Reproducibility and repeatability of foveal avascularzone measurements in healthy subjects by optical coherence tomography angiography

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ABSTRACT

Background/aims To assess the reproducibility andrepeatability of foveal avascular zone (FAZ) area measurements using optical coherence tomography angiography (OCT-A) in healthy subjects.

Methods Sixty healthy volunteers (60 eyes) were subjected to FAZ area measurements by means of highspeed and high resolution spectral-domain OCT
(SD-OCT) XR Avanti with the split-spectrum amplitude decorrelation angiography algorithm by two experiencedexaminers in two different sessions.

Results The mean±SD age of the subjects was 28.9
±7.6 years. Overall FAZ areas measured by the first andsecond observer were 0.251±0.096 mm^2 and 0.252
±0.096 mm^2, respectively. Within subjects, the coefficients of variations were 1.83% (95% CI 1.51% to
2.20%) and 1.86% (95% CI 1.33% to 2.43%) for the
first and second observers, respectively. The coefficient ofrepeatability average measurements of FAZ area were
0.015 mm^2 and 0.013 mm^2 for the first and second observers, respectively. The intraclass correlation coefficient values were 0.997 (95% CI 0.995 to 0.998)and 0.998 (95% CI 0.996 to 0.999) for the first and second observers, respectively. Inter- and intraobserver concordance correlation coefficients ranged from 0.994 (95% CI 0.990 to 0.977) to 0.999 (95% CI 0.997 to 0.999) and from 0.997 (95% CI 0.995 to 0.998) to 0.996 (95% CI 0.994 to 0.998), respectively. Conclusions FAZ area measurements by means of OCT-A showed excellent reproducibility and repeatabilityin healthy eyes. OCT-A is a non-invasive diagnostic method, and its reliability makes it an interesting potential diagnostic tool for disease detection and follow-up in retinal pathologies involving foveal microcirculation.

INTRODUCTION

Fluorescein angiography (FA) is a simple but inva- sive imaging technique that enables the study of the circulating blood in the human retina; it is still con- sidered to be the gold standard for imaging the retinal vascular network and for evaluating the foveal avascular zone (FAZ) in numerous retinovas-cular diseases.[1 2]

In the last 20 years, time domain and spectraldomain (SD) optical coherence tomography (OCT) have radically changed retinal diagnosis.[3 4] However, for many years, a limit of this technique was the inability to provide the functional informa- tion of the retinal microvasculature. Recently, a novel dye-less method for imaging the retinal microvasculature, called OCT angiography(OCT-A), has been introduced in clinical practice. The movement of red blood cells (RBC) within the retinal capillaries is used as an intrinsic contrast medium to generate flow imaging.[5] Two major motion contrast techniques, phase-based andamplitude-based, are used to render depth imaging of retinal and choroidal microvasculature.[5–8]OCT-A provides non-invasive imaging of theretinal capillary network and FAZ that is compar- able to, if not better than, invasive angiographicimaging.[5] The 'in vivo' quantification of FAZ may be a useful tool in detecting and monitoring theprogression of retinovascular diseases.

Reproducibility and repeatability are indicatorsof the applicability of any instrument as a diagnos- tic tool in clinical practice. They refer to the degree of agreement between independent measurements that are obtained with the same method/instrument on identical test material under different conditions (ie, different operators) and under the same condi- tion (ie, same operator performing consecutive measurements), respectively. The aim of this study was to evaluate both the intra- and intersession reproducibility and repeatability of FAZ area mea- surements using XR Avanti AngioVue OCT (Optovue, Inc, Fremont, California, USA) with the split spectrum amplitude decorrelation angiography (SSADA) algorithm in healthy subjects.

METHODS

The study was performed at the Ophthalmology Clinic of the University of Chieti-Pescara, Italy. A total of 60 healthy volunteers aged between 19 and 45 years were enrolled in the study.

The study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board. Informed consent was obtained before the scanning sessions. Each subject underwent a comprehensive ophthalmic examin- ation, including best-corrected visual acuity (using an Early Treatment Diabetic Retinopathy Study chart), slit-lamp biomicroscopy, intraocular pressuremeasurement with Goldmann applanation tonome- try, dilated funduscopic examination using a 78 D (diopters) lens, and visual field testing using the Humphrey 30-2 Swedish Interactive Thresholding Algorithm (Carl Zeiss Meditec Inc, Dublin, California, USA).

