Direct Measurement of Retinal Vessel Diameter: Comparison with Microdensitometric Methods Based on Fundus Photographs

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Abstract. A new method for the direct measurement of retinal vessel diameter was developed and the results were compared with those of two computer-assisted semiautomatic microdensitometric methods. The direct measurement method was based on automated detection of vessel edges by processing the one-dimensional retinal image obtained by a linear image sensor set in a fundus camera; results are obtained in real-time. Thirty-eight points on 36 vessels (15 points on arteries and 23 points on veins) of two normal volunteers (four eyes) were selected as the measurement points. Two observers measured the vessel diameters at these points and the interobserver variation of the direct method and the microdensitometric methods was compared. The coefficient of variation and the interobserver variation of the direct method for all measurements were 1.71 + 1.13% and 2.25 + 1.92%, respectively. There was no significant difference between the measured values for the two observers with the direct method (paired t-test, $P > 0.05$), and the interobserver variation of the direct method was smaller than those of the microdensitometric methods.

This newly developed direct method for measurement of retinal vessel diameter not only avoids systematic errors that result from film development or the characteristics of the film, but also generates reproducible results in real-time and small interobserver variation. **(Surv Ophthalmol 39 [Suppl 1, May]:** S57–S65, 1995)

Key words. fundus photographs • image analysis • linear image sensor • microdensitometry • retinal blood vessel diameter

Retinal blood vessels are the only visible and optically accessible small blood vessels in the human body noninvasively. The information obtained from the examination of retinal vessels offers many useful parameters for the diagnosis or evaluation of ocular or systemic diseases, and vessel diameter is one of the most useful of these parameters. The accuracy of the measurement of retinal vessel diameter markedly influences the reliability of the determination of retinal blood flow, because the latter is calculated as the product of retinal blood flow velocity, measured by laser Doppler velocimetry[6,8,17] or laser speckle velocimetry,[19] and the square of the retinal vessel diameter, assuming a circular cross-section of vessel. Several methods have been described for the measurement of retinal vessel diameter,[1–4,6–13,15–19] almost all of which involve micrometric[1,6,8,12,13,17,19] or microdensitometric[2,4,7,9–11,15,16,18] analysis of fundus photographs. Micrometric methods are reportedly less reproducible and are associated with more interobserver variation than microdensitometric methods,[4,16] whereas microdensitometric image analysis methods are tedious and time-consuming.[4] In addition, both types of method may be associated with systematic error originating from the film characteristics or the film development procedures because they are both based on a film negative taken by a fundus camera. Delori reported a retinal vessel tracking system with capability of measuring the retinal vessel diameter in a direct fashion which was combined with an oximetry system for the retinal blood vessel.[3] Recently, we also reported a new method for measurement of retinal vessel diameter in which vessel diameter is obtained directly from the image of the fundus in real-time.[20] We have now improved this method and

