

# A New Method of Magnification Correction for Accurately Measuring Retinal Vessel Calibers From Fundus Photographs

Aiko Iwase,<sup>1</sup> Akihiko Sekine,<sup>2</sup> Jun Suehiro,<sup>2</sup> Kenji Tanaka,<sup>1</sup> Yumiko Kawasaki,<sup>3</sup> Ryo Kawasaki,<sup>3</sup> Michael J. Sinai,<sup>2</sup> and Makoto Araie<sup>4</sup>

<sup>1</sup>Tajimi Iwase Eye Clinic, Tajimi, Japan

<sup>2</sup>Topcon Corporation, Tokyo, Japan

<sup>3</sup>Department of Public Health, Yamagata University Graduate School of Medical Science, Yamagata, Japan

<sup>4</sup>Kanto Central Hospital of the Mutual Aid Association of Public School Teachers, Tokyo, Japan

Correspondence: Aiko Iwase, Tajimi Iwase Eye Clinic, 3-101-1 Honmachi, Tajimi, Gifu, 507-0033, Japan; aiko-gif@umin.net.

Submitted: November 29, 2016

Accepted: January 30, 2017

Citation: Iwase A, Sekine A, Suehiro J, et al. A new method of magnification correction for accurately measuring retinal vessel calibers from fundus photographs. *Invest Ophthalmol Vis Sci*. 2017;58:1858-1864. DOI: 10.1167/iovs.16-21202

**PURPOSE.** To report a semiautomated retinal vessel caliber measurement system that measures central retinal artery (vein) equivalent (CRAE [CRVE]) with individual correction for magnification errors under conditions assuming optimal focus.

**METHODS.** The focusing condition of the subject eye fundus camera optical system was individually determined by constructing an optical model of each eye applying its refractive error, corneal curvature, and axial length (AL) to Gullstrand's schematic eye, and by adjusting the position of the camera's focusing lenses using each eye's refractive error. Once the focusing condition of the entire optical system was fulfilled, magnification of the fundus images was calculated using paraxial ray tracing. Measurements of CRAE (CRVE) were performed in an annular area centered on the optic disc with magnification-corrected diameter from 1.8 to 2.7 mm. Reproducibility of the measurements of the results using the new method and comparison with those using interactive vessel analysis (IVAN) were performed in normal Japanese eyes.

**RESULTS.** Intra- and interexaminer intraclass correlation coefficient (ICC) for CRAE (CRVE) measurements was greater than 0.978. CRAE (CRVE) using the new method averaged  $148.9 \pm 10.9 \mu\text{m}$  ( $225.0 \pm 13.9 \mu\text{m}$ ; mean  $\pm$  SD,  $N = 99$ ). Differences between the new method and IVAN were greater with increasing AL ( $P < 0.001$ ). The new method yielded CRAE (CRVE) in good agreement with IVAN in eyes with AL of approximately 24 mm. However, the new method yielded smaller values in eyes with shorter AL and vice versa.

**CONCLUSIONS.** A new accurate and reproducible method to measure CRAE (CRVE) from fundus photographs was reported.

**Keywords:** retinal vessel calibers, fundus photographs, magnification correction

Many studies have shown that retinal vessel caliber (RVC) is associated with a variety of systemic disorders.<sup>1,2</sup> Reduced arteriolar caliber is associated with hypertension, a higher risk for development of hypertension and obesity, while increased venular caliber is associated with a risk of diabetes, obesity, renal failure, systemic inflammation, and stroke. Lower arteriolar-to-venular caliber ratio (AVR) is associated with a risk for developing cardiovascular disorders.<sup>1</sup>

Retinal vessel caliber also provides important information on a variety of ocular disorders. Retinal vessel caliber tends to be narrower in glaucoma patients than in healthy control subjects, and this tendency is more evident in advanced stages of the disease.<sup>3-5</sup> A 10-year follow-up study of the Blue Mountains Eye Study participants revealed that people with reduced arteriolar caliber at baseline had a higher risk of developing glaucoma.<sup>6</sup> It has also been reported that increased venular caliber is associated AMD.<sup>7</sup>

A method measuring vessel diameters in a predetermined fundus area (Zone B as defined in the Atherosclerosis Risk In Communities [ARIC] Study) has been developed as a noninva-

sive and simple method of quantitating the RVC of a given eye.<sup>8</sup> The central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE)<sup>8,9</sup> are now more widely used to represent arteriolar and venular caliber in a given eye than AVR, because arteriolar and venular caliber have been shown to vary independently in relation to disease.<sup>10</sup> With recent improvements in image processing technology and digital cameras, semiautomated methods to determine CRAE and CRVE, have been developed such as interactive vessel analysis (IVAN; University of Wisconsin-Madison, Madison, WI, USA),<sup>7,11</sup> Singapore I vessel assessment (SIVA; Singapore National Research Institute, Singapore),<sup>12</sup> and Vesselmap2 (IMEDOS, Jena, Germany).<sup>13</sup> For example, IVAN is a software developed to measure CRAE and CRVE within the Zone B, assuming the average optic disc diameter to be 1.85 mm.<sup>8,11</sup> SIVA has a function that can extend the analysis area up to 2 disc diameters away from the disc margin (extended zone).<sup>12</sup>

For accurate measurements of RVC in individual eyes, it is essential to correct the magnification of the image captured by the fundus camera on an individual basis. For the correction of



magnification effects, Littmann's method and several other simplified methods have been reported.<sup>14-17</sup> In several studies, RVCs were calculated after magnification correction was performed based on the refractive error, corneal curvature, and/or axial length (AL) in each eye.<sup>18-20</sup> Littmann's method, however, can be applied only to fundus cameras constructed on the principle of a telecentric ray path, such as a Zeiss fundus camera (Carl Zeiss Meditec, Dublin, CA, USA).<sup>14</sup> On the other hand, fundus cameras currently on the market are not always constructed based strictly on this principle.

