Axial Length Variation Impacts on Superficial Retinal Vessel Density and Foveal Avascular Zone Area Measurements Using Optical Coherence Tomography Angiography

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PURPOSE. To evaluate the impact of image magnification correction on superficial retinal vessel density (SRVD) and foveal avascular zone area (FAZA) measurements using optical coherence tomography angiography (OCTA).

METHODS. Participants with healthy retinas were recruited for ocular biometry, refraction, and RTVue XR Avanti OCTA imaging with the 3 × 3-mm protocol. The foveal and parafoveal SRVD and FAZA were quantified with custom software before and after correction for magnification error using the Littman and the modified Bennett formulas. Relative changes between corrected and uncorrected SRVD and FAZA were calculated.

RESULTS. Forty subjects were enrolled and the median (range) age of the participants was 30 (18–74) years. The mean (range) spherical equivalent refractive error was −1.65 (−8.00 to +4.88) diopters and mean (range) axial length was 24.42 mm (21.27–28.85). Images from 13 eyes were excluded due to poor image quality leaving 67 for analysis. Relative changes in foveal and parafoveal SRVD and FAZA after correction ranged from −20% to +10%, −5% to +2%, and −20% to +51%, respectively. Image size correction in measurements of parafoveal SRVD and FAZA was greater than 5% in 51% and 74% of eyes, respectively. In contrast, 100% of eyes had less than 5% correction in measurements of parafoveal SRVD.

CONCLUSIONS. Ocular biometry should be performed with OCTA to correct image magnification error induced by axial length variation. We advise caution when interpreting interocular and interindividual comparisons of SRVD and FAZA derived from OCTA without image size correction.

Keywords: optical coherence tomography angiography, OCTA, superficial vessel plexus, normative database, axial length, foveal avascular zone, magnification error, Littman formula, Bennett formula, retina, retinal disease

Quantitative assessment of superficial retinal vessel density (SRVD) and foveal avascular zone area (FAZA) serves as a potential biomarker for macular ischemia in diabetic retinopathy and other retinal vascular diseases. Therefore, it is essential that normal variations in SRVD and FAZA in the healthy population are established definitively. Normative ranges of vessel densities and FAZA measured with optical coherence tomography (OCT) angiography (OCTA) have recently been published. However, current commercial OCTA devices do not correct density or area measurements for changes in image size magnification due to axial length variation.

It is well known that the true size of the fundus area imaged is dependent on the combined magnification of the camera and the eye. Various methods have been introduced to correct for the magnification of the eye. These include methods based on one or more of a combination of variables, including refractive error, keratometry, axial length, anterior chamber depth, and crystalline lens thickness. These methods have been compared by Garway-Heath et al., who concluded that the simplest approach of using only axial length to correct for magnification error was accurate and produced very similar results to the more detailed calculations that incorporated keratometry, refractive error, and lens thickness. Given most

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eye clinics have access to devices that measure axial length and that incorporating axial length has been shown to increase the accuracy of optic nerve head size and retinal layer thickness evaluated by OCT.\textsuperscript{16–23} We hypothesize that OCTA image magnification correction using axial length measurement is feasible and necessary for accurate determination of OCTA-derived variables.

In this study, we sought to investigate the effect of image magnification on quantitative characterization of the retinal vasculature. Specifically, we evaluated the effect of axial length on SRVD and FAZA measurements using OCTA in individuals with healthy eyes.

**METHODS**

This study was approved by The University of Western Australia Human Ethics Research Office (RA/4/1/8570) and was conducted in compliance with the tenets of the Declaration of Helsinki. All patients gave informed consent prior to participation in the study and did not receive financial compensation.

**Study Participants**

Individuals with no history of retinal disease were eligible for the study. Exclusion criteria were: best-corrected visual acuity of worse than 20/20; evidence of macular or retinal disease; and presence of media opacity, such as cataracts, corneal, or vitreous opacities. All participants underwent ophthalmic examination, ocular biometry (IOL Master; Carl Zeiss Meditec, Inc., Dublin, CA, USA), autorefraction (Ark1, Auto Ref/Keratometer; Nidek, Gamagori, Japan), and OCTA imaging in both eyes (RTVue XR Avanti; Optovue, Inc., Fremont, CA, USA). All biometric measurements and OCTA imaging were performed by trained operators.