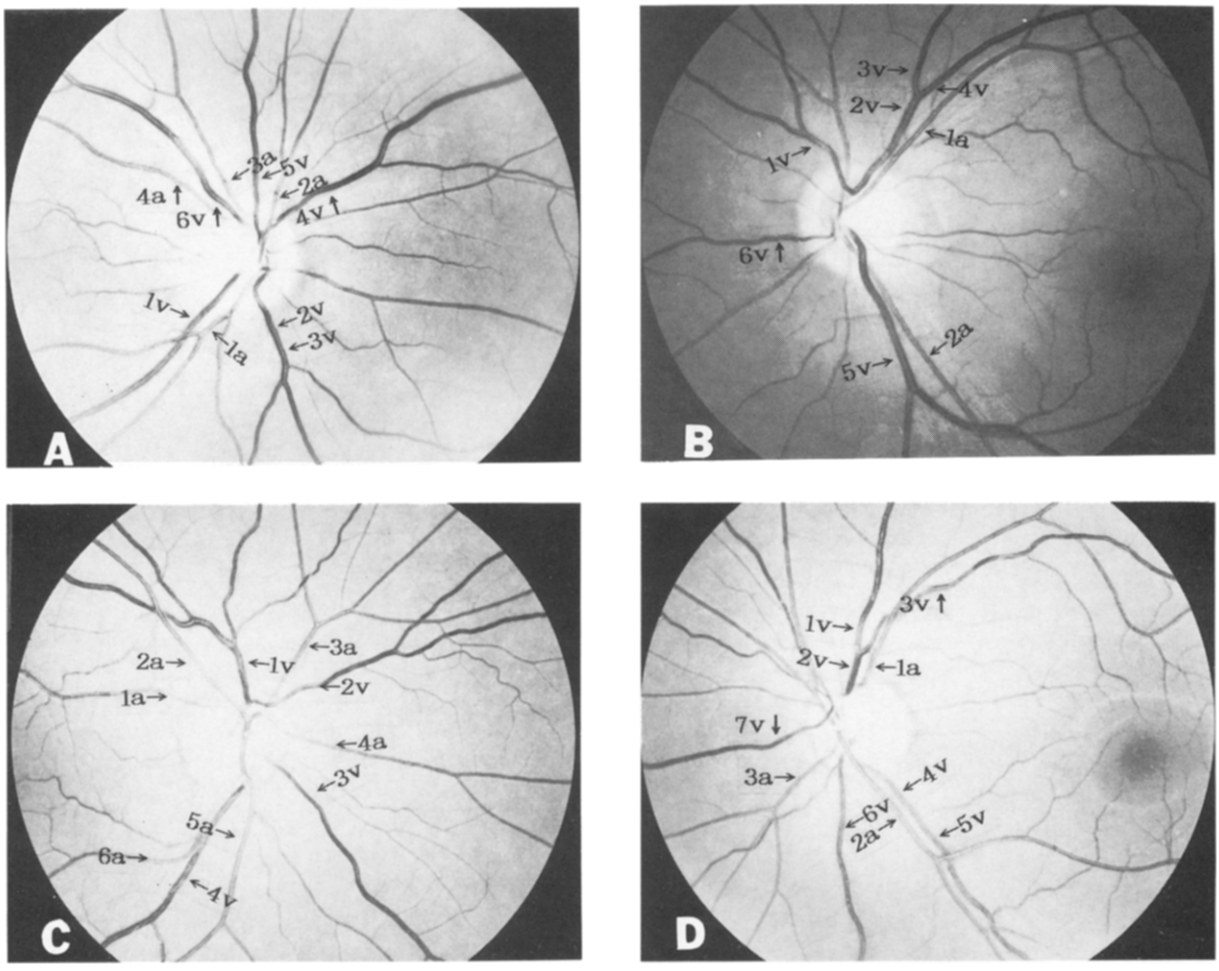

Fig. 1. Fundus photographs of subjects. Measured points on the right eye of subject 1 (A), the left eye of subject 1 (B), the right eye of subject 2 (C), and the left eye of subject 2 (D) are shown. a, artery; v, vein.

compared it with computer-assisted semiautomatic microdensitometric methods. Both reproducibility and ease of use were evaluated.

Subjects and Methods

SUBJECTS

Subjects were two normal male volunteers (four eyes) aged 38 and 36 years, with refracting powers of −3.0 (right eye) and −2.75 (left eye) diopters and +0.25 (right eye) and 0.0 (left eye) diopters, respectively. Before the experiment, informed consent was obtained from both volunteers. Ocular examinations, including fundus examination, slit-lamp examination, and intraocular pressure measurement, as well as systemic examination revealed no ocular or systemic disease. Subjects' eyes were dilated with 0.5% tropicamide and 0.5% phenylephrine hydrochloride ~1 hour before the experiment. Thirty-eight points on 36 vessels (15 points on arteries and 23 points on veins) were selected as the sites of measurement (Fig 1). Two observers measured retinal vessel diameters by the direct method and two other observers measured diameters by microdensitometric methods. All results were masked so that observers were unaware of each other's data.

DIRECT MEASUREMENT OF RETINAL VESSEL DIAMETER

Apparatus

The direct method is based on automated determination of retinal vessel edges from the one-dimensional retinal image obtained by a linear image sensor set in a fundus camera. The apparatus consists of a fundus camera (fx-50R; Kowa, Nagoya, Japan), a MOS (metal oxide seminconductor) linear image sensor (S3904, Hamamatsu Photonics, Hamamatsu, Japan), an A/D (analog to digital) converter, and a microcomputer (Fig. 2). A halogen lamp in the fundus camera serves as a light source and the wavelength of the irradi-