For accurate interindividual comparison of CRAE or CRVE, the following two requirements must be met. First, magnification must be individually corrected under the condition that the proper focus is fulfilled using the entire subject eye fundus camera optical system, and secondly, RVCs must always be measured in an area at the same distance (absolute distance) from a benchmark in the fundus. To measure RVCs in a large group of subjects, an automated measuring system is also desirable. In the previous methods, including semiautomated ones, the magnification was not corrected on an individual eye basis by checking the focusing condition of the subject eye fundus camera optical system.<sup>7,8,11,12,18-20</sup> Therefore, the measure of the RVCs were not completely accurate due to magnification errors in the eye.

The purpose of the current study was to report a semiautomated RVC measurement system meeting the above mentioned two requirements. That is, in all subjects' eyes, magnification correction was done under the condition that the focusing condition in the combined subject eye fundus camera optical system is assumed to be fulfilled, and the annular measurement zone was located at the same predetermined distance from the disc center calculated based on the determined magnification correction. The results obtained with the new method were then compared with those obtained with IVAN<sup>7,11</sup> in the same eyes.

## MATERIALS AND METHODS

### Subjects

At the University of Tokyo Hospital and Tajimi Municipal Hospital, a total of 100 self-reported healthy volunteers 20-years and older were invited. The study was approved by the ethics committees of each facility, adhered to the tenets of the Declaration of Helsinki, and consent forms were obtained from all subjects. Ocular examinations, including automatic refractometer-based measurements of refraction and corneal curvature radius (KR-800; Topcon, Tokyo, Japan), best-corrected visual acuity measurements with the 5-m Landolt chart, IOL Master (Carl Zeiss Meditec, Inc.)-based AL measurements, slit-lamp examinations, Goldmann applanation tonometer-based IOP measurements, Humphrey Field Analyzer 24-2 Swedish Interactive Threshold Algorithm-Standard (SITA-S) program (HFA; Carl Zeiss Meditec, Inc.)-based visual field testing, and dilated fundus examinations were performed. Exclusion criteria included contraindications to pupillary dilation, IOP greater than or equal to 22 mm Hg, best-corrected visual acuity less than 20/25, refractive error less than -6.0 diopters (D) and greater than +5.0 D, unreliable HFA results (fixation loss or false negative >20%, false-positive >15%), abnormal visual fields according to Anderson and Patella's criteria,<sup>21</sup> history of intraocular or refractive surgery, history of ocular or systemic diseases, and any ocular findings suggestive of ocular diseases. Fundus photographs centered on the disc were obtained using a nonmydriatic retinal camera (2048 × 1536 pixels, saved as JPEG 17:1; TRC NW-200; Topcon) with visual angle of 45° in both eyes with dilated pupil.

**Magnification Correction.** In order to use a modification of Littmann's method,<sup>14</sup> a fundus camera-specific table for magnification correction must be prepared and the value of corneal curvature calculated and used on an individual basis.

The position of the focusing lens in the fundus camera must be changed depending on the optical properties of the subject eye in order to focus the fundus image of the eye onto the detector (image sensor plane). That is, the optical properties of the fundus camera system should vary depending on the eye photographed in order to obtain optimal focus. In the current study, we used a Topcon fundus camera (TRC NW-200) for which details of the optical properties were readily available by the manufacturer. An approximate optical model of each subject eye was constructed applying its refractive error, corneal curvature, and AL to Gullstrand's schematic eye,<sup>14</sup> and the positions of the focusing lenses in the fundus camera optical system were adjusted according to the refractive error of each subject's eye. Then, the focusing condition of the entire optical system, including the subject eye and fundus camera, was examined by means of a paraxial ray-tracing algorithm. Because the proper focusing condition of the subject eye fundus camera optical system could not be fulfilled in 90% of the cases, giving a focusing error on the fundus plane greater than 0.25 D (Fig. 1A), the refractive power of the lens in Gullstrand's schematic eye was modified by changing the radii gradually until the focusing error on the fundus plane was within 0.01 D in all cases (Fig.1B). The magnification correction factor was calculated based on this final condition.

With the current method, the magnification correction factor could not be calculated for eyes with a refractive error of less than -13 D or greater than +12 D or those with an intraocular lens, because the parameter values used in the Gullstrand's schematic eye for such eyes were not available. Thus, eyes with a refractive error of less than -13 D or greater than +12 D or those with an intraocular lens were excluded.