Inclusion criteria were best-corrected visual acuityof 20/25 or better, spherical refraction within ±3.0 D, and cylinder correction within ±2.0 D.

Subjects were declared healthy if the visual field mean devi- ation, pattern standard deviation and glaucoma hemifield testwere all within normal limits for at least two reliable visual field tests. Visual fields were considered reliable if fixation losses, false-negative results and false-positive results were <30%.

Subjects were excluded if they had any previous history of ocular disease, lens opacities, surgery, laser or medical treat- ments, or any systemic disease with ocular involvement. Additionally, subjects were excluded if the visual field mean deviation or pattern standard deviation was below 5% cut-offsor the glaucoma hemifield test was outside normal limits repro- ducibly in at least two reliable visual field tests. If the glaucoma hemifield test was borderline, mean and pattern standard devi- ation were <10%, but >5% or defects were inconsistent between visual fields, the subject was categorised as a 'glaucomasuspect' and was removed from the dataset.

Eyes with optical medium opacity, maculopathy, retinal disease, uveitis or glaucomatous or non-glaucomatous optic neuropathy were excluded from the investigation.

Finally, subjects were excluded in the case of an inability of OCT-A to display the FAZ (figure 1).

Imaging with XR Avanti

A commercially available XR Avanti SD-OCT with a high speedof 70 000 axial scans/s, wavelength of 840 nm (band width 45 nm), and an axial resolution of 5 µm was used. AngioVue software (pre-release version: 2014.2.0.68) was based on theSSADA algorithm theory.[8] In brief, it uses flow as the intrinsic contrast that is detected as a variation over time in the speckle pattern formed by the interference of light scattered from RBC and adjacent tissue structures.[8]

Pupils were dilated with a combination of 1% tropicamideand 2.5% phenylephrine. Study participants underwent SD-OCT imaging following a protocol that included an AngioVue OCT 3D volume set of 3×3 mm, consisting of 304×304 pixels in the transverse dimension. An internal fix-ation light was used to centre the scanning area. The OCT signal position and signal quality were optimised by means of the 'Auto All' function that, in sequence, performs the 'Auto Z'to find the best position for acquiring the retina OCT image, the 'Auto F' to find the best focus for the subject's refraction,and the 'Auto P' to find the best polarisation match for the sub- ject's ocular polarisation.

One FastX (horizontal raster) set and one FastY (vertical raster) set were performed for each acquisition scan. Each set took approximately 3 s to complete. After completion of the FastX and FastY sets, the software applied motion correction technology to remove saccades and minor loss of fixation. Low quality scans (ie, if the subject blinked or the scan had signifi- cant motion artefacts) were excluded and repeated until good quality scans were achieved.

Two different measurement sessions were performed in one randomly selected eye of each subject at baseline (T0) and after 30 days (T2). Randomisation was achieved using the random number generator Pro 1.89 (free software that is available online). In the first session, two measurements were acquired using the XR Avanti unit, each by two different trained obser- vers. Thirty minutes later (T1), measurements were repeated in the same fashion but with an inverted observer order. At theend of the first session, each eye received two AngioVue scansby each observer. During the second session, the scans were repeated by the same two observers using the same SD-OCT. Tominimise systematic bias, both examiners were masked withrespect to subject clinical information. After each acquisition, the observers were masked to measurements. FAZ area was mea-sured in mm^2 only at the end of the second session. Briefly, a non-flow measurement on a superficial reference plane (superfi- cial vascular plexus) of en-face projection was selected. The ref- erence plane for determining the superficial plexus was definedat the inner limiting membrane with a 60 µm section. The user manually fine-tuned the plane to maximise the visualisation of the retinal capillary bed. Upon clicking on the centre of theFAZ, the software automatically calculated the area (figure 2and video in the online supplementary appendix).

Sample size and statistical analysis

This study was designed to estimate the reproducibility and repeat-ability of FAZ area measurements. Assuming a within-subject standard deviation of 13% and three measurements per subject bytwo observers, using a Bland formula, the sample size required toestimate the width of the 95% CI within 13% was 60 subjects.

All qualitative characteristics of the subjects were summarised as frequency and percentage; quantitative characteristics were summarised as the mean and standard deviation. Intrasession repeatability for each observer was measured using two measurements (T_0 and T_1). The intraobserver repeatability was evaluated by calculating within-subject standard deviation (Sw), coefficient of repeatability (CR), coefficient of variation (CVw), and intraclass correlation coefficient (ICC). A Bland-Altman plot was used to assess the repeatability of the method by com- paring repeated measurements for each single examiner.[9]

Because the same method is used for the repeated measure- ments, the mean difference should be zero. The CR was calcu- lated as 1.96 (approximately 2) times the SD of the differences between the measurements.