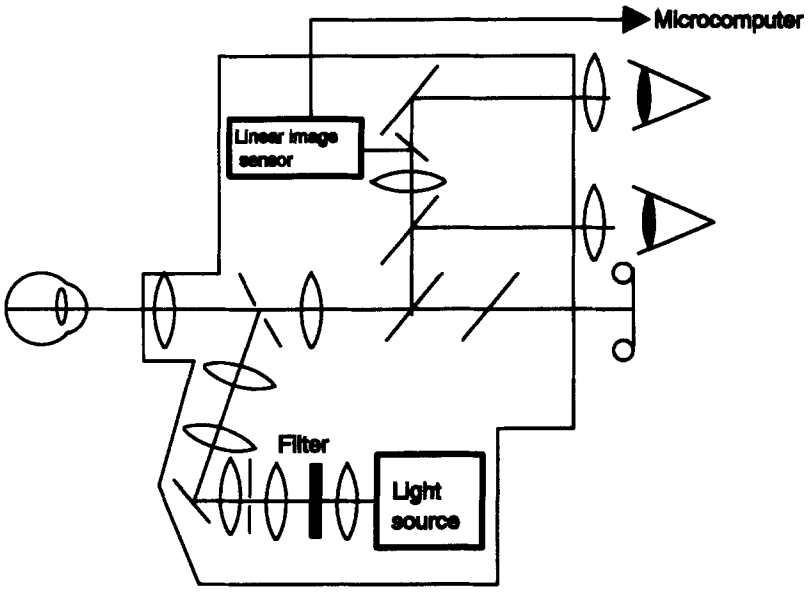

Fig. 2. The apparatus for the direct measurement of retinal vessel diameter.

ating light is filtered to ~570 nm by an interference filter for maximal vessel definition.[5] The irradiated area on the retina is ~1.5 mm in diameter. The target retinal vessel is centered in the irradiated area with the guide of a pointer stick. The measuring point may be further adjusted by moving the lenses in the apparatus with a joystick set on the apparatus table. The reflected light from the fundus is magnified approximately five times by the lenses and focused on the MOS linear image sensor, with sensing elements comprising 512 photodiodes. The center-to-center spacing of the elements corresponds to ~5 μm on the retina of the Gullstrand standard eye. During measurement, the observer watches the irradiated area thorugh an eyepiece. The alignment of the linear image sensor is adjusted by a manipulator situated at the eyepiece to make the direction of the linear image sensor perpendicular to that of the target retinal vessel. The observer can see the direction of the linear image sensor with the indication line in the eyepiece. The image signal obtained by the linear image sensor is transmitted to a microcomputer through an A/D converter and the retinal image is processed. The measuring time is 1.0 s, during which the retinal image is sent to the microcomputer 16 times.

Image Processing Method

The measurement algorithm for retinal vessel diameter consists of two phases: the detection of a retinal vessel and the determination of the vessel edges. First, from the 512 profile data points [x(1), x(2), x(3), . . . x(512)] of the retina obtained with the linear image sensor, five point moving averages [y(i)] and 75 point moving averages [z(i)] are calculated. The amplitude of the z(i) values is reduced to the 80% level [z'(i)] to make the detection of a retinal vessel efficient. Points for which the value of z'(i) is greater than y(i) by an arbitrary predetermined value are recognized as being on a retinal vessel. If a series of more than five points is recognized as being on a retinal vessel, the data are processed further; if not, the data are excluded from further analysis, because the retinal vessel edge cannot be detected from such data.

Second, the edges of the retinal vessel profile are found. y(i) is differentiated [dy(i)/di] and the points corresponding to its extrema are defined as the points near the vascular edge (A and B in Fig. 3), because they indicate the points where the slope of the vessel profile are largest locally. The edge of the retinal vessel is defined as the point with the average height of the y(i) values of the two points with half-height values of the dy(i)/di extrema (C, D and E, F in Fig. 3). The diameter of the target vessel is defined as the distance between the edge points determined by the above calculation, only when the five center points (254th to 258th points) are included between these points. The 16 images obtained in 1.0 s are processed respectively and only the valid values are averaged and corrected for refraction and the axial length of the subject's eye, according to Littmann's method.[14] With this process, the result can be obtained with 0.1 s from the end of the measurement. The theoretically measurable range of retinal vessel diameter

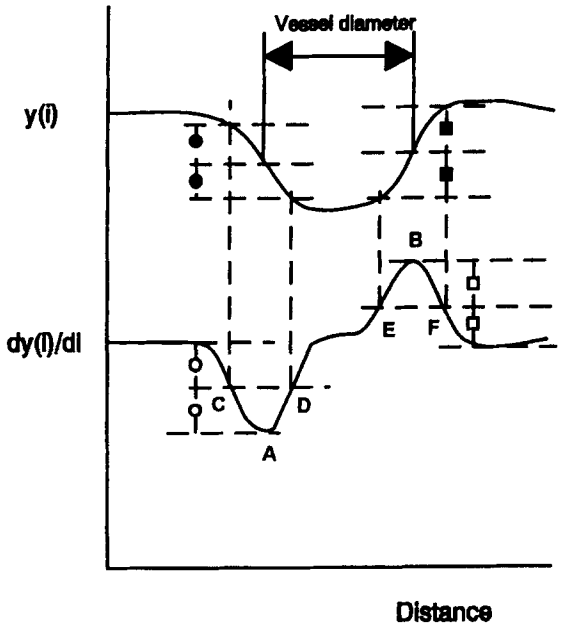

Fig. 3. Schematic diagram of the procedure for detection of the vessel edge in the direct measurement of retinal vessel diameter. Five point moving averages [y(i)] from the 512 image profile data points are calculated and differentiated [dy(i)/di]. The edge of the retinal vessel is defined as the point with the average height of the y(i) values of the two points (C, D and E, F) that have half-height values of the dy(i)/di extrema. A and B are the points corresponding to the dy(i)/di extrema.

