

Intrasession Repeatability and Interocular Symmetry of Foveal Avascular Zone and Retinal Vessel Density in OCT Angiography

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Purpose: To measure intrasession repeatability and interocular symmetry of the foveal avascular zone area (FAZA) and superficial retinal vessel density (SRVD) using AngioVue Analytics optical coherence tomography angiography (OCTA).

Methods: Fifty healthy individuals were prospectively enrolled. OCTA scans (3×3 and 6×6 mm) were acquired twice in right and once in left eyes. FAZA (with and without rescaling) and SRVD for 18 regions (whole, fovea, parafovea, six parafoveal subregions, and nine square zones) were compared between two scans in right eyes (repeatability) and between both eyes (symmetry). Coefficients of repeatability (CRs) and limits of agreement (LAs) were calculated.

Results: Axial length–based image size rescaling had negligible impact on the intrasession CR of FAZA in both 3×3 - and 6×6 -mm images. The intrasession CRs for the foveal SRVD were 3.3% and 6.1% in the 3×3 - and 6×6 -mm OCTA images, respectively. Age and axial length did not influence test–retest variability of FAZA or SRVD. The interocular LAs in FAZA (0.039 – 0.059 mm²) was comparable to its CR. However, the interocular LAs in foveal SRVD were -4.5% to $+3.8\%$, with 13% of the cohort showing an interocular difference greater than the CR.

Conclusions: FAZA repeatability is not influenced by image size correction, and foveal SRVD is more variable in 6×6 - than 3×3 -mm OCTA images. Low image quality may contribute to interocular SRVD asymmetry.

Translational Relevance: CRs and LAs can be used to set a threshold for true changes in FAZA and SRVD in longitudinal studies of healthy individuals.

Introduction

Quantification of retinal vessel density is useful in detecting and monitoring the development of subclinical retinal vascular diseases. Current commercial optical coherence tomography angiography (OCTA) enables noninvasive, depth-resolved, and repeated measurements of the foveal avascular zone area (FAZA) and the superficial retinal vascular density (SRVD) without the risk of dye injections in healthy subjects. Repeated measurements of retinal vessel density at the same retinal location in the same

individual vary around the true value. It is, therefore, crucial to set a threshold of test–retest variability so that true changes in FAZA or SRVD resulting from subclinical retinal vasculopathy can be defined in longitudinal population studies that use OCTA as an end point.¹ This threshold of variability can be estimated by calculating the measurement errors derived from a repeatability study.

The AngioVue Imaging System (RTVue XR Avanti; Optovue, Inc., Fremont, CA) is a spectral-domain optical coherence tomography (SD-OCT) device that enables simultaneous three-dimensional

structural imaging of the retina and generation of en face maps of blood flow through a split-spectrum amplitude decorrelation angiography (SSADA) algorithm.² The AngioAnalytics software (Optovue, Inc.) allows measurement of FAZA and SRVD from selected regions of the retina. Although several studies have examined the intrasession (imaging on same day) and intersession (imaging on different days) repeatability of SRVD measurement from the RTVue XR Avanti SD-OCT, the authors did not calculate the within-subject standard deviation, s_w , required for estimating the threshold of test-retest variability.³⁻⁹ They reported high intraclass correlation coefficients between repeated measurements, but this parameter does not provide a cutoff threshold value for defining disease progression.^{4,5,7,8} Some studies reported the coefficient of variation, but in the absence of a proportional relationship between the s_w and magnitude of measurement, this parameter is not clinically meaningful.¹⁰ A recent large retrospective study of 135 eyes reported the coefficient of repeatability (CR) for FAZA and retinal capillary density measurements as defined by 1.96 times the standard deviation of the differences, s , instead of 2.77 times the within-subject standard deviation, s_w , as recommended by Bland and Altman.^{1,7} Therefore, the reported coefficient is unsuitable for distinguishing true disease progression from fluctuations related to test-retest variability.

The primary objective of this study was to estimate the thresholds for defining true change in FAZA and SRVD and explore factors that may influence test-retest variability. A secondary objective was to determine the interocular limits of agreement (LAS) in FAZA and SRVD and calculate the frequency of interocular asymmetry exceeding measurement error.

Methods

Subjects

This was a prospective observational study of healthy subjects recruited from the ophthalmology clinic at Sir Charles Gairdner Hospital at the Queen Elizabeth II Health Campus and the Lions Eye Institute, Perth, Western Australia, Australia. The study adhered to the tenets of the 1964 Declaration of Helsinki and was approved by The University of Western Australia Human Ethics Research Office (RA/4/1/8570).

Patients attending the eye clinic, who had no history of ocular disease and normal retinal examination as part of routine care, were approached by

one of the investigators. Informed consent was obtained before study procedures were carried out. Study assessments included axial length measurement using the IOL Master 500 (IOL Master; Carl Zeiss Meditec, Inc., Dublin, CA, USA) and auto-refraction using the Ark1 Auto Ref/Keratometer (Ark1, Auto Ref/Keratometer; Nidek, Gamagori, Japan). Retinal imaging was performed without pupil dilatation. Subjects with macular pathology on structural OCT were excluded. Patients with a history of diabetes mellitus or systemic hypertension were eligible provided they did not have macular lesions identified on clinical examination or structural OCT scan. OCTA was performed on all eligible patients as detailed below. Only images with signal strength index of 50 or greater and without significant motion artefacts were included in the study.

OCTA Imaging Protocol

OCTA images were acquired using the RTVue XR Avanti system (version 2016.1.0.26; Optovue, Inc.). The SD-OCT instrument operates at 70,000 scans per second, and its light source is a superluminescent diode with a central wavelength of 840 nm and a bandwidth of 45 nm, providing an axial resolution of 5 μ m in tissue. The instrument uses the SSADA algorithm to create an OCTA map of retinal vasculature.² Each study subject underwent a single session of imaging in which six OCTA images were obtained in the following order:

- 3 \times 3 mm of the right eye (3 \times 3 OD)
- 6 \times 6 mm of the right eye (6 \times 6 OD)
- 3 \times 3 mm of the left eye (3 \times 3 OS)
- 6 \times 6 mm of the left eye (6 \times 6 OS)
- 3 \times 3 mm of the right eye (3 \times 3 OD)
- 6 \times 6 mm of the right eye (6 \times 6 OD)

Each scan set contains a horizontal-priority (fast-x) and a vertical-priority (fast-y) raster OCT volume, and these are combined automatically by the AngioVue software via three-dimensional orthogonal registration (Motion Correction Technology; Optovue, Inc.), thereby removing bulk motion and generating a merged OCTA image for all dimensions with reduced motion artefacts.¹¹

To maximize image quality, subjects were instructed to fixate on the central fixation target and not to blink during the imaging process. Images were discarded if they had signal strength of less than 50 or contained blink or motion artefacts.

