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Intra-session Repeatability and Inter-session Reproducibility of Peripapillary OCTA Vessel Parameters in Non-Glaucomatous and Glaucomatous Eyes

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Abstract

Objective: To compare intra-session repeatability versus inter-session reproducibility of the peripapillary vessel parameters using optical microangiography (OMAG)-based optical coherence tomography angiography (OCTA) in non-glaucomatous and glaucomatous eyes.

Methods: In an observational, longitudinal study, peripapillary OCTA scans were collected to evaluate intra-session repeatability and inter-session reproducibility using within-eye coefficient of variation (CV_W) and intraclass correlation coefficient (ICC). Images were quantified using a custom research-oriented quantification software calculating Vessel Area Density (VAD) and Flux and a commercially-developed, clinic-oriented quantification software (Cirrus 11.0, Zeiss) calculating Perfusion Density (PD) and Flux Index (FI). Effect of signal strength on the reliability of OCTA parameters was also evaluated.

Results: Among 120 non-glaucomatous eyes, intra-session CV_W were 4.2% for VAD, 5.3% for Flux, 1.5% for PD, and 2.0% for FI. The inter-session CV_W were 6.5% for VAD, 8.0% for Flux,

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2.0% for PD, and 3.2% for FI. The intra-session ICC ranged from 0.928–0.945, and inter-session ICC from 0.811–0.866. From 118 glaucomatous eyes, intra-session CV_W was 9.0% for VAD, 10.3% for Flux, 1.7% for PD, and 2.3% for FI. The inter-session CV_W was 12.1% for VAD, 14.2% for Flux, 2.3% for PD, and 3.5% for FI. The intra-session ICC ranged from 0.904–0.972, and inter-session ICC from 0.855–0.955. Signal strength was significantly positively associated with OCTA vessel parameters ($p < 0.0001$) for both groups.

Conclusion: Peripapillary OCTA vessel parameters had greater intra-session repeatability compared to inter-session reproducibility in both non-glaucomatous and glaucomatous eyes. The built-in commercially-developed quantification software demonstrated greater agreement than the custom research-oriented quantification software.

Precis:

Peripapillary vessel parameters of optical coherence tomography angiography in non-glaucomatous and glaucomatous eyes were highly repeatable and reproducible, though repeatability and reproducibility for commercially developed OCTA parameters were higher than that for custom OCTA parameters.

INTRODUCTION

Optical coherence tomography angiography (OCTA) is an evolving non-invasive imaging modality that is used to quantitatively evaluate *in vivo* microvascular perfusion of the retina by detecting blood flow via motion contrast generated by red blood cells.^{1–5} In clinical practice, OCTA vessel parameters have been utilized in evaluating the vascular changes associated with common ocular pathologies. Prior OCTA studies of glaucoma have shown reduced vessel perfusion in the optic nerve head, peripapillary region, and macula, with the highest diagnostic accuracy being in the peripapillary region.^{3–6} OCTA has the potential to assist clinicians with early detection of disease and its progression. It may also provide new insights into the vascular components of glaucoma pathophysiology. However, in order to use OCTA clinically, especially in longitudinal assessments, repeatability and reproducibility of these images need to be established.

Repeatability refers to the variation in repeated measurements made on the same subject under identical conditions, which can be applied to measurements obtained within a given session. Reproducibility refers to variation in measurements made on a subject under changing conditions, which can be applied to measurements obtained on different days, in different sessions.^{7–8} Although there are some studies on either the intra-session repeatability or the inter-session reproducibility of OCTA vessel parameter measurements in various clinic populations^{9–18} and a recent study on the repeatability and reproducibility comparison in healthy eyes,¹⁹ a comparison between repeatability and reproducibility in the same patient population among glaucomatous and non-glaucomatous eyes has not been reported to the best of our knowledge. Understanding the magnitude of any differences may shed light on the extent to which normal physiologic variation may impact the reproducibility of OCTA parameter measurements and will thus inform clinical interpretation of OCTA parameters. For example, studies have shown that use of caffeine,²⁰

nicotine,^{21–22} level of physical activity^{23–24} and cardiovascular health have a direct effect on ocular microcirculation.^{25–26}

In this study, a spectral domain OCTA (SD-OCTA) device with complex signal-based optical microangiography (OMAG^C) was used to generate en face images of the radial peripapillary capillaries (RPC). The en face images were quantified using both research-oriented and clinic-oriented software (CIRRUS 11.0; Zeiss) to measure OCTA vessel parameters. Agreement estimates were calculated to quantify random measurement error of repeated OCTA measurements. The purpose of the study was to compare the intra-session repeatability versus the inter-session reproducibility of both types of OCTA parameters in the same population of glaucomatous and non-glaucomatous eyes.