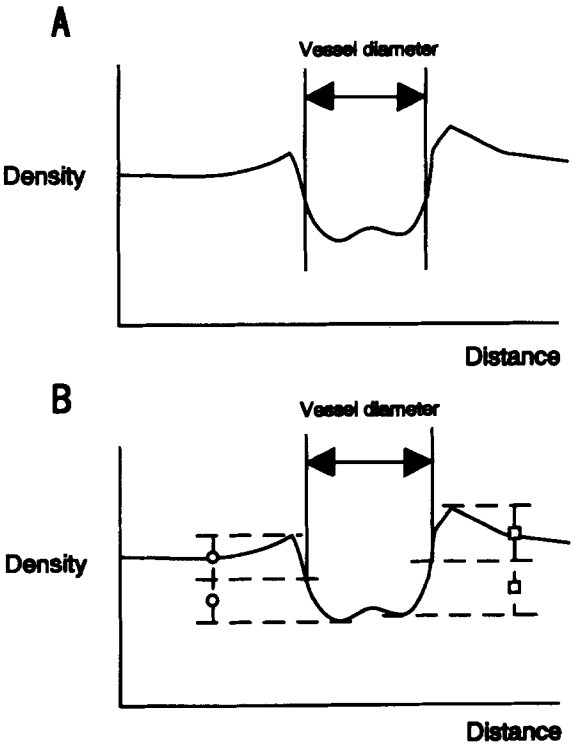

Fig. 4. Schematic diagram of the procedure for detection of the vessel edge in the microdensitometric methods for measurement of vessel diameter. (A) Method 1: An observer marks the points where the change of density is greatest as the vessel edge. (B) Method 2: An observer marks the peak points of the density profile and the half-height point of the peak is regarded as the vessel edge.

is from ~25 μm to ~1480 μm in the Gullstrand standard eye.

Measurements

Before measurement by the direct method, a monochromatic (ware-length of 570 nm) Polaroid instant photograph (Polaroid 600; Polaroid, Cambridge, MA) and a monochromatic 35 mm photograph (Tri-X; Kodak, Rochester, NY) were taken with a fundus camera (fx-500, Kowa). The Kodak film was developed and used as the source image for the microdensitometric methods (Fig. 1). Eight to 10 points on the retinal vessels of each eye were marked on the Polaroid instant photograph as the target points for measurement. Two observers measured the retinal vessel diameter of each target point three to eight times.

MICRODENSITOMETRIC MEASUREMENT OF RETINAL VESSEL DIAMETER

Monochromatic retinal photographs were taken with a fundus camera (fx-500; Kowa) with a field angle of 30° and a 570 nm interference filter for maximal vessel definition.[5] The film used was Tri-X (Kodak) and photographs were taken immediately before measurement of retinal vessel diameter by the direct method. The retinal image on the 35 mm negative was scanned with a slide scanner (Coolscan; Nikon, Tokyo, Japan) and a microcomputer (Macintosh IIci; Apple, Cupertino, CA), and an area of 3.5 mm^2 on the negative, which corresponds to ~0.6 mm^2 on the retina of the Gullstrand standard eye, was digitized to a 200 by 200 array of discrete (256 gray values) pixels, graded according to the film transmittance, and saved on a floppy disk. The scanning contrast was adjusted to give the highest contrast between retinal vessels and the background retina. The digitized retinal image file was then loaded into a microcomputer (PC-9801RA51; NEC, Tokyo, Japan) and the image was reversed and displayed on a monitor. A mouse-controlled cursor was used to mark two points on the target retinal vessel separated by a distance corresponding to ~300 μm on the ret-