**Automatic Detection of Optic Disc Margin, Its Geographical Center, and Setting Analysis Area on Color Fundus Photographs.** Binary images were created from the original color fundus photographs, potential optic disc locations were defined as island-like areas, and the largest dark area was adopted as the primary candidate for the optic disc. Eight equidistant points were automatically located on the outline of the disc candidate and connected with each other using a spline curve as a disc margin candidate. The potential margin thus determined was displayed on a computer display superimposed on the original fundus photograph so that an examiner could manually correct it, if necessary, by moving the positions of the eight equidistant points. The geometrical center of the disc was set as the benchmark in the fundus. After applying the above determined optical magnification correction, the analysis zone was set as an annular area with an absolute diameter from 1.8 to 2.7 mm, which is 1 to 1.5 times the average Japanese disc diameter,<sup>22</sup> centered on the disc center (Fig. 2). The analysis zone was at a constant distance from the disc center regardless of the optic disc size or optical characteristics of the eye. This analysis area was also superimposed on the fundus photographs.

**Measurement of CRAE and CRVE.** The green channel of the original red-green-blue (RGB) color photographs saved as 17:1 JPEG compressed data<sup>23</sup> were processed using a doubling filter in order to detect the major blood vessels within the analysis zone.<sup>24</sup> A binary image where blood vessels are white was constructed by means of the binarizing process, which recognized the second derivative of the edge of a vessel. According to the result where zero cross points exist, this process assigns the value 255 (white) to the vessel area and the value 0 (black) to the area outside the vessels. By means of the thinning processing, blood vessels having a length shorter than

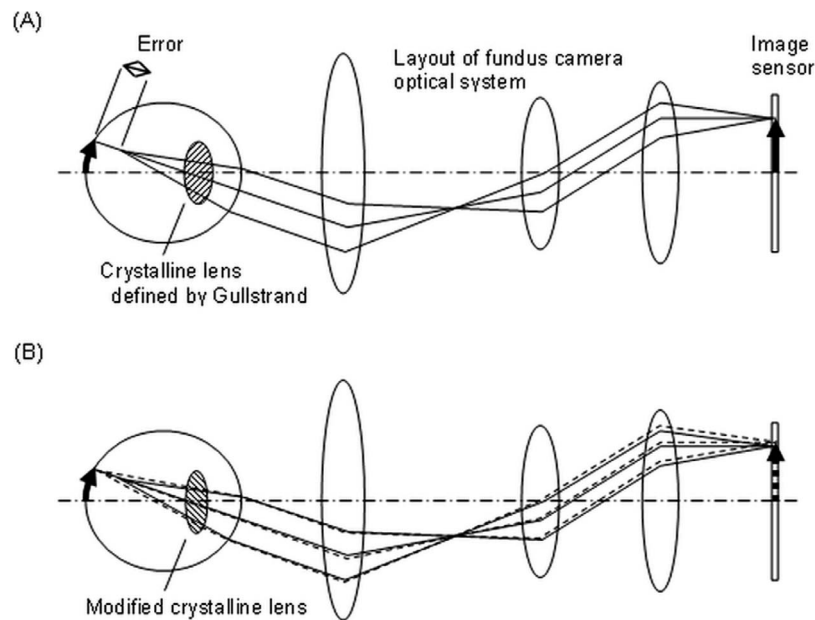


FIGURE 1. Schematic view of model that was used for calculation. (A) Before adjustment. (B) After adjustment.

10 pixels (corresponding to  $\sim 0.1$  mm) and branching parts of blood vessels detected within the analysis zone were eliminated and were excluded from further analysis.<sup>25</sup> Next, the width of the vessel area was measured perpendicular to the direction of blood vessel, and the average width of the vessel was calculated by averaging widths measured along the vessel within the analysis zone.<sup>2,8,9,11,12</sup> All of the vessels within the analysis zone were measured and the vessels with magnification-corrected calibers smaller than  $60 \mu\text{m}$  were excluded. Arterioles and venules were identified by defining characteristics, such as existence and intensity of blood column reflection, hue, and clarity (Fig. 3).

Initially, identification of the optic disc margin, arterioles, and venules was performed automatically, and the obtained results were superimposed onto the original fundus photographs so that an investigator could verify the results. When the optic disc margin was confirmed to be accurate, identifications of branching, arterioles, and venules were checked, and corrected manually if needed. When manual correction of the disc margin was necessary as described above, the annular measurement zone was automatically reset according to the corrected disc margin and its gravity center, and the automatic identification of branching, arteriole, and venules performed again, and manual correction of the results were made, if needed.

Finally, CRAE, CRVE, and CRAE/CRVE (AVR) was calculated using the six biggest arterioles and six biggest venules according to the revised formulas of Knudtson et al.<sup>9</sup>

**Reproducibility of the Results in the New Method and Comparison With the IVAN.** A number of the fundus photographs obtained from randomly chosen subject eyes were used to check measurement reproducibility. Three independent examiners (KT, MM, MT) who had been trained to use the new method measured CRAE, CRVE, and AVR from the same fundus photographs twice each at intervals of 1 day, individually correcting the automatic identification of disc margin, vessel branching, arterioles, and venules, if needed. Inter- and intraindividual intraclass correlation coefficient (ICC) and minimum detectable change (MDC),  $1.96 \times \sqrt{2} \times \sqrt{\{\sum(x_{i1} - x_{i2})^2 / 2n\}}$ , where  $x_{i1}$  and  $x_{i2}$  were the first and second measurement results of the  $i$ -th subject, were calculated.<sup>26</sup>