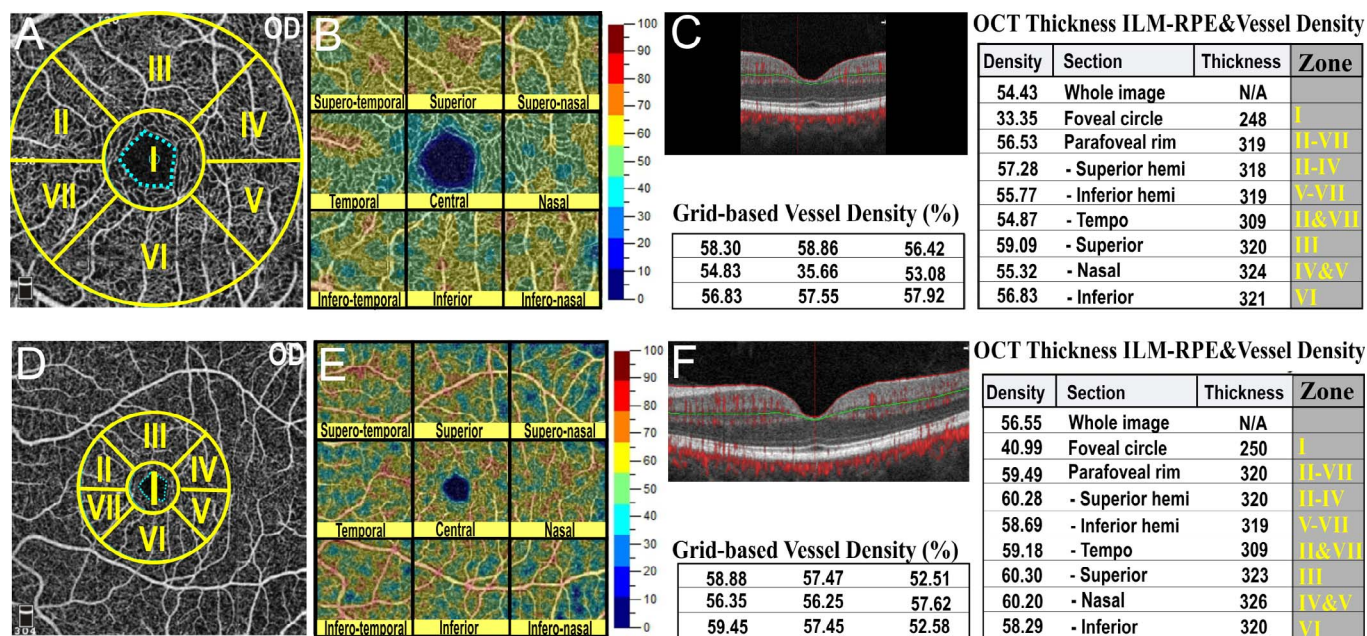


Figure 1. An example of AngioAnalytics output for right eye (OD) for calculation of SRVD and FAZA in 3×3 -mm scan area (A–C) and 6×6 -mm scan area (D–F). SRVD is calculated for the entire image (whole image density), eight regions within the circular grid (A, D) and nine regions within the square grid (B, E). Eight regions within the circular grid include: (1) a foveal circle (zone I), defined as a circular area with 0.5-mm radius centered on the foveal center; (2) a parafoveal region (combined zones II to VII), defined as the annular region between 0.5- and 1-mm radius from the foveal center; (3) a superior hemi-parafoveal rim (combined zones II, III, and IV); (4) an inferior hemi-parafoveal rim (combined zones V, VI, and VII); (5) a tempo-parafoveal rim (combined zones II and VII); (6) a superior-parafoveal rim (zone III); (7) a nasal parafoveal rim (combined zones IV and V), and (8) an inferior parafoveal rim (zone VI). SRVD is expressed as percentage of pixels with flow signals for each of the 18 regions (C, F). FAZA is calculated automatically by the software (*blue contour*) (A, D) and is expressed in square millimeters.

FAZA and SRVD Measurements

The AngioVue software performs automated segmentation by marking the inner boundary at $3 \mu\text{m}$ below the internal limiting membrane and the outer boundary at $15 \mu\text{m}$ below the inner plexiform layer to isolate the inner retinal layers and generate a map of flow signals arising from the superficial capillary plexus. The accuracy of inner retinal layer segmentation and presence of motion artefact were checked in all images.

FAZA (in square millimeters) is measured by the software after an operator manually chooses a point in the middle of the FAZ while using the Nonflow function. It has been recently demonstrated that magnification correction using axial length measurement alone can introduce a significant change in FAZA.^{12,13} Therefore, FAZA values were corrected for image magnification by using Littmann and modified Bennett formulae, as previously reported.¹² The magnification factor for FAZA is linearly related to the deviation of true axial length from the default axial length used by the device. Since the axial length

is known, we have the opportunity to examine test-retest variability and interocular asymmetry in FAZA before and after magnification correction.

Using the “Density” function, SRVD (expressed as the percentage of pixels with intensity greater than a defined threshold) from 18 regions is automatically generated from both 3×3 - and 6×6 -mm OCTA images (Fig. 1). The 18 SRVD variables include whole image density; central 1-mm foveal zone density; 0.5- to 1.5-mm rim of parafoveal zone density; superior hemi-parafoveal and inferior hemi-parafoveal zone densities; temporal, superior, nasal, inferior quadrant parafoveal zone densities, and nine square zone densities. In our recent work on the effect of magnification on OCTA metrics, we showed that the corrected SRVD from the central 1-mm foveal zone increased by 10% in eyes that are as short as 21 mm and decreased by 20% in eyes that are as long as 29 mm.¹² The effect of magnification correction on parafoveal (0.5- to 1.0-mm rim) SRVD was only $\pm 3\%$. Since magnification correction alters the size of the circular and linear grid used to mark the boundary of the 18 SRVD measurements, eyes that are shorter

than the default axial length of 23.95 mm would not have enough area of the retina imaged to provide SRVD values in the parafoveal region (0.5- to 1.5-mm ring) for comparison with other eyes with longer axial lengths.¹² Therefore, it is not possible to determine the impact of image size correction on the variability and asymmetry of AngioVue-derived SRVD in each of the 18 regions. As an exploratory analysis, the effect of axial length on test–retest variability SRVD is examined (see below).

Primary Outcome Measure: Intrasession Repeatability

The primary objective of the study was to estimate the test–retest variability of FAZA and SRVD measurements. A commonly accepted method of quantifying test–retest variability is to use measurement error (*ME*) and coefficient of repeatability (*CR*).

ME is defined by within-subject standard deviation, s_w , according to Bland and Altman.¹ In a study design where only two measurements are taken per subject, the variance of the two observations is half of the square of their difference. Therefore, calculation of the within-subject variance, s_w^2 , can be simplified by using the following formula: $s_w^2 = \frac{\sum_{i=1}^n d_i^2}{2n}$, where n is the number of subjects and d_i is the difference between two observations for subject i . For 95% of the observations, a single density measurement will lie within $ME = 1.96 \times s_w$. However, for two density measurements, the variance of the differences between these two replicates is the sum of the variance for the first and the second measurements. Because variance for each measurement is s_w^2 , the variance of the difference between these measurements is $2s_w^2$, and the standard deviation becomes $\sqrt{2s_w^2}$. For 95% of pairs of observation, the difference in density measurement is expected to lie within $1.96 \times \sqrt{2s_w^2}$ or $2.77 \times s_w$, which is also known as the *CR*. Therefore, if the difference in pairs of FAZA or SRVD measurements exceeded the *CR*, the change can be considered to be a true change because it exceeded the expected test–retest variability. *CR* was calculated for the uncorrected FAZA and SRVD generated by the AngioVue software and the magnification-corrected FAZA.

Secondary Outcome Measure: Interocular Symmetry

The secondary objective was to examine symmetry in FAZA and SRVD between right and left eyes. A commonly accepted method of quantifying interocular difference is the LAs. Having calculated the

expected variability from repeated measurements in one eye, we also wished to examine the frequency of interocular difference exceeding this threshold.

Interocular symmetry between right and left eyes in SRVD is quantified by the LAs as defined by Bland and Altman.¹⁴ The mean (\bar{d}) and standard deviation (s) of the differences between right and left eyes were calculated. It is important to note that s (standard deviation of the differences between right and left eyes) is not the same as s_w (within-subject standard deviation from right eye repeated measurements). We used s to derive the 95% upper and lower LAs between the two eyes, $\bar{d} + 1.96s$ and $\bar{d} - 1.96s$, respectively. Absolute differences between the two eyes were also calculated, and the top 95th percentile is reported.

The percentage (or frequency) of subjects with an interocular difference that exceeded the estimated *CR* for each region was also calculated to examine whether the extent of asymmetry was beyond the variability due to measurement error.

Sample Size Calculation

The precision for determining measurement error or within-subject standard deviation, s_w , is determined by the number of subjects and number of observations per subject. Given s_w^2 is the sum of squares divided by the degrees of freedom, its distribution is that of a χ^2 random variable. For large degrees of freedom (>30), the square root of a χ^2 has an approximately normal distribution.¹⁵ The SE of s_w is $\frac{s_w}{\sqrt{2n(m-1)}}$, where n is the number of subjects and m is the number of repetitions. The 95% confidence limits for s_w is $\pm \frac{1.96 \times s_w}{\sqrt{2n(m-1)}}$. To achieve a confidence interval (CI) of 19.6% on either side of *CR* ($2.77 \times s_w$), a sample size of 50 subjects is required when there are only two observations per subject.¹⁶

The standard error of the Bland-Altman LAs is approximately $\sqrt{3s^2/n}$. Therefore, the 95% CI for the estimated limit is $s \pm 1.96 \times \sqrt{3s^2/n}$.¹⁴ A sample size of 50 subjects will provide a precision $\pm 48\%$ in the upper and lower LAs between the two eyes.

Statistical Analysis

Statistical analysis was conducted using Microsoft Excel (Version 15.21.1; Microsoft, Inc., Redmond, WA) and SPSS Statistics (Version 24.0; IBM, Inc., New York, NY). Normal distribution in the test–retest and interocular difference values were confirmed by visual inspection of the frequency distribu-

Table 1. Intrasection Measurement Error of FAZA in Right Eyes

FAZA, mm ²	3 × 3-mm Scan Pattern			
	Mean (SD) FAZA 1	Mean (SD) FAZA 2	Difference (SD)	CR (95% CI)
Before correction for magnification factor	0.233 (0.104)	0.239 (0.104)	+0.005 (0.025)	0.049 (0.039, 0.059)
After correction for magnification factor	0.234 (0.108)	0.239 (0.108)	+0.004 (0.027)	0.052 (0.042, 0.062)

tion and quantile-quantile plots and the Kolmogorov-Smirnov test. Mean, standard deviation (*s*), range, and percentages were calculated and reported as appropriate. Intraocular differences between the two repeated scans in the right eye (second scan minus first scan) and interocular differences between scans from the right (second scan) and the left (only one scan done) eyes were examined by paired sample *t*-test. For all comparisons, a *P* value of less than 0.05 was considered statistically significant.