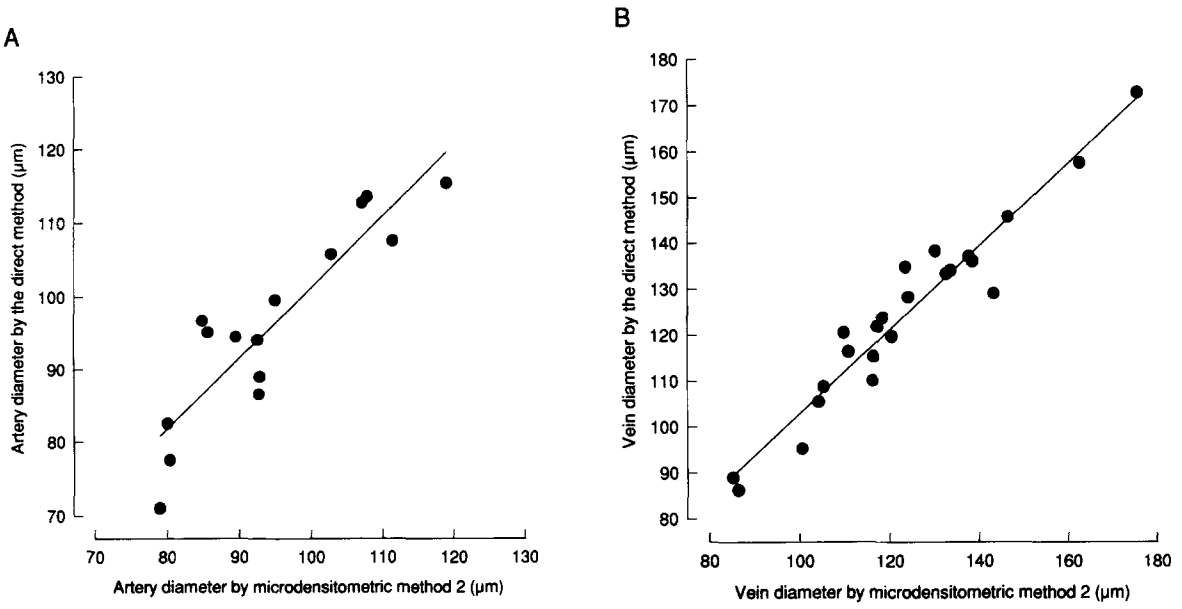

Fig. 5. Relation between the average values of mean vessel diameter determined by two observers for retinal arteries (A) and retinal veins (B) with the direct method and microdensitometric method 2. Linear regression lines are shown.

ina, including the target point at the center of the range, and a line combining the two points that represented the direction of the retinal vessel was drawn. Five parallel lines perpendicular to this direction line were drawn automatically between the two points, with an interval corresponding to ~50 μm on the retina, and the computer showed the gray-level profiles on these lines. The retinal vessel diameter was determined by two methods. In method 1, the observer marked the points where the density changes were locally greatest as the edge of the target retinal vessel (Fig. 4A). In method 2, the observer marked the points of highest and lowest density, which held the edge of the target retinal vessel between them, and the point with half-height density was regarded as the vessel edge (Fig. 4B). The diameter of the target retinal vessel was determined for each profile with both microdensitometric methods and was then corrected for refraction and the axial length of the subject's eye, according to Littmann's method.[14] The values obtained from the five profiles with both methods were averaged and the coefficient of variation was calculated.

Results

INTERMETHOD DIFFERENCES

The results of the direct method and the microdensitometric methods for retinal arteries are shown in Table 1 and those for retinal veins are shown in Table 2. The average of the artery and vein diameters for both observers were significantly smaller with microdensitometric method 1 than with the direct method or microdensitometric method 2 (Sidak paired t-test, $P < 0.05$). There was no significant difference between the values obtained with the direct method and those obtained with microdensitometric method 2 (Sidak paired t-test, $P > 0.05$). When the artery and vein measurements were analyzed separately, there was a statistically significant difference only between microdensitometric methods 1 and 2 (Sidak paired t-test, $P < 0.05$).

The correlation coefficients between the average values of mean vessel diameter obtained by the two observers for the three methods were all highly significant ($P < 0.0001$). Fig. 5 shows the relation between the retinal artery (A) and the retinal veins (B) measurements by the direct method and microdensitometric method 2.

INTRAMEASUREMENT VARIABILITY AND REPRODUCIBILITY FOR EACH METHOD

The coefficient of variation (CV) of the artery and vein measurements obtained with the direct method was 1.76 ± 1.33% (mean ± SD) for the first observer and 1.67 ± 0.91% for the second observer; the CV was 1.71 ± 1.13% for all measurements. The corresponding CV values for microdensitometric method 1 were 1.76 ± 0.85, 2.21 ± 1.52, and 1.99 ± 1.24%, respectively, and those for microdensitometric method 2 were

TABLE 1

Measurements of Artery Diameter
Values Are Means (μm) ± Coefficient of Variation (CV)(%)