**Optical Coherence Tomography Angiography Imaging Protocol**

The instrument used in this study operates at 70,000 A-scans per second. According to the manufacturer, RTVue XR Avanti has an axial resolution of 5 μm and the lateral resolution, defined as full-width at half maximum beam diameter at the retinal plane, is approximately 22 μm. The instrument uses a superluminescent diode light source that has a central wavelength of 840 nm.

Successive OCTA images were taken using a scanning procedure designed to reduce motion artifact. Two volumes scans were acquired sequentially using orthogonal raster scan patterns (denoted, respectively, x-fast and y-fast) over an assumed 3 × 3-mm retinal region centered on the fovea. For the x-fast scan pattern, each B-scan comprised 304 A-scans (along the x-axis) and two consecutive B-scans were captured at each of the 304 locations along the y-axis of the cube. With a scan rate of 209 frames per second, a total of 608 B-scans (304 y-positions × 2 B-scans per position) were acquired in 3 seconds. The flow signal is detected by examining the consecutive B-scans of the same area for differences (decorrelation) using the so-called split-spectrum amplitude decorrelation angiography algorithm. This process allows generation of the nominally 3 × 3-mm x-fast en face OCTA images. The procedure to obtain y-fast en face OCTA images is defined analogously, except that B-scans were acquired parallel to the y-axis. Finally, the Motion Correction Technology (MCT) of the AngioVue software (Optovue, Inc.) automatically aligned and merged the OCTA x-fast and y-fast volume scans to reduce motion artifacts and produce an OCTA en face projection image by automatic segmentation of the superficial retinal capillary plexus and applying maximum intensity projection mapping in the axial direction.

**Optical Coherence Tomography Angiography Fundus Image Magnification**

The nominally 3 × 3-mm retinal scan area is derived from calculation of the linear distance on the surface of the retina subtended by a fixed field of view of approximately $10^\circ \times 10^\circ$ using a defined axial length (for the RTVue XR Avanti system, this length is 23.82 mm) (Optovue, Inc., personal communication, 2017). However, the true physical linear dimension of the image ($D_\text{m}$) varies proportionally with the axial length of the eye being imaged (Fig. 1). Therefore, we used the Littman and the modified Bennett formulae to calculate the image size; thereby, determining the relationship between measurements with and without correction.\textsuperscript{10}

According to the Littman formula, the relationship between the measured OCTA image diameter, $D_\text{m}$, and the true diameter on the fundus, $D_1$, can be expressed as:

\[
D_1 = \frac{D_\text{m} \cdot q \cdot p \cdot C_1}{C_0}
\]

The factor $q$ can be determined from the Bennett formula:

\[
q = 0.01306 \cdot (AL – 1.82)
\]

where 1.82 is a constant related to the distance between the corneal apex and the second principal plane and $AL$ is the axial length.\textsuperscript{10} The factor $p$, omitting any effect arising from image distortion, can be readily calculated from the Bennett formula if the axial length at which $D_1 = D_\text{m}$ is known (i.e., 23.82 mm here). When $D_1 = D_\text{m}$, then $p = 1/q$ and, therefore, $p = 1/[0.01306(23.82 – 1.82)] = 3.48$. Thus, the
Validation of Custom-Written Vessel Segmentation

The highest resolution en face OCTA image exported from AngioVue software is 72 dpi (Fig. 2A). This image is first resized to 300 dpi (Corel Photo-Paint X6, Corel, Ottawa, Ontario, Canada) by using nearest-neighbor interpolation prior to automated analysis with our custom-written algorithm in MATLAB (vR2012a: The MathWorks, Inc., Natick, MA, USA). Resizing and interpolation is necessary so that our custom software can accurately identify the pixel intensity threshold used by the AngioVue Analytics software, since the raw decorrelation value for each pixel is not accessible. Vessel density is defined as the percentage of the number of pixels with intensity greater than a fixed threshold to the total number of pixels in the region of interest and expressed in units of percent pixels. The threshold value for each nominally 3 × 3-mm image is set individually by benchmarking against the values obtained from AngioVue Analytics software (version 2016.1.0.26). The optimal threshold for each image is chosen when the sum of the absolute differences in vessel densities (SRVD from our custom software minus SRVD from AngioVue software), across all nine square zones, reaches a minimum (Fig. 2B). This procedure is repeated so that an image-specific threshold is determined and used for calculating our custom-derived vessel density from each OCTA image.