Test-retest variability (in right eye) and interocular differences (between right and left eyes) may vary with the magnitude or other ocular or patient variables (axial length, FAZA, signal strength index, and age). We examined these by plotting absolute difference against these independent variables as recommended by Bland and Altman.¹ Linear regression was performed and Pearson product-moment correlation coefficients were reported. All abbreviations and formulae used in this study are summarized in [Supplementary Tables S1 and S2](#).

Results

Study Cohort Demographics and OCTA Parameters

A total of 50 volunteers with no clinical retinal pathology were enrolled in the study between February and November 2016. The mean age (standard deviation, range) was 36.6 (13.6, 19–69) years with the majority (40 subjects) aged 50 years or under. Fifty-six percent (*n* = 28) of the participants were female. The mean (standard deviation) foveal thicknesses were 261 (24) and 261 (23) μm in the right and left eye, respectively.

The mean (standard deviation, range) spherical equivalent was −1.66 (2.46, −8.00 to +3.25) and −1.68 (2.72, −8.25 to +4.88) diopters for the right and left eyes, respectively. The mean (standard deviation, range) axial lengths were 24.26 (1.43, 21.45–27.88) and 24.40 (1.63, 20.99–28.85) mm for right and left

eyes, respectively. There was no relationship between axial length (right eye) and participant age, and the axial lengths between right and left eye were strongly correlated ($r^2 = 0.95$, [Supplementary Fig. S1](#)).

The mean (standard deviation, range) signal strength index for the right (first and second tests) and the left eyes were 74 (9, 52–86), 75 (9, 52–92), 75 (7, 54–94), respectively, for 3 × 3-mm OCTA images and 71 (7, 54–84), 71 (7, 52–85), 70 (7, 53–90), respectively, for 6 × 6-mm OCTA images. There was a weak negative relationship between signal strength index and age, but the variability in the index (between two tests in the right eye and between two eyes) is not related to age ([Supplementary Fig. S2](#)). Longer axial length is only weakly predictive of lower signal strength index ([Supplementary Fig. S3](#)).

The mean FAZA and whole image SRVD on the 3 × 3-mm OCTA images (right eye, first scan, no scaling) were 0.233 mm² and 53.80% ([Tables 1 and 2](#)). SRVD (3 × 3 mm) in the foveal and parafoveal zones were 34.08% and 55.84%, respectively. The 6 × 6-mm OCTA images (right eye, first scan, no scaling) provided a similar mean FAZA of 0.248 mm² and whole image SRVD of 51.22% ([Tables 1 and 2](#)). SRVD (6 × 6 mm) in the foveal and parafoveal zones were 37.56% and 54.21%, respectively. Image size correction did not alter mean FAZA significantly. The mean FAZA from the right eye after image scaling was 0.234 mm² and 0.259 mm² on the 3 × 3- and 6 × 6-mm OCTA images, respectively.

Repeatability of FAZA: Right Eye

The uncorrected FAZA obtained from the first and second OCTA scans in the right eye were similar for 3 × 3-mm OCTA images ($t = -1.2$, $df = 38$, $P = 0.2$) and the 6 × 6-mm OCTA images ($t = 0.6$, $df = 35$, $P = 0.6$). This difference remained nonsignificant after FAZA was corrected for image magnification. Bland-Altman plots showed no relationship between corrected FAZA test-retest variability (absolute difference within the same eye) and its magnitude ([Fig. 2](#)).

Table 1. Extended

FAZA, mm ²	6 × 6-mm Scan Pattern			
	Mean (SD) FAZA 1	Mean (SD) FAZA 2	Difference (SD)	CR (95% CI)
Before correction for magnification factor	0.248 (0.097)	0.250 (0.096)	−0.005 (0.051)	0.099 (0.079, 0.118)
After correction for magnification factor	0.259 (0.095)	0.254 (0.095)	−0.005 (0.051)	0.099 (0.080, 0.118)

The CR (95% CI) for uncorrected FAZA on the 3 × 3-mm OCTA image was 0.049 (0.039–0.059) mm². The CR (95% CI) for uncorrected FAZA on the 6 × 6-mm OCTA image was almost twice as large as that of 3 × 3-mm OCTA: 0.099 (0.079–0.118) mm². Image size correction did not have a significant impact on variability of FAZA (0.049 mm² vs. 0.052 mm², [Table 1](#)). [Figure 3](#) illustrates two cases that had large differences in FAZA between two consecutive OCTA scans in the right eye. The top panel illustrates FAZA segmentation in a 29-year-old female with axial length 23.29 mm. In her case, the difference in segmented area could be due to attenuation of the flow signal in one of the capillaries adjacent to the FAZ. The bottom panel demonstrates variability in the FAZA measurement of the right eye from a 26-year-old female with axial

length 23.10 mm. The differences in FAZ margin segmentation may be related to the lower signal strength index in the second scan. Bland-Altman plots showed no relationship between corrected FAZA variability (absolute difference within the same eye) and age, axial length, and signal strength index ([Supplementary Fig. S4](#)). Signal strength index may contribute up to 25% of the variability in the corrected FAZA ([Supplementary Fig. S4](#)).

Repeatability of SRVD: Right Eye

The 3 × 3-mm whole image SRVDs were similar between first and second OCTA scans in the right eye ($t = -0.7$, $df = 49$, $P = 0.5$). Similarly, there was no significant change in the whole image SRVD in the

Table 2. Intrasection Measurement Error in Right Eyes

SRVD (%)	3 × 3-mm Scan Pattern			
	Mean (SD) SRVD 1	Mean (SD) SRVD 2	Difference (SD)	CR (95% CI)
Whole scan area	53.80 (2.76)	54.03 (2.75)	+0.23 (2.38)	4.64 (3.73, 5.55)
Foveal circle (1 mm)	34.08 (5.71)	34.44 (5.85)	+0.36 (1.64)	3.26 (2.62, 3.90)
Parafoveal rim (1–3 mm)	55.84 (2.96)	55.98 (2.81)	+0.14 (2.50)	4.85 (3.90, 5.80)
<i>Superior hemi-parafovea</i>	56.08 (3.06)	56.23 (2.87)	+0.15 (2.60)	5.05 (4.06, 6.04)
<i>Inferior hemi-parafovea</i>	55.60 (3.15)	55.85 (3.02)	+0.25 (2.55)	4.97 (3.99, 5.94)
<i>Temporal parafovea</i>	54.99 (2.91)	54.67 (3.43)	−0.32 (2.80)	5.47 (4.40, 6.54)
<i>Superior parafovea</i>	56.88 (3.33)	57.01 (2.90)	+0.13 (2.91)	5.64 (4.53, 6.74)
<i>Nasal parafovea</i>	55.48 (3.32)	56.12 (3.26)	+0.64 (3.24)	6.40 (5.15, 7.66)
<i>Inferior parafovea</i>	55.99 (3.46)	56.53 (2.94)	+0.54 (2.56)	5.08 (4.09, 6.08)
Superotemporal square	54.93 (3.62)	55.00 (3.83)	+0.07 (3.12)	6.06 (4.87, 7.24)
Superior square	57.15 (3.04)	57.12 (2.49)	−0.03 (2.82)	5.46 (4.39, 6.53)
Superonasal square	57.08 (3.65)	57.16 (3.31)	+0.08 (3.37)	6.53 (5.25, 7.81)
Temporal square	54.81 (2.80)	54.59 (2.70)	−0.22 (2.49)	4.84 (3.89, 5.79)
Central square	37.74 (4.94)	38.08 (4.89)	+0.34 (1.76)	3.40 (2.80, 4.17)
Nasal square	55.45 (3.22)	56.07 (2.76)	+0.62 (3.18)	6.28 (5.05, 7.51)
Inferotemporal square	53.72 (3.90)	54.14 (4.61)	+0.42 (3.57)	7.03 (5.66, 8.41)
Inferior square	56.30 (3.65)	56.94 (3.16)	+0.63 (2.61)	5.22 (4.19, 6.24)
Inferonasal square	56.43 (3.57)	56.81 (3.53)	+0.38 (3.12)	6.10 (4.90, 7.29)

Italicized terms indicate parafoveal subregions.

* $0.01 < P < 0.05$.

right-eye 6×6 -mm OCTA images ($t = -0.66$, $df = 46$, $P = 0.5$). Bland-Altman plots of absolute difference against mean showed no relationship between variability and magnitude for whole image, foveal, or parafoveal SRVD (Fig. 4).

CR for the whole scan, foveal, and parafoveal SRVD in the 3×3 -mm OCTA images were 4.64%, 3.26%, and 4.85%, respectively. The CRs for foveal and parafoveal SRVD from the 6×6 mm were significantly larger at 6.13% and 6.38%, respectively (Table 2). The Bland-Altman plot showed no relationship between foveal or parafoveal SRVD test-retest variability (absolute difference within the same eye) and other variables such as axial length and age (Supplementary Fig. S5). Signal strength index may contribute up to 17% and 12% of the variability in foveal and parafoveal SRVD, respectively (Supplementary Fig. S5).