	Direct Method				Microdensitometric Method							
					Method 1				Method 2			
	Observer 1		Observer 2		Observer 1		Observer 2		Observer 1		Observer 2	
Position	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
S1R-1a	108.2	1.55	107.0	1.55	115.9	0.77	103.2	1.92	114.0	0.84	108.7	1.40
S1R-2a	91.5	1.34	86.5	0.64	94.6	1.06	86.2	4.28	92.9	0.45	92.7	1.68
S1R-3a	91.1	0.78	99.2	2.24	89.3	2.04	75.3	1.03	89.8	1.50	81.4	0.48
S1R-4a	96.1	2.63	92.1	2.29	92.5	0.70	87.2	2.05	91.6	0.49	93.4	1.64
S1L-1a	112.5	1.29	113.0	1.63	111.9	3.80	96.7	1.37	113.4	1.36	100.6	1.00
S1L-2a	115.5	0.78	111.7	1.20	103.9	0.65	104.3	2.14	108.9	1.92	106.6	1.44
S2R-1a	78.4	2.11	76.8	0.46	80.9	2.99	81.4	2.17	80.4	2.62	80.2	1.98
S2R-2a	99.9	1.47	93.6	2.36	85.0	2.08	86.2	0.69	84.9	0.71	84.8	0.90
S2R-3a	93.8	3.07	95.2	1.50	92.4	1.43	88.4	1.34	90.9	1.15	88.1	0.98
S2R-4a	73.0	1.56	69.2	2.11	85.1	2.51	74.4	2.89	85.4	1.06	72.5	2.55
S2R-5a	107.6	1.53	104.0	1.88	103.1	3.00	97.9	0.60	104.7	2.26	100.8	0.86
S2R-6a	87.4	4.49	85.8	1.76	103.4	3.17	76.3	8.97	103.0	1.76	82.4	4.58
S2L-1a	98.6	0.68	100.6	1.48	95.5	0.63	90.0	3.40	96.6	1.05	93.2	1.64
S2L-2a	115.8	0.77	115.1	0.92	123.2	1.84	110.1	2.62	122.2	2.03	115.4	1.89
S2L-3a	83.2	2.54	81.9	2.81	82.2	2.95	78.1	1.37	79.5	1.23	80.4	2.04
Average	96.8	1.77	95.4	1.66	97.3	1.97	89.0	2.46	97.2	1.36	92.1	1.67
SD	13.3	1.05	13.7	0.66	12.7	1.07	11.3	2.07	13.2	0.65	12.3	0.97

S1R, S1L: right eye and left eye of subject 1, respectively.
S2R, S2L: right eye and left eye of subject 2, respectively.

1.20 ± 0.59, 1.37 ± 0.85, and 1.28 ± 0.73%, respectively (Table 3). The CV values of the vein measurements and all measurements were significantly smaller for microdensitometric method 2 than for the direct method and microdensitometric method 1 (Sidak t-test, $P < 0.05$); there were no significant corresponding differences between the direct method and microdensitometric method 1 (Sidak t-test, $P > 0.05$). There were no significant differences between the three methods for the artery measurements.

INTEROBSERVER DIFFERENCES FOR EACH METHOD

Comparison between the measurements obtained by the different observers revealed no significant differences for the direct method with regard to artery, vein, or all measurements (paired t-test, $P > 0.05$). Significant differences (paired t-test, $p < 0.05$) between the results obtained by the two observers were apparent for both microdensitometric methods, except in the comparison of vein measurements with microdensitometric method 2. The interobserver variation, defined as the absolute value of the difference between the mean measurement values of two observers divided by their average value, was 3.21 ± 2.38% for artery measurements, 1.63 ± 1.24% in vein measurements, and 2.25 ± 1.92% for all the measurements by the direct method. The corresponding values for microdensitometric method 1 were 9.08 ± 7.77, 3.70 ± 3.91, and 5.82 ± 6.25%, respectively, and those for microdensitometric method 2 were 5.81 ± 6.52, 2.89 ± 2.28, and 4.04 ± 4.61%, respectively (Table 4). The interobserver variation for the direct method was significantly smaller than that for microdensitometric method 1 with regard to artery, vein, and all measurements; it was also significantly smaller than that for microdensitometric method 2 with regard to vein measurements (Sidak paired t-test, $P < 0.05$). The difference in interobserver variation between microdensitometric methods 1 and 2 was statistically significant with regard to artery measurements and all measurements (Sidak paired t-test, $P < 0.05$), but not vein measurements.

Discussion

We have shown that the direct method for determination of retinal vessel diameter is associated with smaller interobserver variability than the two microdensitometric methods used. For all measurements, the interobserver variation of the

TABLE 2

Measurements of Vein Diameter
Values Are Means (μm) ± Coefficient of Variation (CV)(%)