The interobserver reproducibility was evaluated with a two-way mixed model with the subjects as a random effect and the observers as the fixed effect. The observer-effect estimates indicated the magnitude for bias between observers. In addition, Lin's concordance correlation coefficient (CCC) was calculated along with its 95% CI.[10] Statistical analysis was performed using the computing environment R.[11]

RESULTS

A total of 60 eyes of 60 subjects (24 males and 36 females) underwent FAZ area measurements with the previously described protocol.

All measurements provided high-quality scans and were prospectively included in the study. The sample age ranged from 19–44 years (mean±SD 28.9±7.6 years).

Additional demographic and clinical characteristics of the enrolled subjects are reported in table 1.

The mean±SD FAZ area measurements are shown in table 2. Overall FAZ areas measured by the first and second observers were 0.251±0.096 mm^2 and 0.252±0.096 mm^2, respectively. As calcu-lated from the means of the Bland-Altman plots (figure 3A, B),

CRs for average FAZ area were 0.015 mm^2 and 0.013 mm^2 for the first and second operators, respectively.

The indicators of repeatability, CVw, CR and ICC, resulting from statistical analysis are shown in table 3.

Interobserver intrasession CCCs of FAZ area measurements were 0.994 (95% CI 0.990 to 0.997) at T_0, 0.998 (95% CI 0.997 to 0.999) at T_1 and 0.999 (95% CI 0.997 to 0.999) at T_2 (table 4).

The estimate of the effect of the observer, which indicates the bias between observers, was not statistically significant for all time points (p=0.951, p=0.929, and p=0.968).

Intraobserver intersession CCCs of FAZ area measurements were 0.997 (95% CI 0.995 to 0.998) at T_0 and 0.996 (95% CI 0.994 to 0.998) at T_2 (table 5).

DISCUSSION

FAZ is the macular capillary-free zone that is used as an ana- tomic landmark for locating the retinal point of fixation. It i s surrounded by interconnected retinal capillary beds. Its size reflects the condition of the microcapillary circulation in thefoveal area.[12] The FAZ dimension has a strong positive correl- ation with the severity of capillary non-perfusion (drop-out) in several retinovascular diseases.[13] Vision-threatening retinovascu-lar diseases such as diabetic retinopathy, retinal vein occlusion and macular telangiectasia involve retinal microcirculation by modifying the FAZ size.[14] Mansour *et al*[15] found significantly smaller FAZ dimensions in control eyes (median value 0.405 mm^2) than in eyes with a background diabetic retinopathy (median value 0.737 mm^2) or eyes with a proliferative diabetic retinopathy (median value 0.866 mm^2). FAZ was found to be smaller in subjects with idiopathic juxtafoveolar telangiectasia than in a healthy control group.[16]

Studies[15–17] using FA to analyse the FAZ size demonstrated large variability of FAZ measurements in healthy subjects, with diameters data ranging from 500–600 µm and area data ranging from 0.205–0.405 mm^2.

Recently, John *et al*[18] hypothesised that the use of the contrast-adjusted method could be a better tool for studyingdigital angiogram pictures. They found that the FAZ area out- lined by the conventional method was larger than by the contrast-adjusted method (0.6241±0.177 mm^2 vs 0.2753 ±0.074 mm^2, respectively).

The high variability of measurements using FA cast doubt on the reliability of this diagnostic method to measure FAZ size.

OCT-A is a new dye-less method of imaging the retinal circu- lation[8] that provides a clean and continuous microvascular network around the FAZ.[7] In contrast to the FA imaging tech- nique that is two-dimensional and can explore the retina onlyon a single plane, OCT-A provides a non-invasive approach for assessing the three-dimensional microcirculation imaging of the retina.[19 20]

It can image the two vascular plexuses (superficial and deep) separately, showing different features that cannot be distin- guished with FA.[8 21]

Huang *et al*[22] first measured FAZ diameter in four healthy subjects by means of OCT-A using a modified Cirrus prototype with a swept-source laser provided by Carl Zeiss Meditec (Dublin, California, USA). The diameter of FAZ was 588 ±104 mm, as the mean value of size in the nasal-temporal and superior-inferior directions. The FAZ area was not detected.