Interocular Asymmetry of FAZA: Right Versus Left Eye

The FAZA was highly symmetrical between the two eyes with before ($t = -2.2$, $df = 35$, $P = 0.03$) and after ($t = -2.2$, $df = 35$, $P = 0.03$) magnification correction in the

3×3 -mm OCTA images (Table 3). This was similar in the 6×6 -mm OCTA images before ($t = 0.14$, $df = 35$, $P = 0.9$) and after ($t = 0.78$, $df = 35$, $P = 0.4$) magnification correction (Table 3). Bland-Altman plots of absolute interocular difference in FAZA against mean of FAZA between the two eyes showed no obvious relationship between asymmetry and magnitude (Fig. 5).

The lower and upper bounds of the LA in FAZA (3×3 -mm OCTA) were -0.039 to $+0.059$ mm^2 (Table 3). In 95% of the cohort, the absolute difference in FAZA (3×3 -mm OCTA) was as large as 0.05 mm^2 between the two eyes. For a mean FAZA (3×3 -mm OCTA) of 0.233 mm^2 , a 0.05 - mm^2 interocular difference is equivalent to a 22% relative difference between the two eyes. This threshold is very similar to the CR for FAZA (0.05 mm^2). Only 6% of the participants had interocular difference in uncorrected FAZA (3×3 -mm OCTA) exceeding CR for FAZA derived from 3×3 -mm OCTA (Supplementary Table S3).

Interocular Asymmetry of SRVD: Right Versus Left Eye

The whole image SRVD was highly symmetrical between the two eyes in both the 3×3 -mm ($t = 1.49$,

Table 2. Extended

SRVD (%)	6 × 6-mm Scan Pattern			
	Mean (SD) SRVD 1	Mean (SD) SRVD 2	Difference (SD)	CR (95% CI)
Whole scan area	51.22 (3.40)	51.65 (2.66)	+0.43 (2.53)	4.97 (3.99, 5.94)
Foveal circle (1 mm)	37.56 (6.52)	38.15 (6.21)	+0.59 (3.11)	6.13 (4.93, 7.33)
Parafoveal rim (1–3 mm)	54.21 (3.86)	54.58 (3.41)	+0.37 (3.27)	6.38 (5.13, 7.63)
Superior hemi-parafovea	54.43 (3.82)	55.02 (3.39)	+0.59 (3.51)	6.90 (5.54, 8.25)
Inferior hemi-parafovea	53.99 (4.11)	54.14 (3.62)	+0.15 (3.27)	6.35 (5.10, 7.59)
Temporal parafovea	54.67 (4.45)	54.35 (4.42)	−0.32 (3.53)	6.86 (5.51, 8.20)
Superior parafovea	54.21 (4.02)	54.90 (3.61)	+0.69 (4.03)	7.92 (6.37, 9.47)
Nasal parafovea	54.48 (4.04)	54.90 (3.24)	+0.42 (3.71)	7.24 (5.82, 8.65)
Inferior parafovea	53.50 (4.42)	54.10 (3.84)	+0.60 (3.35)	6.60 (5.31, 7.89)
Superotemporal square	47.42 (4.44)	48.44 (3.54)	+1.02 (3.12)*	6.37 (5.12, 7.62)
Superior square	51.77 (3.28)	52.59 (2.59)	+0.82 (2.78)*	5.62 (4.52, 6.72)
Superonasal square	53.85 (3.08)	54.36 (2.69)	+0.51 (2.73)	5.38 (4.33, 6.44)
Temporal square	51.87 (4.60)	51.59 (3.93)	−0.28 (3.36)	6.53 (5.25, 7.81)
Central square	51.78 (3.53)	52.10 (3.11)	+0.32 (3.29)	6.40 (5.14, 7.65)
Nasal square	54.28 (3.26)	54.80 (2.53)	+0.52 (2.67)	5.26 (4.23, 6.30)
Inferotemporal square	46.25 (4.87)	46.33 (4.23)	+0.08 (3.36)	6.51 (5.24, 7.79)
Inferior square	50.45 (3.96)	50.81 (3.32)	+0.36 (2.68)	5.25 (4.22, 6.28)
Inferonasal square	53.40 (3.32)	53.90 (3.00)	+0.50 (2.25)	4.47 (3.59, 5.35)

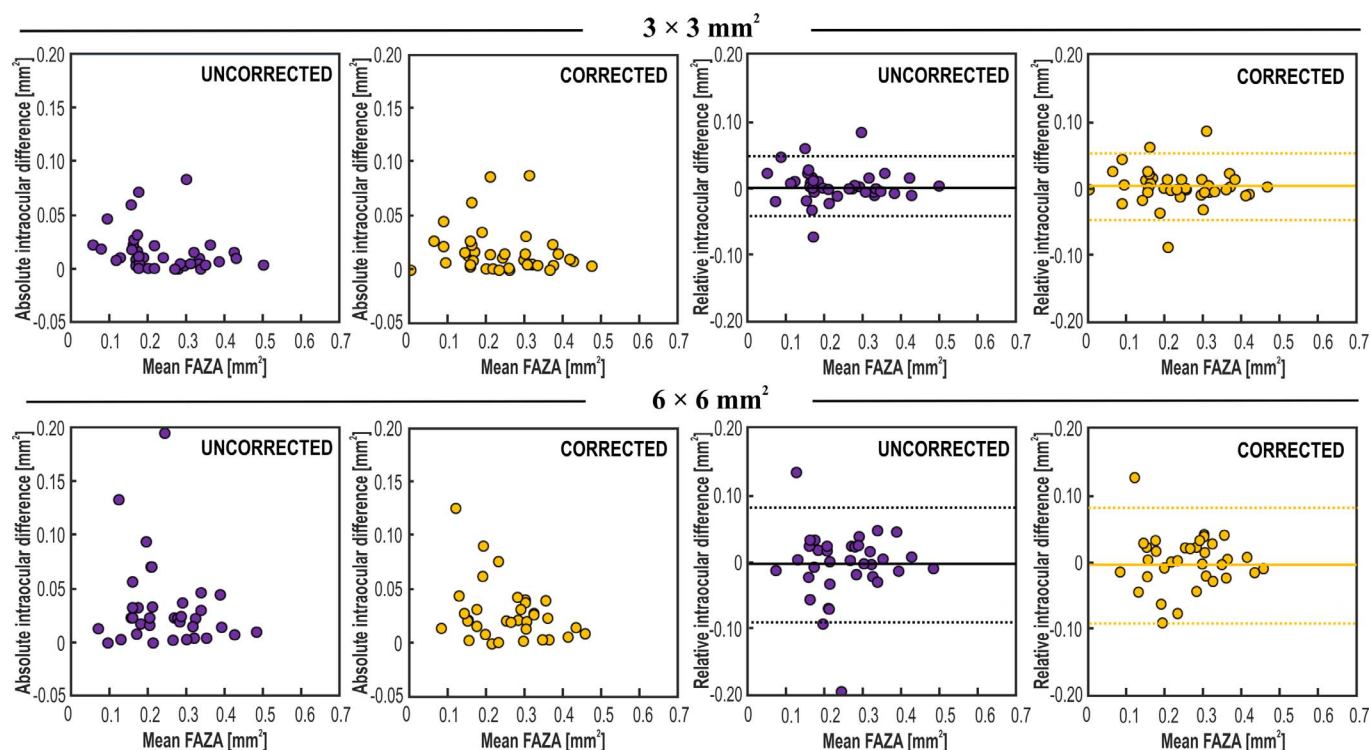


Figure 2. Bland-Altman plots of absolute difference and relative difference between the two measurements in the right eye against mean of FAZA before correction for magnification factor (purple dots) and after correction for magnification factor (yellow dots) in the 3×3 - and 6×6 -mm images. Plots of absolute difference against mean show no significant increase in variability with magnitude for the 3×3 and the 6×6 mm. Plots of relative difference against mean show similar LAs between the different regions.

$df = 47$, $P = 0.14$) and the 6×6 -mm ($t = -1.8$, $df = 48$, $P = 0.07$) OCTA images (Table 4). Bland-Altman plots of absolute interocular difference in SRVD against mean of SRVD between the two eyes showed no obvious relationship between asymmetry and magnitude (Fig. 6).