	Direct Method				Microdensitometric Method							
					Method 1				Method 2			
	Observer 1		Observer 2		Observer 1		Observer 2		Observer 1		Observer 2	
Position	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
S1R-1v	130.0	0.33	128.0	0.52	141.5	1.48	138.6	1.41	143.7	0.58	142.6	0.87
S1R-2v	136.8	1.17	132.8	1.83	130.6	1.54	114.3	3.70	128.6	1.20	118.3	0.89
S1R-3v	127.8	0.83	128.6	1.80	127.3	1.29	119.7	3.35	124.1	1.38	124.0	1.20
S1R-4v	118.9	1.07	120.5	1.48	123.6	1.22	118.2	1.19	120.8	0.80	120.1	0.36
S1R-5v	110.0	0.69	110.1	1.09	112.8	1.23	113.2	2.84	114.1	0.41	118.3	0.51
S1R-6v	136.6	1.71	137.5	1.22	132.4	0.89	133.1	2.98	135.1	0.35	140.1	0.79
S1L-1v	135.0	3.50	132.9	2.10	128.8	2.98	133.9	2.01	130.8	1.49	136.2	1.37
S1L-2v	174.4	0.42	171.2	0.67	176.8	1.58	175.0	0.93	177.7	0.66	173.0	0.77
S1L-3v	118.0	1.72	112.6	1.54	114.8	2.15	115.0	0.73	117.1	1.36	115.6	1.14
S1L-4v	132.6	1.08	134.0	0.68	135.4	2.18	124.9	1.33	136.1	0.73	129.0	0.46
S1L-5v	159.2	1.05	156.0	1.11	160.1	1.87	158.9	1.81	165.6	1.26	159.4	1.44
S1L-6v	94.8	2.07	95.6	0.78	97.4	0.79	101.2	1.07	99.9	1.51	101.1	0.60
S2R-1v	145.1	2.17	146.3	1.89	145.9	0.63	145.4	1.39	146.9	0.25	145.8	0.78
S2R-2v	105.4	1.05	105.6	1.81	104.1	2.50	104.1	1.46	104.5	0.81	103.8	0.93
S2R-3v	88.5	1.48	89.5	0.37	83.3	2.34	84.4	1.49	84.6	0.92	85.5	0.95
S2R-4v	137.6	1.40	134.4	0.75	137.1	1.13	139.7	1.21	137.9	1.57	139.0	1.50
S2L-1v	123.3	1.47	124.2	1.27	117.9	2.05	117.1	1.08	118.4	0.43	118.3	0.65
S2L-2v	137.9	3.04	138.6	2.81	131.4	2.05	126.9	1.16	132.1	0.81	128.1	0.88
S2L-3v	122.6	1.44	118.5	4.58	114.6	1.12	108.0	3.81	111.7	1.88	107.9	2.79
S2L-4v	121.4	2.02	122.2	3.49	119.7	2.12	111.2	3.29	119.6	2.03	114.8	2.01
S2L-5v	114.2	7.75	118.5	3.03	107.8	1.36	110.6	2.14	107.2	2.03	114.4	1.02
S2L-6v	108.0	1.83	109.5	2.81	103.2	0.48	106.4	2.51	102.6	0.91	108.0	1.98
S2L-7v	84.8	1.08	87.7	0.89	89.7	2.43	78.1	4.12	88.7	1.65	83.9	3.17
Average	124.5	1.76	124.1	1.67	123.3	1.63	120.8	2.04	123.8	1.09	122.9	1.18
SD	21.1	1.51	20.0	1.06	21.7	0.66	21.9	1.05	22.3	0.54	21.3	0.71

S1R, S1L: right eye and left eye of subject 1, respectively.
S2R, S2L: right eye and left eye of subject 2, respectively.

direct method was 2.25 ± 1.92% (mean ± SD), whereas that of microdensitometric method 1 and 2 was 5.82 ± 6.25% and 4.04 ± 4.61%, respectively. On the other hand, the coefficient of variation of the direct method was as good as that of microdensitometric method 1, but greater than that of microdensitometric method 2. The CV for all measurements was 1.71 ± 1.13% by the direct method, 1.99 ± 1.24% by microdensitometric method 1, and 1.28 ± 0.73% by microdensitometric method 2. In the direct method, the observers adjusted the position of the apparatus to obtain the retinal image in clear focus, and then corrected the position and the direction of the linear image sensor for every measurement to compensate for eye movements of the subject between measurements. In addition, we used simulated repeated measurements for the evaluation of the reproducibililty of the microdensitometric methods; i.e., we calculated the CV using measured values on five profiles with an interval corresponding to ~50 μm on the retina of one image instead of actually performing repetitive measurements on a point. Therefore, these values do not precisely mean the reproducibility of the methods, but they are reference values indicating the reproducibility of the methods. In contrast, the CV of the direct method was

TABLE 3

The Coefficient of Variation of Each Measurement Method Calculated with All the Measured Data Including Those of Arteries and Veins
Values Are Means ± Standard Deviation (SD), (%)