### Comparison With Results Obtained Using IVAN

One trained examiner (JS) measured CRAE, CRVE, and AVR from the photograph with the new method twice at intervals of 1 day by individually correcting magnification, automatic identification of disc margin, vessel branching, arterioles, and venules, if needed, and the results were averaged. Additionally,

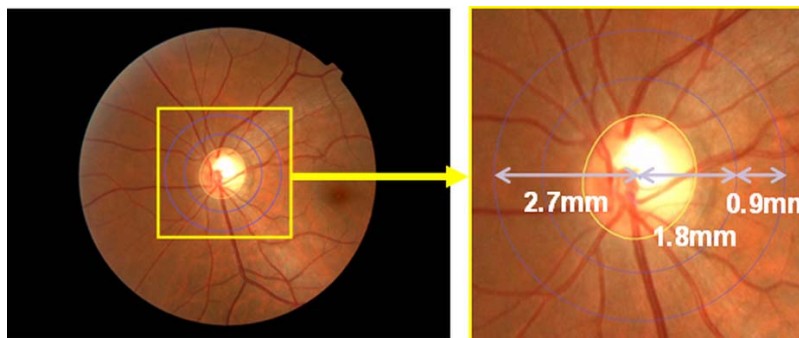


FIGURE 2. Setting of analysis area. Analysis area is set at the same distance in absolute value from disc center.

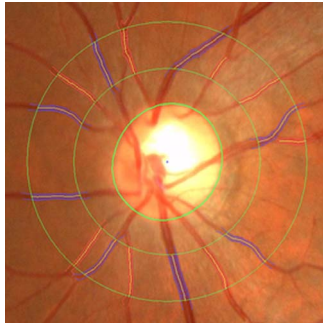


FIGURE 3. Detection and identification of retinal vessels. Red, arteries; blue, veins.

a certified examiner (RK) measured the same photograph twice with the IVAN method following standardized protocols with magnification correction based on the assumption that the mean disc size was 1.85 mm.<sup>8,11,24</sup> The means of the twice-measured CRAE, CRVE, and AVR were calculated.

The time needed to measure the CRAE and CRVE from one eye (photograph) was also recorded for both methods.

For magnification correction in the current study, three ocular factors, namely AL, refractive error, and corneal curvature, were adopted in the new method. To study how these factors affect the differences between the new method and IVAN, the effects of these factors and other ocular factors that may correlate with RVC measurement results such as IOP and disc area,<sup>6,27</sup> on the intergroup differences of CRAE and CRVE were analyzed by means of multiple regression analysis. However, refractive error and corneal curvature were excluded from explanatory variables in consideration of multicollinearity because of high intercorrelations with AL in the subject eyes (Pearson's correlation coefficient = 0.71 for refractive error and  $r = 0.58$  for corneal curvature, respectively).

Statistical analysis was performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).<sup>28</sup>

## RESULTS

Two hundred eyes of 100 subjects meeting the inclusion criteria were reviewed for inclusion. It was necessary to manually correct the automatically detected optic disc margin 58% of the time, and from one to four of eight points were found to be not correctly located on the optic disc margin. While observing automatically drawn disc margin superimposed on the original fundus photograph on a computer display, an examiner moved the positions of these incorrectly located points onto the clinically determined correct disc margin, and the points were reconnected with each other to yield a corrected disc margin using a spline curve. Automatic detection of branching of vessels was unsuccessful in 75% of the photographs, while most failures in branch detection were due to blurred images of one of the branches. The misidentification of vessels detected in the analysis area was 20%, from a total of 3197 vessels. An examiner on a computer display superimposed on the original fundus photograph also manually corrected misidentification of arterioles or venules and failures in branch detection found. Twenty photographs from 20 eyes had to be excluded from further analysis because the new method could not identify six arterioles and venules of which the diameters were greater than 60  $\mu\text{m}$ . These eyes were also excluded from analysis using IVAN. After exclusions, a total of

TABLE 1. Subject Characteristics

Number of Eyes, Subjects	180 (99)
Right/left	96/84
Male/female	51/48
Age, y	51.9 $\pm$ 15.9
Spherical equivalent refraction, D	-0.67 $\pm$ 1.88
Axial length, mm	23.8 $\pm$ 1.1
Corneal curvature, mm	7.7 $\pm$ 0.3
Disc area, mm <sup>2</sup>	2.78 $\pm$ 0.68

180 photographs from 180 eyes of 99 persons (96 right eyes, 84 left eyes) were analyzed in the current study (Table 1).

## Reproducibility

Twenty fundus photographs from 20 randomly selected eyes of 20 subjects were used to assess reproducibility. For CRAE, the interindividual ICC was 0.994 (95% confidence interval [CI] was 0.988–0.998), and the intraindividual ICC for the three examiners ranged from 0.979 (0.946–0.992) to 0.994 (0.984–0.997) with a mean of 0.986. For CRVE, the results were 0.996 (0.992–0.998) for the interindividual ICC, and the intraindividual ICC ranged from 0.991 (0.978–0.996) to 0.996 (0.991–0.999), with a mean of 0.993. For AVR, the ICC results were 0.993 (0.985–0.997) for interindividual ICC, 0.966 (0.916–0.987) to 0.992 (0.98–0.997) for intraindividuals ICC, with a mean of 0.978. The MDC for the three examiners ranged from 2.8 to 5.4  $\mu\text{m}$  with a mean of 4.2  $\mu\text{m}$  for CRAE, while the results ranged from 3.1 to 4.9  $\mu\text{m}$  with a mean of 4.2  $\mu\text{m}$  for CRVE, and ranged from 0.015 to 0.032 with a mean of 0.024 for AVR (Table 2).