The lower and upper bounds of the LA in foveal SRVD (3×3 -mm OCTA) were -4.53% to $+3.77\%$. In 95% of the cohort, the absolute difference in foveal SRVD was as large as 4.60% between the two eyes. For a mean foveal SRVD of 34.08%, a 4.60% interocular difference is equivalent to a 13% relative difference in SRVD. This threshold is slightly larger than the CR for whole image SRVD (3.26%). The interocular difference in foveal SRVD (3×3 -mm OCTA) is not explained by asymmetry in uncorrected FAZA (Supplementary Fig. S6). Thirteen percent of pairs of foveal SRVD (3×3 -mm OCTA) measurement had interocular differences that exceeded the CR for foveal SRVD (Supplementary Table S3). In contrast, the greatest percentages are seen in 6×6 -mm OCTA images with 33% of the cohort having differences that exceeded CR in the superotemporal square zone.

Discussion

We examined the test–retest variability and interocular symmetry of FAZA and SRVD measurement in healthy subjects. Test–retest CRs and interocular LAs for FAZA and SRVD were reported. Our main findings were (1) image size rescaling based on axial length had little impact on CR for FAZA in the 3×3 -mm image (0.049 mm^2 vs. 0.052 mm^2), (2) CR for foveal SRVD was lower in 3×3 -mm than 6×6 -mm OCTA images (3.26% vs. 6.13%), and (3) interocular LAs in FAZA were comparable to the thresholds of test–retest variability, but SRVD asymmetry was much greater in certain regions of the macula.

Mean FAZA and SRVD Measurements

The mean FAZA (3×3 mm) in our cohort was 0.233 mm^2 , which is lower than 0.29 mm^2 reported by Iafe et al.,⁹ higher than 0.17 mm^2 reported by Hwang et al.,⁶ and comparable to 0.23 mm^2 reported by Linderman et al.¹³ and 0.25 to 0.28 mm^2 reported by Shahlaee et al.¹⁷ The mean foveal SRVD (3×3 mm) in our cohort was 34.08%, which is higher than 31.9%

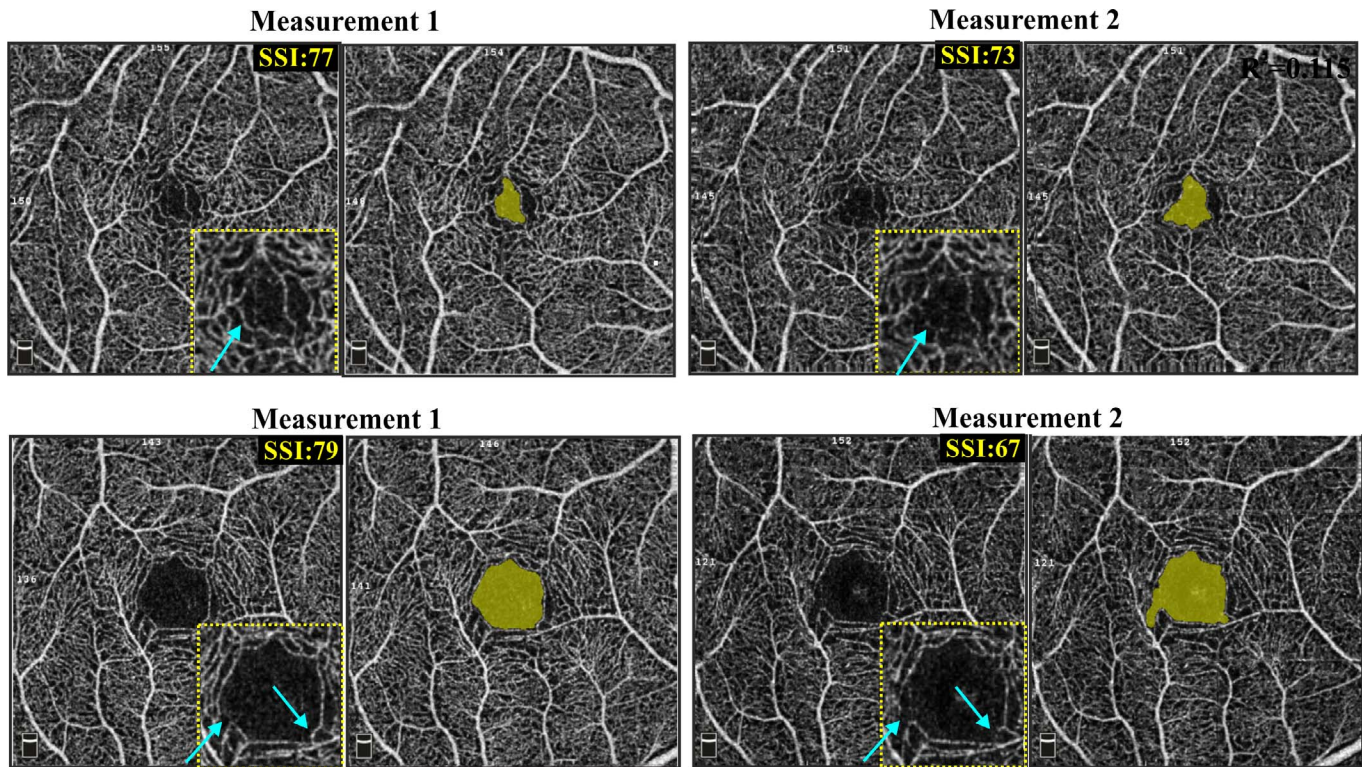


Figure 3. The effect of segmentation error and differences in signal strength index between two measurements on repeatability. Blue arrow indicates a vessel for which attenuated flow signal is visible in the second measurement. Top row: 29-year-old female (AL = 23.29 mm), bottom row: 26-year-old female (AL = 23.10 mm). The FAZA for case 1 was 0.067 and 0.114 mm² in measurements 1 and 2. The FAZA for case 2 was 0.310 and 0.345 mm² in measurements 1 and 2. AL, axial length.

reported by Shahlaee et al.⁴ and 31.5% reported by Coscas et al.⁷ Similarly, our cohort also had a higher mean parafoveal SRVD of 55.84% compared to the 46.0% and 54.3% reported by Shahlaee et al.⁴ and Coscas et al.⁷ Several groups have found increasing FAZA and decreasing SRVD with age.^{4,7,17} However, factors other than age may contribute to the variation in FAZA and SRVD, including (1) ethnicity and gender, (2) definition of foveal and parafoveal zones, (3) avascular zone boundary segmentation algorithms, (4) flow signal detection threshold algorithm, and (5) sample size. For example, Hwang et al.⁶ defined parafoveal region as an annular zone from a 0.30- to 1.25-mm radius, whereas Cosas et al.⁷ defined parafoveal region as from 0.5- to 1.0-mm radius from the foveal center and used the 2015 version of AngioAnalytics software for density measurement. Shahlaee et al.¹⁷ used ImageJ software (<http://imagej.nih.gov/ij/>; provided in the public domain by the National Institutes of Health, Bethesda, MD) to analyze OCTA images and defined foveal zone as the central 120-pixel (≈ 1.2 mm) parafoveal zone as the surrounding 91 pixels (≈ 0.9 -mm-wide ring). This

study used the 2016 AngioAnalytics software, and our foveal and parafoveal SRVD measurements were taken directly from the output by the software. These factors have to be taken into consideration when comparing or applying our thresholds of test–retest variability or interocular LAs.

Intrasession Repeatability of FAZA

The test–retest variability of FAZA using the RTVue XR Avanti OCTA device has been examined in several studies.^{6,7,9,18} These studies reported consistency in FAZA measurements within and between observers and imaging sessions. In the study by Carpineto et al.,¹⁸ a total of sixty 3×3 -mm OCTA images of 60 subjects (mean age 29 years) were analyzed by two trained observers at 30-minute intervals. The observers were allowed to fine-tune the plane of inner retinal segmentation prior to clicking on the center of the FAZ for automated calculation of FAZA. The intraobserver CRs of FAZA were 0.015 and 0.013 mm² for the first and second observers, respectively. Their CR cannot be compared to our CR because they used the formula