	Direct Method		Microdensitometric Method			
			Method 1		Method 2	
	Mean	SD	Mean	SD	Mean	SD
Observer 1	1.76	1.33	1.76	0.85	1.20	0.59
Observer 2	1.67	0.91	2.21	1.52	1.37	0.85
All	1.71	1.13	1.99	1.24	1.28	0.73

TABLE 4

The Interobserver Variation of Each Method Values Are Means ± Standard Deviation (SD), (%)

	Direct Method		Microdensitometric Method			
			Method 1		Method 2	
	Mean	SD	Mean	SD	Mean	SD
Artery	3.21	2.38	9.08	7.77	5.81	6.52
Vein	1.63	1.24	3.70	3.91	2.89	2.28
All	2.25	1.92	5.82	6.25	4.04	4.61

calculated from three to eight measurements for one target point and therefore truly represents the reproducibility of the method. This difference may account for the larger CV of the direct method than that of microdensitometric method 2, in spite of the small interobserver variability. The reproducibility of the direct method (1.71 ± 1.13%) was comparable to or better than that of microdensitometric methods in other studies.[2,4,15,16]

The fact that the two observers in the direct method were not the same as those in the microdensitometric methods may be one source of the discrepancy in the interobserver variability. However, this possibility is considered to be very small because the procedure used in the direct method was much different from those in the microdensitometric methods and most of the difference of interobserver variability is considered to reflect the difference of measurement procedures rather than that arising from the difference of observers.

Several algorithms have been used for the microdensitometric measurement of retinal vessel diameter from fundus photographs.[2,4,7,9–11,15,16,18] We used the half-height method (method 2), which has been one of the most popular methods in previous studies,[2,4,9,10,16] and a simple observer-driven edge detection method (method 1), which is similar to micrometric methods. The CV of method 2 was smaller than that of method 1, which was compatible with previous reports.[4,16] However, the measured values with method 1 were smaller than those with method 2, which contrasts with the results of previous studies,[4,16] showing that observers tended to overestimate vessel width with micrometric methods.

Several investigators have described automatic microdensitometric methods for the measurement of retinal vessel diameter.[9,10,15] These methods not only reduce the optical aberration of projection in micrometric methods, but also avoid observer bias. Therefore, these automatic microdensitometric methods may share similar advantages over the micrometric methods as with our direct method, although the systematic errors associated with film development and film characteristics cannot be avoided and real-time measurement is not possible with microdensitometric methods.

The most significant merit of the direct method for determination of retinal vessel diameter is that it offers the possibility of simultaneous measurement of the retinal vessel diameter and retinal blood flow velocity. Such simultaneous measurements could be achieved by installing the image detection instruments in the apparatus used for measuring retinal blood flow velocity. Ocular movement is one of the largest obstacles to measurement of retinal blood flow velocity; consequently, repeated measurements at exactly the same point are almost impossible. However, if the simultaneous measurement of retinal vessel diameter is possible, the accuracy of retinal blood flow measurement will be markedly increased because blood flow is calculated as proportional to the square of the retinal vessel diameter.[6,8,17,19] Moreover, the directness of the method avoids those errors that originate from the use of film or from the projection of the negative of fundus photographs. Additionally, the disparity between the intensity of the original image and the film density can be avoided.[4] Recently, a retinal vessel tracking system combined with the direct measurement of the retinal vessel diameter was developed by Delori[3] and this system may further facilitate the exact measurement of retinal blood flow. In either method, however, the assumption of a circular cross-section of vessel should be further investigated and verified, especially in measurement of blood flow in retinal veins, because there is high possibility that the cross-section of retinal veins are ellipsoid rather than circular. When the cross-section of the retinal veins are ellipsoid rather than circular, the blood flow calculated on the assumption of a circular cross-section may be larger than the real values. Therefore, care should be taken to evaluate the measured values of retinal blood flow in these methods.

The application of the MOS linear image sensor in place of a PCD (plasma-coupled device) image sensor and the optimization of the software for image detection were the main improvements in the direct method since our previous report.[20] The MOS linear image sensor has a higher sensitivity than the PCD linear image sensor, which, together with the improved soft-

ware, allowed measurement of the image 16 times in 1.0 s, instead of eight times in the apparatus previously described.

Comparison of the reproducibility between artery measurements and vein measurements with the direct method showed that the CV values were approximately the same. The difference in vessel size between retinal arteries and veins would have been expected to result in a larger CV for artery measurements than vein measurements, because fluctuations in the measured values should be greater in the measurement of smaller vessels. Moreover, the reproducibility of artery measurements should have been less than that of vein measurements because of retinal artery pulsation.[10,16] Although the reason for the similar degrees of reproducibility in artery measurements and vein measurements is unclear, the measurement algorithm or the measurement time of 1.0 s, which is approximately the same as the pulsation cycle in humans, may have masked the influence of artery pulsation.

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