## Comparison With IVAN

Randomly selected photographs from one eye of 81 subjects where photographs of both eyes were available, and 18 photographs from 18 subjects where one photograph from one eye was available, were used for analysis (99 eyes from 99 subjects).

The CRAE using the new method averaged  $148.9 \pm 10.9$   $\mu\text{m}$  (mean  $\pm$  SD), while the CRVE averaged  $225.0 \pm 13.9$   $\mu\text{m}$ , and the AVR  $0.66 \pm 0.05$ . The corresponding values using IVAN were  $152.0 \pm 14.0$   $\mu\text{m}$  for the CRAE,  $216.2 \pm 18.5$   $\mu\text{m}$  for the CRVE, and  $0.71 \pm 0.06$  for the AVR. With the new method, the average CRAE was smaller by 2% ( $P = 0.073$ ), the average CRVE larger by 4.1% ( $P < 0.001$ ), and the average AVR smaller by 5.9% ( $P < 0.001$ ) compared with IVAN (Table 3).

The grading times for the two methods, including manual correction for one eye, were  $6.4 \pm 2.7$  minutes for IVAN and  $3.6 \pm 2.9$  minutes for the new method ( $P < 0.001$ ).

CRAE and CRVE differences between the two methods became significantly greater along with greater AL (Table 4; Fig. 4).

Because the difference in RVC measurement results between the two methods depended on the AL, an intergroup comparison was done using varied ALs (Table 5). In eyes with AL of approximately 24 mm, the CRAE using the new method had good agreement with IVAN; however, for CRVE, the new method was similar to IVAN in eyes with AL of approximately 23 mm. Both the CRAE and CRVE using the new method tended to be smaller in eyes with shorter AL, while they tended to be larger in eyes with greater AL. In eyes with AL of less than or equal to 25 mm, correlation of CRAE, CRVE, and AVR between the two methods was significant ( $r = 0.41$ – $0.77$ ,  $P = 0.027 \sim < 0.001$ ), while in eyes with AL of greater than 25 mm,

TABLE 2. Measurement Repeatability

Variable	Interindividual ICC (95% CI)	Intraindividual ICC Range (mean)	MDC Range (mean)
CRAE	0.992 (0.985–0.997)	0.979–0.994 (0.988)	2.8–5.4 (4.2) $\mu\text{m}$
CRVE	0.996 (0.992–0.998)	0.991–1 (0.995)	3.1–4.9 (4.2) $\mu\text{m}$
AVR	0.99 (0.981–0.996)	0.966–0.996 (0.981)	0.015–0.032 (0.024)

For intraindividual ICC and MDC, range of the results obtained from three independent examiners and its mean were presented.

correlation of CRAE and AVR was not significant. However, this finding may be partly attributed to a relatively large dispersion of the data and the small number of eyes ( $n = 10$ ) in this group.

## DISCUSSION

We have developed a new and accurate method of magnification correction for RVC measurements from fundus photographs, whereby magnification of fundus images was corrected on an individual eye basis. The new method utilizes a paraxial ray-tracing algorithm on a combined individualized subject eye-fundus camera optical system. Although the current method was developed based on the optical system of a TRC NW-200 Topcon fundus camera, the new method of magnification correction should be readily applicable to any type of fundus camera or fundus imaging system, as long as details of the optical properties of the device are available from the manufacturer. The new method showed good repeatability and had an MDC of approximately 4  $\mu\text{m}$ , which corresponds to 0.5 pixels on an image sensor, and yielded CRAE, CRVE, and AVR measurement results similar to those previously reported in healthy eyes, that is, CRAE equals 133.6 to 169.8  $\mu\text{m}$ , CRVE equals 202.0 to 242.6  $\mu\text{m}$ , and AVR equals 0.64 to 0.71.<sup>6,7,9,10,12,20,27</sup>

Accurate measurements of RVCs in an individual eye by correcting magnification of the image captured by a fundus camera on an individual eye basis is important for interindividual or intergroup comparison of RVCs and for studying effects of various factors on RVCs.

When the measurement results using the new method were compared to those using IVAN, interesting findings were obtained: (1) correlation of the RVC measurement results between the new method and IVAN was substantial (Pearson's correlation coefficients  $> 0.45$ ) except for CRAE in eyes with AL greater than 25 mm, (2) the agreement was dependent on AL such that in eyes with AL of approximately 23 to approximately 24 mm, the results with the new method agreed with those using IVAN, while in eyes with shorter AL, the new method yielded RVCs smaller than IVAN, and in eyes with longer AL the new method yielded RVCs larger than the IVAN method, and (3) the total measurement time needed for each fundus photo using the new method was on average shorter than that using the IVAN method.