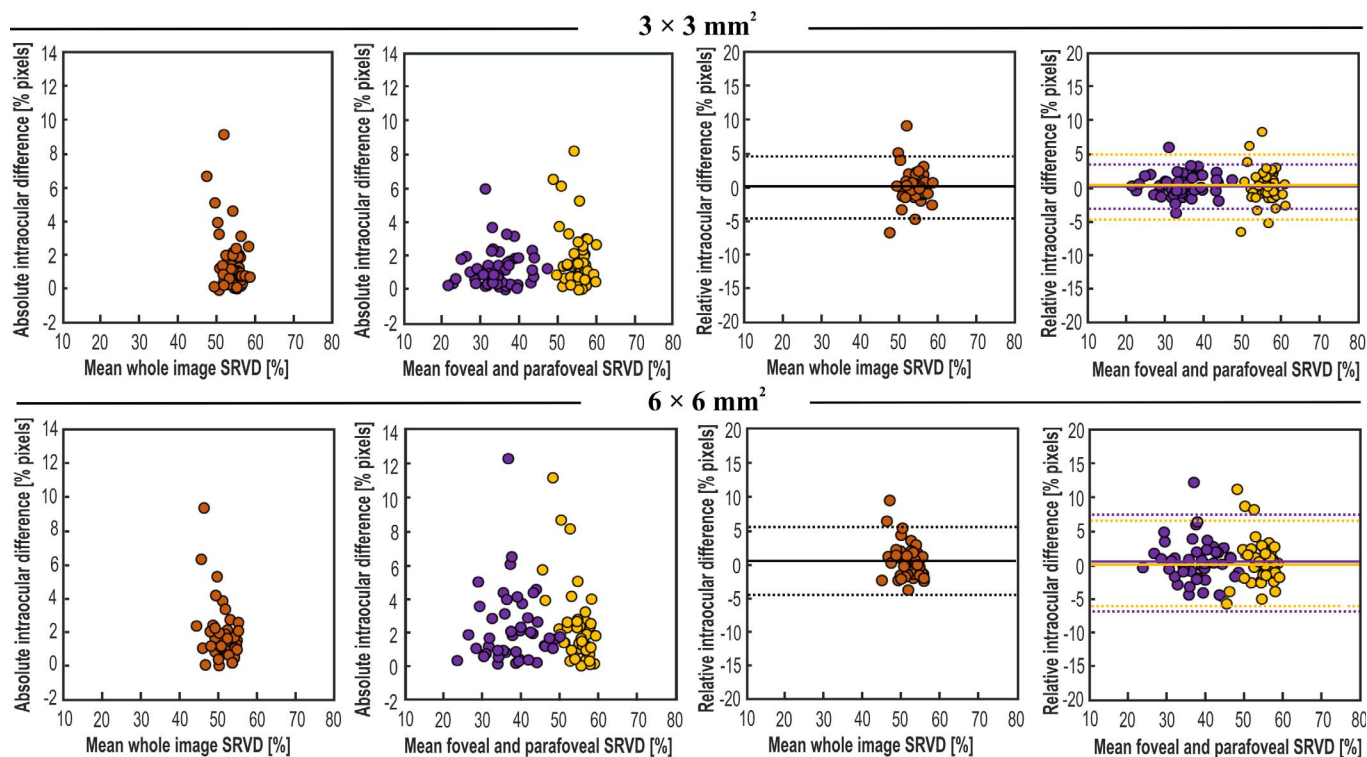


Figure 4. Bland-Altman plots of absolute difference and relative difference between the two measurements in the right eye against mean of SRVD for whole image (orange dots), foveal (purple dots) and parafoveal (yellow dots) regions in the 3×3 - and 6×6 -mm images. Plots of absolute difference against mean show no significant increase in variability with magnitude for the 3×3 and the 6×6 mm. Plots of relative difference against mean show similar LAs between the different regions. The LAs are wider for whole image (orange dots) and foveal (purple dots) and parafoveal (yellow dots) regions in 6×6 -mm images.

$1.96 \times \text{SD}$ of the differences between the measurements rather than $2.77 \times s_w$, and the same OCTA image was used for repeated FAZA measurement. Linderman et al.¹³ published repeatability of FAZA measurement for two sets of 3×3 -mm OCTA images using one of three methods: (1) manual segmentation of superficial plexus and FAZ boundary, (2) semi-automated measuring tool within version 2016.2.0.16 AngioVue software, or (3) fully automated measuring

tool within version 2016.200.0.37 AngioVue software. After correction for magnification error, their reported CRs were 0.022², 0.046, and 0.060 mm², similar to our reported CR of 0.049 and 0.052 mm² before and after image size correction. In another study, Lupidi et al.¹⁹ used the Heidelberg OCT2 instrument to analyze FAZA of 47 subjects (mean age 39 years). However, their results are not comparable to ours because they used a different OCTA imaging system

Table 3. LAs of FAZA Between Right and Left Eyes

FAZA, mm ²	3 × 3-mm Scan Pattern			50th and 95th Percentile in Absolute Difference
	Mean Difference (SD) ^a	Lower LA (95% CI)	Upper LA (95% CI)	
Before correction for magnification factor	0.010 (0.027)	−0.043 (−0.069, −0.018)	+0.063 (+0.037, +0.088)	0.019, 0.048
After correction for magnification factor	0.009 (0.031)	−0.052 (−0.081, −0.023)	+0.069 (+0.040, +0.098)	0.020, 0.039

^a difference = right (second test) – left.

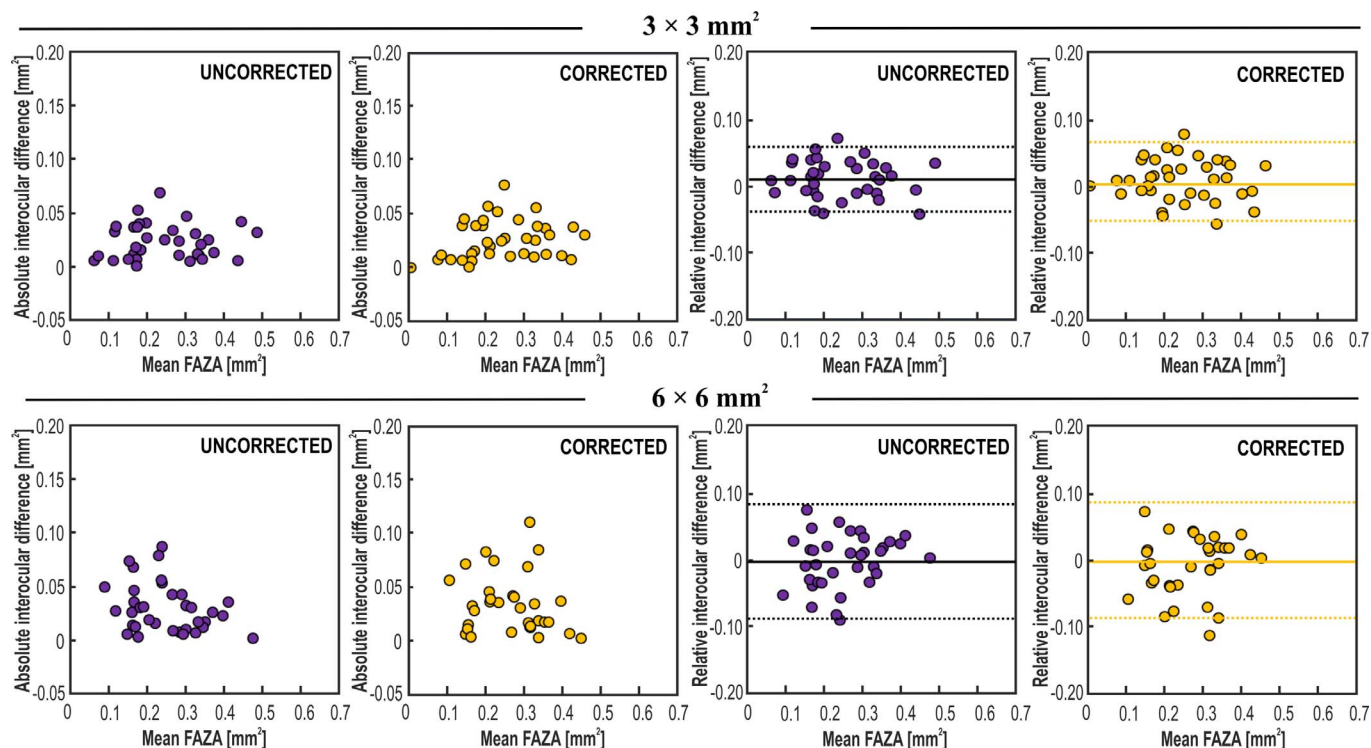


Figure 5. Bland-Altman plots of absolute difference and relative difference between the two eyes against mean of FAZA before correction for magnification factor (purple dots) and after correction for magnification factor (yellow dots) in the 3×3 - and the 6×6 -mm images. Plots of absolute difference against mean show no significant increase in variability with magnitude for the 3×3 and the 6×6 mm. Plots of relative difference against mean show similar LAs between the different regions.

and FAZA segmentation method. There has been, to our knowledge, no previous study that reported FAZA repeatability in the 6×6 -mm OCTA images. Based on our results showing that CR of FAZA derived from 6×6 mm is twice that of 3×3 -mm images, we would recommend against the use of 6×6 -mm images for FAZA measurements. The reason for this difference in variability is probably related to pixel density and definition of FAZ boundary with lower image resolution. We explored the relationship

between FAZA variation and other factors such as age, axial length, and signal strength index, but none of these were important in predicting variability. This observation undermines the importance of optimizing image quality in reducing FAZA measurement variability.

