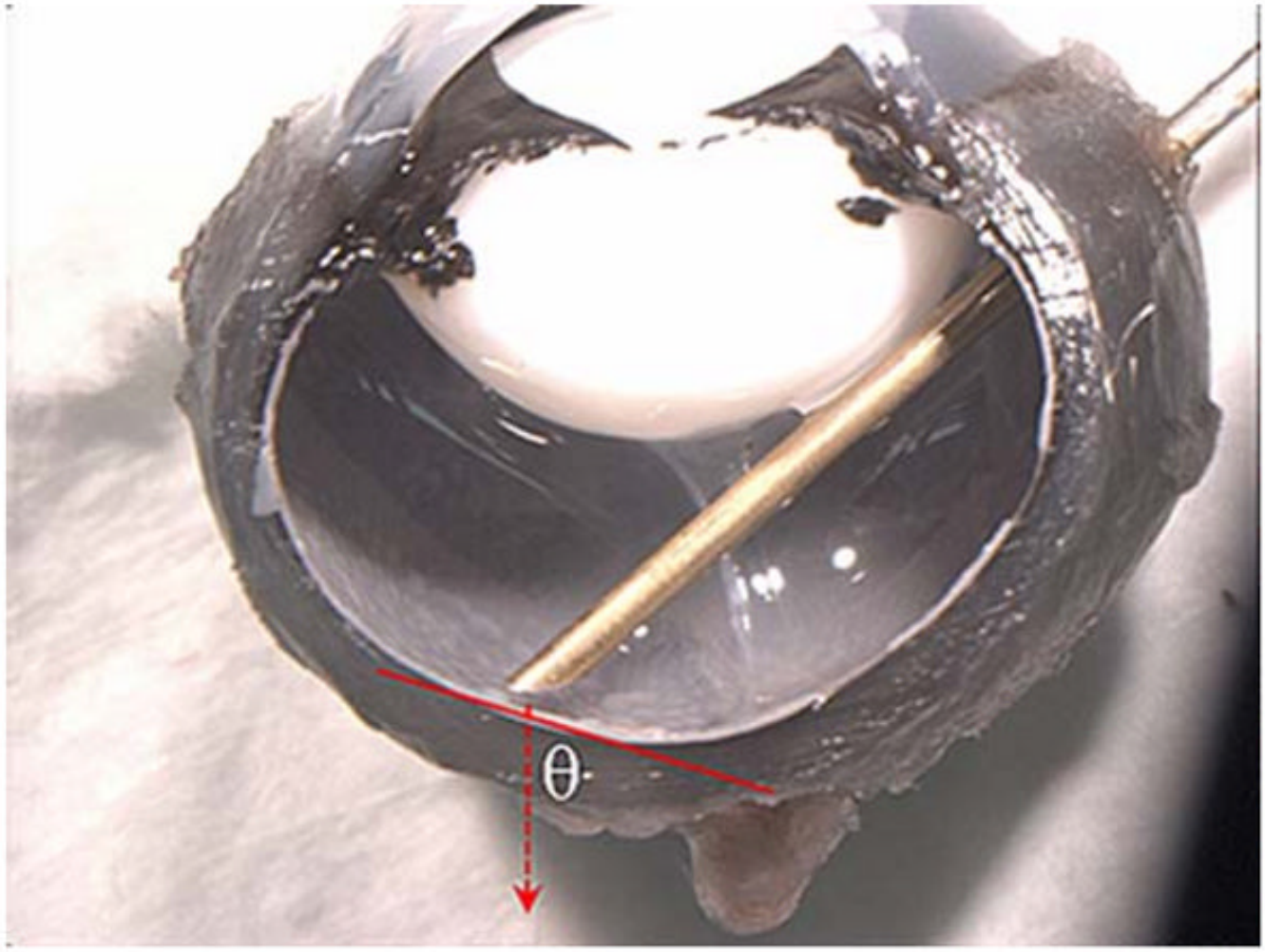

Fig. 5.
Doppler angle θ estimation. The *red solid line* indicates the estimated flow direction and the *red dashed line* indicates the direction of ultrasound

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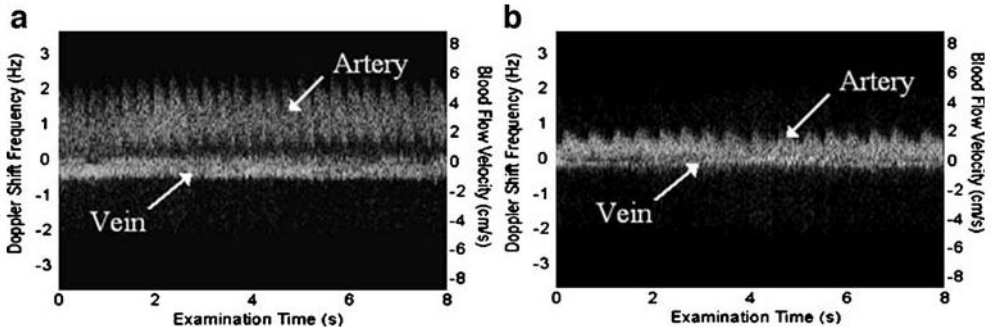

Fig. 6.

The typical Doppler spectrogram of retinal vessel: the optic disc edge (**a**), and distal retinal vessel (**b**)

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Table 1

The result of measured retinal blood flow velocities at different positions in the rabbit eyes (cm/s)

Measurement site			Artery (peak)	Artery (mean)	Artery (Minimum)	Vein
Left Eye	Nasal	Position 0	6.95±0.87	5.37±1.08	3.91±0.65	3.47±0.65
		Position 1	5.72±1.06	4.45±0.87	3.28±0.71	2.89±0.47
		Position 2	4.25±0.82	3.06±0.67	2.63±0.45	2.28±0.59
		Position 3	3.73±0.69	2.62±0.73	1.97±0.52	1.73±0.53
	Temporal	Position 0	7.01±0.98	5.52±0.94	4.25±0.74	3.61±0.35
		Position 1	6.45±0.83	4.89±0.73	3.62±0.53	3.17±0.68
		Position 2	5.47±0.89	3.63±0.55	2.96±0.62	2.69±0.36
		Position 3	4.18±0.46	3.05±0.85	2.45±0.36	2.13±0.49