Because the new method yielded smaller RVCs in eyes with shorter AL and larger RVCs in eyes with longer AL than IVAN, it is difficult to attribute this difference to the difference in the

determination of the location of the vessel walls between the two methods. If the magnification is not corrected on an individual eye basis taking AL into consideration, the size of an object in question on the fundus will appear relatively smaller on the image (thus, measured to be smaller) in eyes with longer AL, and relatively larger on the image in eyes with shorter ALs. Further, an analysis area set based on the assumed disc diameter tends to shift relatively more peripherally (farther from the disc center) in eyes with longer AL, and vice versa in eyes with shorter AL. Thus, AL-dependent differences in the measured RVCs between the new method and IVAN is thought to be mainly attributable to the difference in the treatment of the AL in each subject eye between the two methods, which was confirmed by multiple regression analysis. In the new method, AL in an individual eye was incorporated into the calculation of magnification correction, which was not the case using IVAN. On the other hand, good agreement of the RVC measurement results between the two methods in eyes with AL of approximately 24 mm indicates the validity of IVAN in eyes with normal and average optical properties. The new method required less measurement time ( $\sim$ half of that using IVAN). Thus, the new method will provide faster grading processes and facilitate not only more accurate interindividual comparison of RVCs, especially in a group with a high prevalence of myopia (a long AL),<sup>29</sup> but also in studies of RVCs using a large group (where even small differences can be significant).

There are several limitations of the new method. First, we found that the optimum focusing condition of the subject eye Topcon fundus camera optical system as checked by means of a paraxial ray-tracing algorithm was not always fulfilled when we only use refractive error, corneal curvature, and AL to represent the optical characteristics of the subject's eye.<sup>14</sup> Furthermore, Littmann's method can be applied only to fundus cameras constructed on the principle of a telecentric ray path,<sup>14</sup> and Topcon fundus cameras are not constructed based on this principle. To address this issue, the refractive power of the lens in Gullstrand's schematic eye must be modified. A better approximation of the optical properties of each subject eye deserves future studies. Secondly, the current method of magnification correction not only required the refraction error, corneal curvature, and AL of each subject eye, but also information of the optical properties of the fundus camera used, which is available only with cooperation of the

TABLE 3. Results Obtained With the Current Method and IVAN

Variable	Current Method	IVAN	P Value*
CRAE, $\mu\text{m}$	148.9 $\pm$ 10.9	152.0 $\pm$ 14.0	0.073
CRVE, $\mu\text{m}$	225.0 $\pm$ 13.9	216.2 $\pm$ 18.5	<0.001
AVR	0.66 $\pm$ 0.05	0.71 $\pm$ 0.06	<0.001

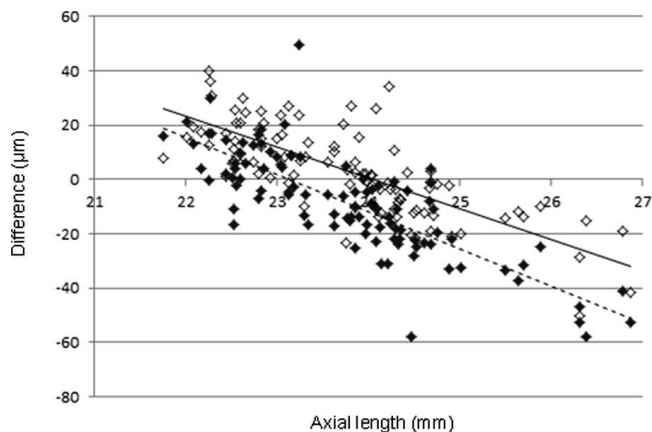
Figures are mean  $\pm$  SD ( $n = 99$ ).

\* Intergroup difference.

TABLE 4. Results of Multiple Regression Analysis of Difference in Values Obtained Between the Current Method and IVAN

Variable	Difference in CRAE	Difference in CRVE
Axial length, mm	-11.3 ( $P < 0.001$ )	-14.3 ( $P < 0.001$ )
IOP, mm Hg	-0.148 ( $P = 0.784$ )	0.138 ( $P = 0.802$ )
Disc area, mm <sup>2</sup>	-0.667 ( $P = 0.743$ )	1.674 ( $P = 0.420$ )

Figures indicate partial regression coefficient. Difference in CRAE (CRVE) obtained with IVAN minus that with the current method. Adjusted  $R$ -squared values were 0.567 for difference in CRAE ( $P < 0.001$ ) and 0.636 for difference in CRVE ( $P < 0.001$ ).



**FIGURE 4.** Differences between two methods (IVAN - Current method). Open (solid) diamonds indicates CRAE (CRVE) differences. Solid (dashed) line indicates regression line for CRAE (CRVE) difference.

manufacturer. This knowledge may not always be available. Thirdly, performance of automatic identification of the fundus photograph-based optic disc margin was not satisfactory, which is partly attributable to the presence of peripapillary atrophy. Identification of the optic disc margin is likely to have some variation. This variation, however, does not seem to significantly affect the results of the RVCs, because inter- and intraindividual reproducibility of RVC measurements with the new method were found to be good. In the future, a better benchmark in the fundus may be the center of Bruch's membrane opening determined using optical coherence tomography rather using fundus photography.<sup>30</sup> Another limitation is that in approximately 10% of fundus photographs, the system failed to identify six retinal arterioles and venules with diameter greater than 60 μm, and these photographs had to be excluded from the analysis. A lower limit of 60 μm is mainly based on the resolution of the image sensor installed in the camera currently used, and this point can be easily

improved by using an image sensor with far more pixels. Also, the performance of identification of branching, arterioles, and venules was not satisfactory, and manual correction was needed in approximately 25% of the photographs, which must be improved in the future.<sup>31</sup> Finally, there are reports of imaging methods that reduce variations in measurement caused by differences in image quality, and also reports of larger variation in vessel caliber due to heartbeat. It may be helpful to create a standard protocol, for example, the synchronization of imaging to the subject's pulse, for measuring RVCs from fundus photographs.<sup>32,33</sup>