Superficial Retinal Vessel Density and Foveal Avascular Zone Area Quantification

Following validation of the custom-written segmentation software, SRVD was measured in two regions: the foveal region, defined as a circular area with 0.5-mm radius centered on the foveal center (Fig. 2C yellow region labeled i), and the parafoveal region, defined as the annular region between 0.5- and 1-mm radius from the foveal center (Fig. 2C, pale blue region labeled ii). The foveal avascular zone (FAZ) was delineated in uncorrected images by image segmentation using the Chan-Vese approach (Fig. 2E).24 A contour within the FAZ defined by four points, manually selected by the user, initiated the search routine. FAZA was calculated as the total number of black pixels enclosed by the segmentation contour and expressed in millimeters squared. This procedure was repeated for each image. Finally, the area values were scaled by the magnification correction factor to obtain the corrected FAZA.

The impact of axial length on deviation of true SRVD or FAZA from uncorrected values was examined by plotting their relative changes against axial length. The relative change in SRVD was defined as: 100 × (SRVD after correction − SRVD before correction) / SRVD before correction. The relative change in FAZA was defined analogously as a percentage of uncorrected FAZA. Image magnification correction factor was defined as: image area after correction for magnification error divided by image area before correction. Bland-Altman analysis was also performed to determine limits of agreement between corrected and uncorrected SRVD and FAZA, as described in detail below.

Statistical Analysis

Agreement between the results from custom-written and AngioVue Analytics software and the SRVD and FAZA measured before and after correction for magnification error were evaluated using Bland-Altman plots and limits of agreement.25–27 First, a scatter plot of absolute differences in values between the two methods against their means was plotted to confirm that there was no relationship between error and magnitude. If this condition was met, then the bias (mean difference), standard deviation of the differences (SD of diff.) and confidence limits for the bias (i.e., the limits of agreement, LA, defined as: mean difference ± 1.96 SD of the differences) were calculated. The bias and LA are displayed in Figures 3 and 4 as solid and dashed lines, respectively. The LA represents the range of values for the differences between the methods that
can be expected 95% of the time. Paired $t$-tests were used to examine the significance of the bias. The $t$-statistic ($t_{\text{stat}}$) is given by $t_{\text{stat}} = \frac{d}{SE(d)}$, where $d$ is the mean difference, and $SE(d) = \frac{s_d}{\sqrt{n}}$ is the standard error of the mean difference calculated under the null hypothesis. A $P$ value of 0.05 or less was considered statistically significant.

RESULTS

Demographics
Forty individuals were enrolled in this study (24 women, 16 men). The median (range) age of the participants was 30 (18–74) years. Nineteen subjects were under 30 years, eight between 30 and 40, four between 40 and 50, and nine individuals older than 50 years. Of the subjects, 30 were Caucasian and 10 were Asian. Sixty-seven eyes from 40 volunteers produced adequate OCTA image quality for analysis. Images from the remaining 13 eyes were excluded from the study due to poor image quality. Among the 67 eyes, only 62 images had an identifiable FAZ for measurement of FAZA. The mean (SD, range) spherical equivalent refraction was $1.65 (2.54, -8.00 to +4.88)$ diopters (D). The mean (SD, range) axial length was $24.42 (1.59, 21.27–28.85)$ mm.

Validation of Custom-Written Vessel Segmentation Software
There was good overall agreement between our custom software and the AngioVue Analytics software for the
The mean (SD) difference in SRVD before and after correction (vessel density in units of percentage: number of bright pixels/total number of pixels in each image [% pixels]) were −0.6 (2.5) and 0 (0.5) in the fovea (P = 0.05) and parafovea (P = 0.28 Figs. 4A–B), respectively. The lower and upper bounds of the LAs in SRVD before and after correction were −5 and +4, and −1 and +1 in the fovea and parafovea, respectively. The mean (SD) difference in FAZA before and after correction (in units of mm² in each case) was 0 (0.03) and the respective lower and upper bounds of LAs were −0.06 and +0.06 (P = 0.08, Fig. 4C).

Relative change in foveal and parafoveal SRVD and FAZA, before and after correction for magnification error at various axial lengths and refractive errors, are shown in Figure 5. While the relative change in parafoveal SRVD (% of the uncorrected value) was minimal (−3 to +2), the relative change in foveal SRVD (% of the uncorrected value) ranged from −20 to +10. The relative change in FAZA (% of uncorrected value) ranged from −20 to +51 after image size correction.