Intrasession Repeatability of SRVD

Intrasession repeatability of SRVD measurement using the RTVue XR Avanti OCTA device has been

Table 3. Extended

6 × 6-mm Scan Pattern				
FAZA, mm ²	Mean Difference (SD _{DIF}) ^a	Lower LA (95% CI)	Upper LA (95% CI)	50th and 95th Percentile in Absolute Difference
Before correction for magnification factor	−0.001 (0.039)	−0.077 (−0.113, −0.040)	+0.075 (+0.039, +0.111)	0.029, 0.075
After correction for magnification factor	−0.008 (0.043)	−0.093 (−0.134, −0.052)	+0.077 (+0.036, +0.117)	0.020, 0.085

Table 4. LAs Between Right and Left Eyes

SRVD, %	3 × 3-mm Scan Pattern			50th and 95th Percentile in Absolute Difference
	Mean Difference (SD) ^a	Lower LA (95% CI)	Upper LA (95% CI)	
Whole scan area	−0.50 (2.30)	−5.00 (−7.16, −2.84)	+4.00 (+1.84, +6.16)	1.28, 5.95
Foveal circle (1 mm)	−0.38 (2.12)	−4.53 (−6.52, −2.54)	+3.77 (+1.78, +5.76)	1.48, 4.56
Parafoveal rim (1–3 mm)	−0.75 (2.36)	−5.37 (−7.59, −3.15)	+3.88 (+1.66, +6.10)	1.33, 5.77
<i>Superior hemi-parafovea</i>	−0.23 (2.86)	−5.83 (−8.52, −3.14)	+5.37 (+2.68, +8.06)	1.29, 5.91
<i>Inferior hemi-parafovea</i>	−0.92 (2.56)*	−5.95 (−8.36, −3.53)	+4.11 (+1.69, +6.52)	1.41, 5.96
<i>Temporal parafovea</i>	−1.06 (2.69)	−6.33 (−8.87, −3.80)	+4.22 (+1.69, +6.76)	1.39, 7.91
<i>Superior parafovea</i>	−0.40 (2.93)	−6.14 (−8.89, −3.38)	+5.34 (+2.59, +8.10)	1.51, 6.20
<i>Nasal parafovea</i>	−0.32 (3.42)	−7.01 (−10.23, −3.80)	+6.38 (+3.17, +9.59)	1.92, 7.83
<i>Inferior parafovea</i>	−0.66 (2.80)	−6.14 (−8.77, −3.51)	+4.82 (+2.19, +7.45)	1.62, 5.43
Superotemporal square	−0.19 (3.59)	−7.23 (−10.60, −3.85)	+6.84 (+3.47, +10.20)	1.68, 6.67
Superior square	−0.97 (2.79)	−6.43 (−9.05, −3.81)	+4.49 (+1.86, +7.11)	1.46, 6.07
Superonasal square	−0.04 (2.73)	−5.40 (−7.97, −2.83)	+5.32 (+2.75, +7.89)	1.97, 5.24
Temporal square	−0.67 (2.29)	−5.16 (−7.31, −3.00)	+3.82 (+1.67, +5.98)	1.51, 3.77
Central square	−0.75 (2.60)	−5.84 (−8.29, −3.40)	+4.35 (+1.90, +6.80)	1.90, 4.91
Nasal square	+0.04 (2.92)	−5.69 (−8.44, −2.94)	+5.77 (+3.02, +8.52)	1.77, 5.43
Inferotemporal square	−0.63 (3.79)	−8.07 (−11.64, −4.50)	+6.81 (+3.24, +10.38)	2.18, 5.96
Inferior square	−0.33 (2.97)	−6.15 (−8.94, −3.35)	+5.49 (+2.70, +8.28)	2.07, 5.41
Inferonasal square	−0.58 (3.43)	−7.29 (−10.52, −4.07)	+6.14 (+2.92, +9.37)	1.90, 6.59

Italicized terms indicate parafoveal subregions. RE, right eye; LE, left eye.

^a difference = right (second test) – left.

* 0.01 < *P* < 0.05.

** 0.005 < *P* < 0.010.

*** 0.001 < *P* < 0.005.

examined in small cohorts of patients ranging from 5 to 17 subjects.^{3,4,6,8,9} These studies generally showed good consistency in SRVD values between imaging sessions and an overall standard deviation of 4%, the equivalent of 2% to 5% of the mean vessel density.^{3,6} More recently, a large retrospective study of healthy individuals (135 eyes) undergoing routine OCTA reported a standard deviation of the differences between two SRVD measurements ranging from 2.1% to 3.4% and 1.7% to 2.6% for inter- and intraobserver variability, respectively.⁷ We also calculated the standard deviation of the differences between the two intrasession OCTA images, and our range is similar: from 1.64% to 3.57% and 2.25% to 4.03% for the equivalent regions in 3 × 3- and 6 × 6-mm scan protocols (Table 2). Other studies have also examined the repeatability of the Nidek and Heidelberg OCTA devices.^{19,20} However, their results are not comparable to ours because the OCTA algorithm, vessel segmentation, and density analysis

methods used were different. Unlike any of the previous studies in OCTA repeatability, we also reported the measurement error of SRVD, s_w . In contrast to the standard deviation of the differences between two measurements, s_w is derived from averaging the within-subject variance across the cohort, and this is used to define CR, an estimate of the cutoff limit for a change in the SRVD value that can be considered to exceed test–retest variability. The CRs (95% CI) for foveal SRVD (0.5-mm radius) were 3.26% (2.62%–3.90%) versus 6.13% (4.93%–7.33%) in 3 × 3- and 6 × 6-mm OCTA images respectively. This difference could be due to lower image resolution of the 6 × 6-mm OCTA scans and hence reduced clarity of vessel structures and lower repeatability. We did find a weak correlation ($r^2 = 0.11$ – 0.17) between signal strength index (mean or differences) and SRVD variability, but this requires further studies to confirm. Other factors such as axial length and age did not exert significant influence on

Table 4. Extended

SRVD, %	6 × 6-mm Scan Pattern			50th and 95th Percentile in Absolute Difference
	Mean Difference (SD) ^a	Lower LA (95% CI)	Upper LA (95% CI)	
Whole scan area	+0.84 (2.96)	−4.96 (−7.75, −2.17)	+6.65 (+3.86, +9.44)	1.54, 5.31
Foveal circle (1 mm)	+0.48 (3.59)	−6.55 (−9.92, −3.17)	+7.52 (+4.14, +10.89)	2.22, 7.31
Parafoveal rim (1–3 mm)	+0.05 (3.69)	−7.19 (−10.66, −3.72)	+7.28 (+3.81, +10.76)	2.65, 7.90
Superior hemi-parafovea	+0.37 (4.02)	−7.51 (−11.30, −3.73)	+8.26 (+4.47, +12.04)	2.79, 9.32
Inferior hemi-parafovea	−0.26 (3.59)	−7.29 (−10.67, −3.92)	+6.78 (+3.40, +10.15)	1.90, 8.18
Temporal parafovea	−0.88 (4.15)	−9.02 (−12.92, −5.11)	+7.25 (+3.34, +11.15)	1.75, 8.30
Superior parafovea	+0.31 (4.25)	−8.02 (−12.02, −4.02)	+8.65 (+4.65, +12.65)	3.16, 9.34
Nasal parafovea	+0.59 (4.38)	−7.99 (−12.11, −3.87)	+9.17 (+5.05, +13.29)	2.35, 8.88
Inferior parafovea	+0.13 (4.06)	−7.82 (−11.63, −4.00)	+8.08 (+4.27, +11.90)	2.81, 9.02
Superotemporal square	+2.05 (5.66) ^{***}	−9.04 (−14.36, −3.71)	+13.14 (+7.82, +13.14)	3.53, 11.25
Superior square	+1.32 (3.51) ^{**}	−5.57 (−8.87, −2.26)	+8.20 (+4.90, +11.51)	2.30, 7.71
Superonasal square	+0.86 (3.40)	−5.80 (−9.00, −2.60)	+7.53 (+4.33, +10.73)	1.63, 7.64
Temporal square	−0.42 (4.30)	−8.85 (−12.90, −4.80)	+8.01 (+3.96, +12.05)	2.16, 11.55
Central square	+0.00 (3.50)	−6.86 (−10.15, −3.56)	+6.86 (+3.57, +10.16)	2.17, 5.89
Nasal square	+1.15 (3.06) [*]	−4.85 (−7.73, −1.97)	+7.15 (+4.27, +10.03)	1.94, 6.89
Inferotemporal square	+1.19 (4.68)	−7.97 (−12.37, −3.58)	+10.35 (+5.95, +14.75)	3.23, 9.66
Inferior square	+0.65 (3.23)	−5.68 (−8.72, −2.65)	+6.98 (+3.94, +10.02)	2.15, 6.28
Inferonasal square	+0.87 (3.18)	−5.36 (−8.35, −2.37)	+7.10 (+4.11, +10.09)	1.69, 6.42

SRVD variability. Table 2 provides a list of the cutoff boundaries for true change for SRVD in each of the 18 regions for both 3 × 3- and 6 × 6-mm OCTA scans. We would consider that a change of greater than CR to represent a true change in SRVD.