Many ophthalmic instruments introduced into clinical prac- tice for biometric measurements were later withdrawn becauseof poor reliability. Reliability refers to the property of a meas- urement instrument that enables it to give similar results for similar inputs.

We investigated the reliability of OCT-A to measure FAZ areain healthy subjects using the high-speed (70 000 A-scans/s) 840 nm wavelength SD-OCT XR Avanti (Optovue, Inc) withthe SSADA algorithm. The SSADA algorithm can be used to dis-tinguish blood flow by calculating the decorrelation of the signal amplitude from consecutive B-scans at the same retinal location. It creates a contrast between static and non-static tissue that allows the visualisation of the blood flow in the retinal capillary bed.[7]

In this observational instrument validation study evaluating reproducibility and repeatability of FAZ area measurements using OCT-A in young healthy subjects, two experienced opera- tors independently performed two measurements in the first session and repeated the measurements in the second session30 days later.

Repeatability was tested by means of CVw, ICC and CR.

The within-subject coefficient of variation is the ratio between the within-subject standard deviation and the mean; the smaller the CVw, the better the repeatability.

The ICC is the ratio of the intersubject component of the variance to the total variance. The higher the ratio, the better the repeatability; the variability of measurements is primarily the result of interindividual differences.

There are some basic differences between the two measures. The ICC is scaled relative to the population mean, while CVwis scaled relative to the between-subject variance. CVw, unlike the intraclass correlation, is sensitive to a shift in scale. This is because it combines both precision (variation) and accuracy (bias) measures so that it measures the repeatability in bothsenses.[23]

The CR is the value below which the absolute differences between two measurements would lie with 0.95 probability; it is directly related to the 95% limits of agreement proposed byBland and Altman that contain 95% of the differences between repeated measurements on the same subjects.[9]

Intrasession and intersession reproducibility were evaluated by means of CCC. The CCC evaluates the degree to which pairs of observations fall on the 45° line through the origin.[14] It contains a measurement of precision ρ (the Pearson correlation coefficient, which

measures how far each observation deviates from the best-fit line) and accuracy Cb (a bias correction factor that measures how far the best-fit line deviates from the 45° line through the origin): ρc=ρ Cb.

The study showed both promising reproducibility and repeat- ability results for both operators. The within-subject coefficients of variations were 1.83% and 1.86% for the first and second observers, respectively. The ICCs were 0.997 for the first obser- ver and 0.998 for the second observer. The coefficients of repeatability of average FAZ area measurements were excellent, resulting in 0.015 mm^2 for the first observer and 0.013 mm^2 for the second observer.

Both interobserver intrasession and intraobserver intersession reproducibilities were very high, with CCC values ranging from 0.994 to 0.999.

In addition, FAZ area mean value (0.251 mm^2) was similar to the results obtained by John *et al*[18] using contrast-adjusted FA and Kim *et al*[24] using phase-variance OCT compared to FA.

To our knowledge, this is the first study assessing the reliabil- ity of FAZ area measurements using OCT-A with the SSADA algorithm in healthy subjects. We found a high reproducibility and high repeatability of the FAZ area measurements.

Technically, the high reproducibility may be a result of the automatic detection and calculation of the FAZ area using the SSADA algorithm that is included in the AngioVue software. These results confirm the reliability of the measurements obtained by means of automated systems described by Zheng*et al*[17] using the FA computerised technique for automated seg- mentation of the FAZ.

Reliability of FAZ measurements makes OCT-A an interesting potential diagnostic tool for retinal pathologies involving micro-circulation because the FAZ size is correlated to vision- threatening diseases.[13–16]

A limitation of the present study is that the population included only healthy young subjects. Additionally, given the relatively young age of our healthy population, the examination time in this study may be shorter than that encountered in older patients or patients who are affected by retinovascular diseases. Moreover, patients affected by maculopathies with poor fixation represent another limit for the reliability of the examination. Further studies will be needed to confirm this supposition andto test the reproducibility and repeatability in older and diseased patients.

In conclusion, the results presented in this study showed excellent reproducibility and repeatability of FAZ area measure- ments in normal eyes using a novel non-invasive method, making the OCT-A a potential diagnostic tool for disease detec- tion and progression in retinal pathologies involving microcirculation.