The relative change in foveal SRVD was less than 5% (relative to uncorrected values) for 33 of 67 eyes (49%), with axial lengths ranging from 22.28 to 24.71 mm (Fig. 5A). This contrasts with parafoveal SRVD (Fig. 5B), which exhibited changes of less than 5% in 67 eyes (100%) with axial lengths ranging from 21.27 to 28.85 mm. Only 16 of 62 eyes (26%), with axial lengths ranging from 23.29 to 24.33 mm, had less than 5% change in FAZA after correction (Fig. 5C). There was no specific range of refractive error that predicted less than 5% change in foveal and parafoveal SRVD (Figs. 5D, 5E), as well as in FAZA (Fig. 5E).

**TABLE.** Bland-Altman Analysis and Paired t-test Showing the Agreement Between Custom-Written Software and the AngioVue Analytics Software for the Measurement of Retinal Vessel Density

<table>
<thead>
<tr>
<th>Square</th>
<th>Mean SRVD, % Pixels</th>
<th>SD SRVD, % Pixels</th>
<th>Lower LA, % Pixels</th>
<th>Upper LA, % Pixels</th>
<th>t_{total}</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.1</td>
<td>1.8</td>
<td>−3.4</td>
<td>+3.7</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>0.6</td>
<td>−1.2</td>
<td>+1.2</td>
<td>0</td>
<td>0.96</td>
</tr>
<tr>
<td>III</td>
<td>−0.1</td>
<td>0.6</td>
<td>−1.4</td>
<td>+1.0</td>
<td>−1.8</td>
<td>0.08</td>
</tr>
<tr>
<td>IV</td>
<td>0.1</td>
<td>0.5</td>
<td>−0.9</td>
<td>+1.1</td>
<td>1.5</td>
<td>0.14</td>
</tr>
<tr>
<td>V</td>
<td>0.4</td>
<td>0.4</td>
<td>−0.5</td>
<td>+1.4</td>
<td>7.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>VI</td>
<td>0.1</td>
<td>0.5</td>
<td>−0.8</td>
<td>+1.0</td>
<td>1.6</td>
<td>0.1</td>
</tr>
<tr>
<td>VII</td>
<td>−0.3</td>
<td>0.9</td>
<td>−2.1</td>
<td>+1.4</td>
<td>−0.3</td>
<td>&lt;0.05</td>
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<tr>
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<td>−0.7</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**DISCUSSION.**

We found that, after correction, relative changes in foveal and parafoveal SRVD and FAZA ranged from −20% to +10%, −3% to +2%, and −20% to +51%, respectively. Image size correction was greater than 5% in 51% and 74% of eyes, respectively, in measurements of foveal SRVD and FAZA. By contrast, 100% of eyes exhibited less than 5% correction of parafoveal SRVD.

The differential effects of image magnification on foveal versus parafoveal SRVD can be explained as follows: the foveal region (central 1-mm circle) incorporates the FAZ (black pixels) surrounded by dense capillaries (white pixels) at the edge of the image. A small change in the image boundary location, as a result of magnification correction, will affect bright pixel density disproportionately; thus, having greater impact on foveal SRVD (Fig. 2D). In contrast, the parafoveal region has a relatively uniform vascular network in healthy eyes; therefore, small changes in its boundary from image size correction are less likely to induce significant change in overall density. If we excluded the FAZ from the calculation of vessel density. If we excluded the FAZ from the calculation of vessel density.
density in the foveal region, the error in foveal SRVD would be smaller and may even be comparable to that of parafoveal SRVD. However, our main purpose is to illustrate the extent of the impact of image magnification on readily accessible parameters, such as foveal and parafoveal SRVD without the need to exclude an arbitrarily defined avascular zone, as this may introduce another source of variability to the definition of normative intervals in SRVD. Furthermore, we believe it is necessary to include the FAZ in characterization of the foveal SRVD because pathologic retinal capillary dropout can alter the size of FAZA and also lead to the development of new regions of avascular zones in the parafovea. Such arbitrary exclusion of avascular regions from the measurement of foveal and parafoveal SRVD may result in reduced sensitivity of OCTA in detecting retinal vascular disease. In addition, the impact of image size correction on parafoveal SRVD may be greater in diseased eyes, where significant ischemia (black pixels) may occur in the parafoveal region simulating the pattern seen in the healthy fovea.