Interocular Symmetry in FAZA and SRVD

Interocular symmetry of FAZA was reported by Shahlaee et al.,¹⁷ and they found no significant differences in FAZA among 17 healthy subjects. Although they suggested that FAZA or the fellow healthy eyes can be used as controls, they did not report on the limits of interocular variation. We found good concordance in FAZA (3 × 3-mm OCTA) between the two eyes, with a difference in FAZA of up to 0.05 mm² in 95% of the cohort, which is also the threshold of test–retest variability. It is worth noting that a small proportion (Supplementary Table S3) of healthy subjects will have interocular difference exceeding this limit; therefore FAZA asymmetry of greater than 0.05 mm² is not always due to pathology. Symmetry in SRVD was examined in one previous study using ImageJ for vessel segmentation rather than AngioAnalytics

software.⁴ These investigators reported a mean (standard deviation) parafoveal SRVD of 46.61% (2.29) and 46.17% (1.55) in right and left eyes of 41 healthy subjects, respectively. Although they showed no statistically significant differences between the eyes, they unfortunately did not report the LAs or the 95th percentile of absolute interocular difference, both of which are clinically useful when interpreting OCTA. We showed no statistically significant differences between the two eyes for all regions except for two of the nine zones in the 6 × 6-mm image. Our data on LAs and the 95th percentile in absolute interocular differences (Supplementary Table S3) are clinically useful for detecting unilateral pathology resulting in subtle asymmetrical capillary dropout. We further investigated whether the range of interocular differences observed can be attributed to measurement error by OCTA. We used the CRs derived from repeated measurement in the right eye to determine the proportion of subjects with an interocular difference that exceeded the test–retest variability for a given region. According to Bland and Altman,¹ 95% of pairs of observations within the same individual for the same variable will lie within

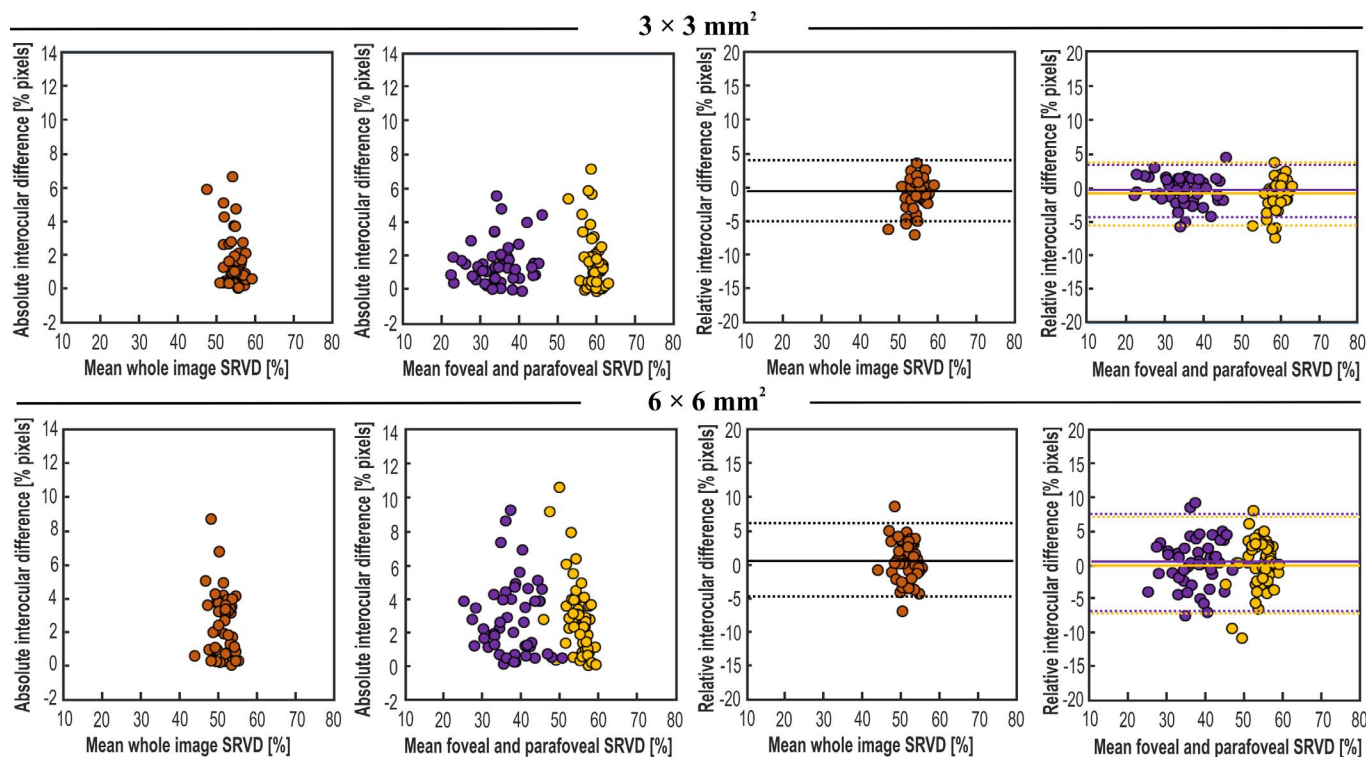


Figure 6. Bland-Altman plots of absolute difference and relative difference between the two eyes against mean of SRVD for whole image (orange dots), foveal (purple dots), and parafoveal (yellow dots) regions in the 3×3 - and the 6×6 -mm images. Plots of absolute difference against mean show no significant increase in variability with magnitude for the 3×3 and the 6×6 mm. Plots of relative difference against mean show similar LAs between the different regions. The LAs are wider for whole image (orange dots) and foveal (purple dots) and parafoveal (yellow dots) regions in 6×6 -mm images.

the limits set by the CR. Therefore, assuming that there is no interocular difference in all healthy individuals, we would expect two to three pairs of measurements (5%) to have a difference that exceeded the CR for that particular region. However, we observed that 13% of cohort had foveal SRVD in the 3×3 -mm scans that exceeded the CR. Notably, 33% of measurements in the superotemporal zone of 6×6 -mm image exceeded its CR of 6.4%. The higher frequency of large interocular difference relative to CR in certain regions may be due to underestimating the CR itself for that region, interocular difference in FAZA, subclinical pathology, or image artifact. The calculated CR has an error margin of 20% due to the sample size and number of repetition. We found no direct relationship between interocular difference in FAZA and asymmetry in SRVD (Supplementary Fig. S6). Although we excluded patients with retinal vascular disease by indirect biomicroscopic retinal examination and structural OCT, we cannot rule out that our cohort contains patients with undiagnosed systemic hypertension or diabetes mellitus who may have

subclinical retinal vascular abnormalities contributing to the observed asymmetry in FAZA and SRVD. Given the prevalence of diabetes and hypertension in Australia is around 5% and 11%, respectively (National Health Survey 2014–2015),²¹ the impact of these systemic diseases on the observed asymmetry in FAZA and SRVD is likely to be small. However, further studies are warranted. The most likely cause for the observed asymmetry in SRVD is probably poor image quality since higher frequency is found in 6×6 -mm compared to 3×3 -mm OCTA scans (Supplementary Table S3).

Study Limitations

Despite being, to our knowledge, the first large prospective study on the threshold of intrasession test–retest variability and limits of interocular agreement in FAZA and SRVD, there are several drawbacks in our study. First, we examined only healthy subjects. Patients with retinal disease have poorer fixation, and therefore image quality may succumb to motion and segmentation artefacts. However, our main purpose was to determine the

threshold for detecting development of subclinical change in vessel density, and therefore our results cannot be readily applied in a retinal vascular disease clinic in which patients may have larger FAZA and reduced SRVD. Second, we examined only the superficial plexus and not the deep plexus vessel density. We feel that the current algorithm provided by RTVue XR Avanti cannot reliably remove projection artefact within the deep vascular network for accurate determination of the vessel density. Third, the sample size of our study is relatively small, and only two scans were performed in one eye and one scan in the fellow eye. By increasing the sample size from 50 to 100 subjects and the number of scans from two to three, the 95% CI for the s_w can be reduced from $\pm 19.6\%$ to $\pm 9.8\%$. Increasing the number of scans in both eyes will also provide more power in examining interocular asymmetry. Fourth, our cohort may include undiagnosed hypertensive or diabetic patients who have not developed clinically visible macular lesions or altered structural OCT profile. It is possible that these and other systemic diseases may alter FAZA and SRVD and thus contribute to interocular asymmetry. The impact of systemic disease on the repeatability of FAZA and SRVD measurements need to be examined in future studies with a much larger sample size.

Conclusions

We showed that image size rescaling based on axial length did not impact on FAZA variability. Foveal SRVD variability was greater in 6×6 -mm compared to 3×3 -mm OCTA images. Limits of interocular asymmetry in FAZA are comparable to thresholds of test-retest variability, but SRVD asymmetry can be much greater in certain regions of the macula. The reported intrasession CRs and interocular LAs are useful in clinical settings for defining thresholds of subclinical FAZA and SRVD change in longitudinal population studies of healthy subjects.

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