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Figure 1 Optical coherence tomography angiography in the right eyeof a young healthy subject showing the absence of a foveal avascular zone

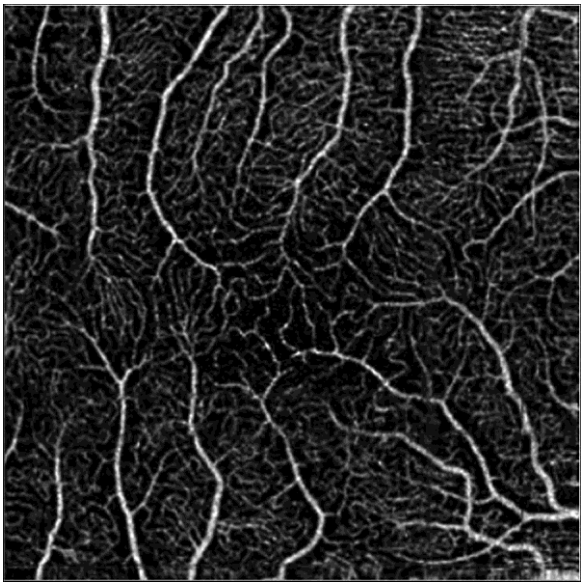

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Figure 2 Optical coherence tomography (OCT) angiography in the left eye of a young healthy subject showing the foveal avascular zone as a 'noflow area' within the superficial vascular plexus (top, left). The foveal avascular zone area is indicated in mm^2 (top, right). Longitudinal OCT scan showing the reference plane of the superficial vascular plexus between red and green lines (bottom).