In summary, a new and accurate method of magnification correction for RVC measurements from fundus photographs was reported. It corrects magnification on an individual eye basis, utilizing a paraxial ray-tracing algorithm on a combined individual subject eye fundus camera optical system. It showed reasonable RVC measurement repeatability and yielded results compatible with those using IVAN in the same eyes, with a shorter time needed to obtain RVC values from one fundus photograph, suggesting its clinical usefulness. An AL-dependent difference in the measured RVC values between the new method and IVAN can be explained by the fact that the AL of each subject eye is not incorporated into calculation when using IVAN, and suggests that the new method should be especially useful in accurately measuring RVCs in a population with a high prevalence of myopia such as Far Eastern Asians including Japanese.<sup>29</sup>

**Acknowledgments**

Disclosure: **A. Iwase**, Otsuka (R), Carl Zeiss Meditec (R), Topcon (R), Santen (R), Pfizer (R), Senju (R), Kowa (R), P; **A. Sekine**, Topcon (E), P; **J. Suehiro**, Topcon (E); **K. Tanaka**, None; **Y. Kawasaki**, Senju (F), Novartis (F), Japan Health Research Institute (F), Pfizer Japan (F), Rohto (F); **R. Kawasaki**, Santen (R), Senju (F, R), Novartis (F, R), Bayer (F), Japan Health Research Institute (F), Pfizer Japan (F, R), Rohto (F), Predictive Analytics Group Future, Inc. (C), Office Future, Inc. (C), Kowa (R), Bayer (R), Takeda (R), Novo Nordisk (R), Astellas (R); **M.J. Sinai**, Topcon (E); **M. Araie**, B&M (C), Senju (C), Santen (C), Kowa (C), Pfizer,

**TABLE 5.** Results of Comparison Between the Current Method and IVAN Based on Varied AL

Variable	Current Method	Mean Value		Pearson's Correlation Coefficient	
		IVAN	P Value*	Coefficient	P Value
AL <23 mm, n = 28					
CRAE, μm	139.3 ± 7.2	156.1 ± 12.0	<0.001	0.538	0.003
CRVE, μm	215.5 ± 11.8	222.9 ± 16.1	<0.001	0.767	<0.001
AVR	0.65 ± 0.04	0.70 ± 0.06	<0.001	0.663	<0.001
AL 23~24 mm, n = 29					
CRAE, μm	149.8 ± 10.5	157.1 ± 10.8	0.002	0.410	0.027
CRVE, μm	221.1 ± 9.4	217.1 ± 18.3	0.152	0.605	<0.001
AVR	0.68 ± 0.06	0.73 ± 0.06	<0.001	0.495	0.006
AL 24~25 mm, n = 32					
CRAE, μm	153.2 ± 8.9	148.2 ± 15.4	0.028	0.626	<0.001
CRVE, μm	232.8 ± 12.4	215.4 ± 18.0	<0.001	0.735	<0.001
AVR	0.66 ± 0.03	0.69 ± 0.07	0.002	0.627	<0.001
AL >25 mm, n = 10					
CRAE, μm	159.8 ± 6.5	137.3 ± 10.7	<0.001	-0.205	0.571
CRVE, μm	238.2 ± 12.2	197.2 ± 15.6	<0.001	0.709	0.022
AVR	0.67 ± 0.04	0.70 ± 0.06	0.165	0.434	0.210

Figures are mean ± SD.  
\* Intergroup difference.

(C), Topcon (C), Allergan (C), Alcon (C), Heidelberg Engineering (C), Aerie (C), Nitten (R), Otsuka (R), Carl Zeiss Meditec (R), P