The impact of correction for magnification error on the interpretation of OCT findings in a variety of retinal disease conditions has been previously shown by others. The effect of axial length on peripapillary retinal nerve fiber layer (RNFL) thickness in myopic, hyperopic, and emmetropic eyes has been investigated using different OCT instruments. For example, Kang et al.\textsuperscript{18} and Savini et al.\textsuperscript{22} used the Cirrus HD OCT instrument to evaluate the influence of axial length on RNFL measurements in adult eyes. In other studies, the RTVue OCT was employed to evaluate the same relationship in a group of adults\textsuperscript{20} and, separately, in a group of children.\textsuperscript{23} Those studies showed that RNFL thickness was significantly different between myopic, hyperopic, and emmetropic eyes in adults and children; however, these differences disappeared after correction for magnification error using the modified Bennett formula.\textsuperscript{10} Similarly, measurement of the optic disc size has an important bearing on the evaluation of optic nerve diseases and anomalies. Leung et al.\textsuperscript{20} used a Stratus OCT instrument to evaluate associations between optic disc area and axial length/spherical equivalent. The relationship between the measured size of the OCT image and the actual fundus size imaged was also described using Littmann’s formula.\textsuperscript{20} The main finding of that study was that the optic disc area increases with axial length and myopic refraction. For each millimeter increase in axial length, the optic disc area increased by 0.095 mm\textsuperscript{2}.\textsuperscript{20} Huang et al.\textsuperscript{14} also studied the relationship between RNFL thickness, optic disc size, and image magnification using an approach based on axial length only. In that study, after accounting for the effect of magnification, RNFL thickness was no longer dependent on axial length. Our observation is in agreement with previously described reports that axial length affects the assessment of retinal vascular biomarkers and should be taken into account in their quantification. Furthermore, we report here the range of axial lengths (22.28–24.71 mm) that occurred in 49% of the...
eyes that result in a less than 5% change in foveal SRVD following image size correction. Theoretically, axial lengths in the range 23.29 to 24.33 mm would be predicted to correspond to less than 5% change in FAZA, but only 26% of the eyes in our cohort had axial lengths within this range. Therefore, 51% and 74% of eyes may have significant error (>5%) in foveal SRVD and FAZA, respectively, if image size correction is not applied.

There are several limitations in the present study. First, our sample size is small and accordingly our range of axial lengths is smaller than expected in the general population. Further studies with a larger number of healthy subjects, encompassing a wider range of axial lengths, age, and spherical equivalents, are necessary to fully evaluate vessel density as a biomarker of retinal health. Second, we only examined healthy subjects with no clinical evidence of retinal disease based on indirect fundoscopy. The relative error in SRVD measurement may be greater in patients with capillary dropouts arising from diseases, such as diabetic retinopathy, radiation retinopathy, macular telangiectasia, and retinal vein occlusion. Third, we did not examine the impact of magnification on deep retinal vessel density (DRVD). Quantitative analysis of the deep capillary plexus is not reliable using the AngioVue software because of projection artifacts cause the FAZ to be not as clearly defined in the deep capillary plexus as in the superficial capillary plexus. For these reasons, we recommend further studies on DRVD once projection artifacts can be removed in commercial instruments.29,30

Further work is required to design a method for vessel density calculation that is independent of the AngioVue software and to optimize FAZA segmentation. There were no cases in our study population where the FAZ was not continuous (i.e., divided by bridging vessels into separate zones). However, this phenomenon is not uncommon in diseased eyes where discrete avascular regions can be separated by capillaries requiring manual adjustment. An important related issue, which did not arise in our study, is the need for segmentation and calculation of not only the FAZA but also of total avascular zone areas in the macula. 

Hwang et al.31 demonstrated that objective quantification of total capillary nonperfusion can be a better biomarker of retinal vascular disease than FAZA, because of FAZA’s relatively high variability in healthy individuals. Thus, an accurate and automated method for measurement of both FAZA and total avascular area would be advantageous.

CONCLUSIONS

We have demonstrated the impact of image magnification due to axial length variation on SRVD and FAZA measurement in OCTA. Large errors in these parameters can arise in the absence of image size correction. Our results show correction of foveal SRVD is required in 51% of eyes and of FAZA in 74% of eyes. We advise caution when interpreting interocular and interindividual comparisons of SRVD and FAZA derived from OCTA without image size correction.

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