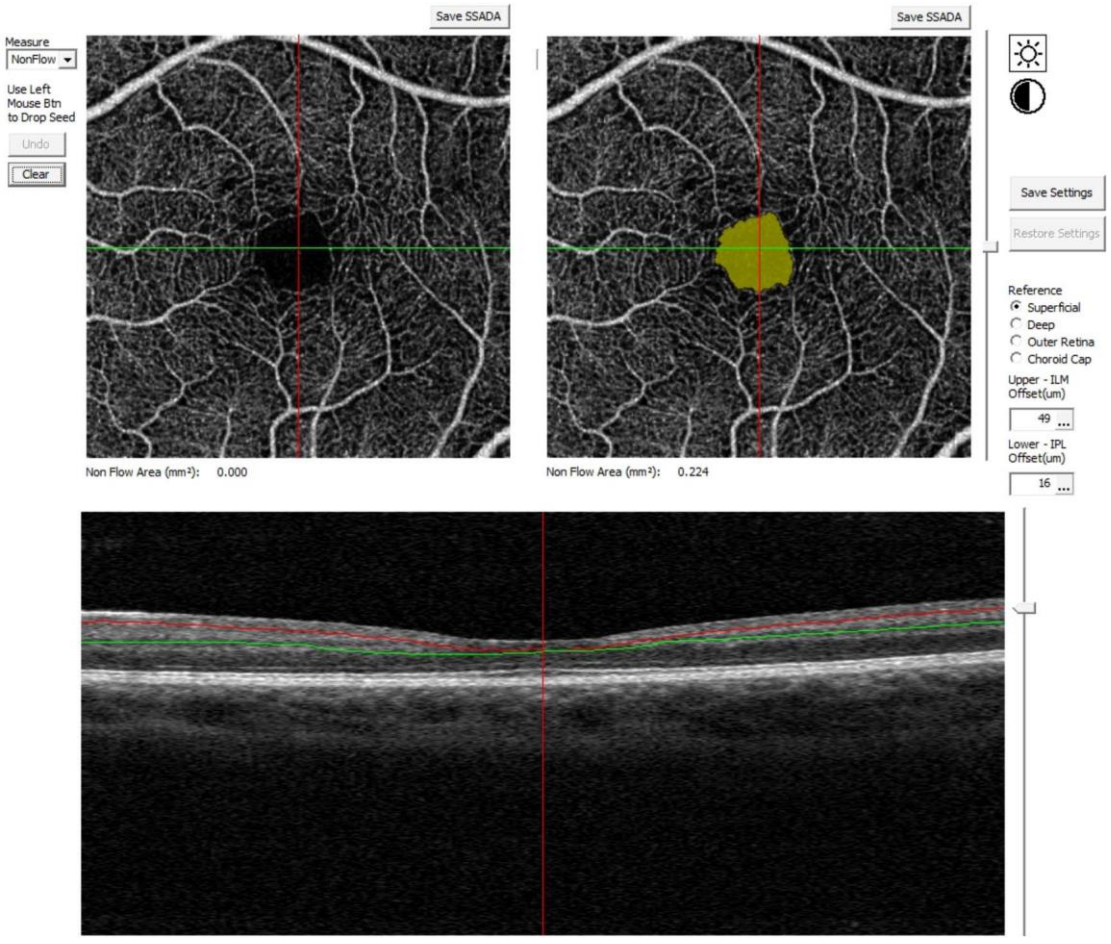

Figure 3 Bland-Altman plots showing the intraobserver (observer 1, panel A; observer 2, panel B) deviation of the foveal avascular zone area at T_0 from T_1. T_0 represents measurements by the observer during the first session; T_1 represents repeated measurements by the same observer duringthe same session.

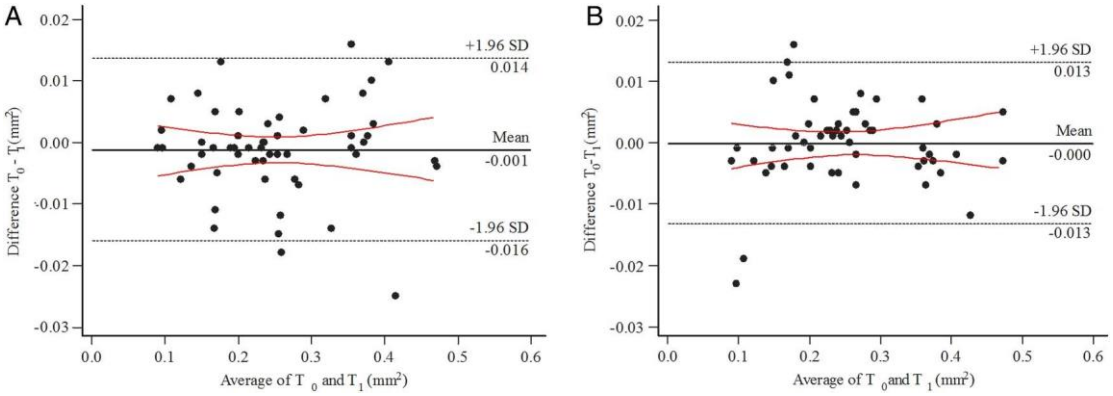
A
+1.96 SD
0.014
Mean
-0.001
-1.96 SD
-0.016
Difference T0 - T1 (mm2)
Average of T 0 and T 1 (mm2)
B
+1.96 SD
0.013
Mean
-0.000
-1.96 SD
-0.013
Difference T0-T1 (mm2)
Average of T 0 and T 1 (mm2)

Table 1 Patient characteristics

Variable	
Age (years)	28.9±7.6
Gender	
Female	36 (60.0)
Male	24 (40.0)
Eye	
Right	31 (51.7)
Left	29 (48.3)
Intraocular pressure (mm Hg)	16.2±1.5
Visual field mean deviation (dB)	+0.5±0.9

Data presented as mean±SD or n (%).

	T_0	T_1	T_2	Overall
Observer 1	0.251±0.097	0.254±0.096	0.252±0.097	0.251±0.096
Observer 2	0.252±0.097	0.252±0.096	0.253±0.096	0.252±0.096

Table 3 Intrasession repeatability of measurements

CVw (%) (95% CI)	CR (95% CI)	ICC (95% CI)
1.83 (1.51 to 2.20)	0.015 (0.013 to 0.017)	0.997 (0.995 to 0.998)
1.86 (1.33 to 2.43)	0.013 (0.011 to 0.014)	0.998 (0.996 to 0.999)

CR, coefficient of repeatability; CVw, within-subject coefficient of variation; ICC, intraclass correlation coefficient.

Table 4 Interobserver intrasession reproducibility of foveal avascular zone area measurements

	CCC	95% CI
T_0 (observer 1 vs observer 2)	0994	0.990 to 0.997
T_1 (observer 1 vs observer 2)	0998	0.997 to 0.999
T_2 (observer 1 vs observer 2)	0999	0.997 to 0.999

CCC, concordance correlation coefficient.

Table 5 Intraobserver intersession reproducibility of fovealavascular zone area measurements

	CCC	95% CI
Observer 1 (T_0 vs T_2)	0997	0.995 to 0.998
Observer 2 (T_0 vs T_2)	0996	0.994 to 0.998

CCC, concordance correlation coefficient.