## References

- Sun C, Wang JJ, Mackey DA, Wong TY. Retinal vascular caliber: systemic, environmental, and genetic associations. *Surv Ophthalmol*. 2009;54:74-95.
- Ikram MK, Cheung CY, Lorenzi M, Klein R, Jones TLZ, Wong TY. Retinal vascular caliber as a biomarker for diabetes microvascular complications. *Diabetes Care*. 2013;36:750-759.
- Jonas JB, Nguyen XN, Naumann GOH. Parapapillary retinal vessel diameter in normal and glaucoma eyes I. Morphometric Data. *Invest Ophthalmol Vis Sci*. 1989;30:1599-1603.
- Jonas JB, Naumann GOH. Parapapillary retinal vessel diameter in normal and glaucoma eyes. II. Correlations. *Invest Ophthalmol Vis Sci*. 1989;30:1604-1611.
- Harris A, Chung HS, Ciulla TA, Kagemann L. Progress in measurement of ocular blood flow and relevance to our understanding of glaucoma and age-related macular degeneration. *Prog Ret Eye Res*. 1999;18:669-687.
- Kawasaki R, Wang JJ, Rochtchina E, Lee AJ, Wong TY, Mitchell P. Retinal vessel caliber is associated with the 10-year incidence of glaucoma: the Blue Mountains Eye Study. *Ophthalmology*. 2003;120:84-90.
- Jeganathan VSE, Kawasaki R, Wang JJ, et al. Retinal vascular caliber and age-related macular degeneration: the Singapore Malay Eye Study. *Am J Ophthalmol*. 2008;146:954-959.
- Hubbard LD, Brothers RJ, King WN, et al. Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the Atherosclerosis Risk in Communities Study. *Ophthalmology*. 1999;106:2269-2280.
- Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BEK. Revised formulas for summarizing retinal vessel diameters. *Curr Eye Res*. 2003;27:143-149.
- Ikram MK, de Jong FJ, Vingerling JR, et al. Are retinal arteriolar or venular diameters associated with markers for cardiovascular disorders? The Rotterdam Study. *Invest Ophthalmol Vis Sci*. 2004;45:2129-2134.
- Wong TY, Knudtson MD, Klein R, Klein BEK, Meuer SM, Hubbard LD. Computer-assisted measurement of retinal vessel diameters in the Beaver Dam Eye Study: methodology, correlation between eyes, and effect of refractive errors. *Ophthalmology*. 2004;111:1183-1190.
- Cheung CY, Hsu W, Lee ML, et al. A new method to measure peripheral retinal vascular caliber over an extended area. *Microcirculation*. 2010;17:495-503.
- Nagel E, Vilser W, Fink A, Riemer T. Static vessel analysis in nonmydriatic and mydriatic images [in German]. *Klin Monatsbl Augenheilkd*. 2007;224:411-416.
- Littmann H. Determination of the true size of an object on the fundus of the living eye [in German]. *Klin Monatsbl Augenheilkd*. 1982;180:286-289.
- Bengtson B, Krakau CET. Correction of optic disc measurements on fundus photographs. *Graefes Arch Clin Exp Ophthalmol*. 1992;30:24-28.
- Bennett AG, Rudnicka AR, Edgar DF. Improvements on Littmann's method of determining the size of retinal features by fundus photography. *Graefes Arch Clin Exp Ophthalmol*. 1994;32:361-367.
- Mansour AM. Measuring fundus landmarks. *Invest Ophthalmol Vis Sci*. 1990;31:41-42.
- Cheung N, Tong L, Tikellis G, et al. Relationship of retinal vascular caliber with optic disc diameter in children. *Invest Ophthalmol Vis Sci*. 2007;48:4945-4948.
- Cheung N, Huynh S, Wang JJ, et al. Relationships of retinal vessel diameters with optic disc, macular and retinal nerve fiber layer parameters in 6-year-old children. *Invest Ophthalmol Vis Sci*. 2008;49:2403-2408.
- Lim LS, Cheng CY, Lin X, Mitchell P, Wong TY, Saw SM. Influence of refractive error and axial length on retinal vessel geometric characteristics. *Invest Ophthalmol Vis Sci*. 2011;52:669-678.
- Anderson DR, Patella VM. *Automated Static Perimetry*. St. Louis, MO: Mosby; 1999.
- Tsutsumi T, Tomidokoro A, Araie M, Iwase A, Sakai H, Sawaguchi S. Planimetrically determined vertical cup/disc and rim width/disc diameter ratios and related factors. *Invest Ophthalmol Vis Sci*. 2012;53:1332-1340.
- Lee MS, Shin DS, Berger JW. Grading, image analysis, and stereopsis of digitally compressed fundus images. *Retina*. 2000;20:275-281.
- Muramatsu C, Hatanaka Y, Iwase T, Hara T, Fujita H. Automated detection and classification of major retinal vessels for determination of diameter ratio of arteries and veins. *Proc SPIE*. 2010;7624: 76240J.
- The Atherosclerosis Risk in Communities (ARIC) Study Research Group. *ARIC Retinal Reading Center Protocol, Manual 14B*. Chapel Hill, NC: ARIC Coordinating Center; 1996.
- Araie M. Test-retest variability in structural parameters measured with glaucoma imaging devices. *Jpn J Ophthalmol*. 2013;57:1-24.
- Lee KE, Klein BEK, Klein R, Meuer SM. Association of retinal vessel caliber to optic disc and cup diameters. *Invest Ophthalmol Vis Sci*. 2007;48:63-67.
- Kanda Y. Investigation of the freely-available easy-to-use software "EZR" (Easy R) for medical statistics. *Bone Marrow Transplant*. 2013;48:452-458.
- Sawada A, Tomidokoro A, Araie M, Iwase A, Yamamoto T; for the Tajimi Study Group. Refractive errors in an elderly Japanese population: the Tajimi Study. *Ophthalmology*. 2008;115:363-370.
- Chauhan BC, Burgoyne CF. From clinical examination of the optic disc to clinical assessment of the optic nerve head: a paradigm change. *Am J Ophthalmol*. 2013;156:218-227.
- Relan D, MacGillivray T, Ballerini L, Trucco E. Retinal vessel classification: sorting arteries and veins. *Conf Proc IEEE Eng Med Biol Soc*. 2013;2013:7396-7399.
- Ikram MK, Ong YT, Cheung CY, Wong TY. Retinal vascular caliber measurements: Clinical significance, current knowledge and future perspectives. *Ophthalmologica*. 2012;229:125-136.
- Knudtson MD, Klein BEK, Klein R, et al. Variation associated with measurement of retinal vessel diameters at different points in the pulse cycle. *Br J Ophthalmol*. 2004;88:57-61.