METHODS

An observational, longitudinal study was performed on consecutive willing patients presenting to the glaucoma service at University of Southern California Roski Eye Institute between February 2016 and November 2019. The research protocol was approved by the Health Science Institutional Review Board of the University of Southern California, conducted in accordance with the Health Insurance Portability and Accountability Act of 1996, and adhered to the tenets of the Declaration of Helsinki. Subjects were informed of the nature and intent of the study, and their written consent was obtained.

All subjects underwent the standard comprehensive ophthalmic examination, including visual acuity measurement, slit lamp biomicroscopy, intraocular pressure (IOP) measurement (applanation tonometry), Visual Field (VF) testing (24–2 SITA Standard, HFATM II-I; Carl Zeiss Meditec, Dublin, CA, USA), OCT imaging of retinal nerve fiber layer (RNFL) thickness (CirrusTM HD-OCT 5000; Carl Zeiss Meditec), and 6×6mm OCTA imaging (CirrusTM HD-OCT 5000 with AngioPlex® OCT Angiography 9.5.2; Carl Zeiss Meditec). Triplicate OCTA scans centered on the optic nerve head (ONH) were obtained for each subject on each session.

A fellowship-trained glaucoma specialist diagnosed glaucoma by identifying characteristic optic nerve rim defects such as notching or localized thinning on clinical examination. Additionally, RNFL thickness and deviation maps from OCT (Cirrus HD-OCT 5000; Zeiss, Dublin, CA) were reviewed for all patients. Glaucomatous eyes had RNFL thinning consistent with fundusoscopic disc findings. Staging of glaucomatous damage was determined based on Hodapp-Parrish-Anderson criteria.^{27–28} Preperimetric glaucoma presented with optic nerve rim defect and consistent RNFL thinning without visual field defects. Glaucomatous eyes included preperimetric glaucoma and all types of glaucoma. Non-glaucomatous eyes ranged from normal to glaucoma suspects based on the clinical exam results without glaucomatous optic discs. For subjects with a glaucomatous eye and a non-glaucomatous eye, each eye was included in its respective group.

Inclusion criteria included age ≥ 21 years, images with signal strength ≥ 7, at least 2 good quality intra-session images and an additional good quality inter-session image (Fig. 1a), and clinically stable disease for the glaucoma group. The exclusion criteria for

non-glaucomatous eyes were inter-sessions greater than 12 months apart. The exclusion criteria for glaucomatous eyes was any evidence of progression based on the glaucoma specialist assessment and inter-sessions greater than 6 months apart. A shorter inter-session time criterion was used for the glaucomatous group to minimize any potential effect of subclinical glaucomatous progression on OCTA parameters.²⁹ A supplemental analysis applied the same inter-session criteria to both groups. Other exclusion criteria include history of ocular surgery between inter-session images; ocular trauma; comorbid, non-glaucomatous optic neuropathies; severe non-proliferative diabetic retinopathy; proliferative diabetic retinopathy; and poor image quality due to motion artifacts, media opacities, or vitreous floaters (Fig. 1b). Demographic information collected from the clinical chart included age, sex, diagnoses of hypertension or diabetes, and history of ocular surgeries. For subjects with both eyes meeting the inclusion and exclusion criteria, both eyes were included to the study.

OCTA IMAGE ANALYSIS

Commercially available, FDA approved, OMAG^C algorithm based on SD-OCTA platform (CIRRUS 9.5.2; Zeiss) was used to generate angiographic images, where the intensity and phase information between sequential B-scans at the same position generated the motion signal, which indicated blood flow. After image acquisition with the CIRRUS 9.5.2 software, automatic segmentation and quantification were performed by the updated CIRRUS 11.0 software (Zeiss). This software was used to detect the boundaries of the RNFL extending superficially from the inner limiting membrane to the outer boundary of the RNFL from structural OCT cross-sectional images, by measuring the gradient of OCT signals. This 11.0 software is commercially available in several regions outside the US, including the European Union and Japan, but is currently awaiting FDA approval. The defined RNFL boundaries were used to create the 2-dimensional RPC angiographic en face image.

Subsequently, two different types of quantification software were applied to the RPC images. A custom quantification software³⁰ with an interactive interface was used to quantify the microvasculature for the annular peripapillary region (MATLAB R2019a; MathWorks, Natick, MA). An annulus with inner diameter of 2mm and outer diameter of 6mm centered on the ONH of the 6×6mm RPC scan was used to delimit that portion of the retina that was measured for the annular OCTA parameters (Vessel Area Density and Flux). The original grayscale en face images were converted to a binarized, black and white vessel map using a three-way combined method consisting of global thresholding, Hessian filter, and adaptive threshold. The area within the ONH was used to establish the baseline background noise for global thresholding. Then, the Hessian filter and adaptive threshold were combined to generate a binarized vessel map and to obtain the vessel area. Vessels larger than 32 μm were removed from the image area that was quantified. From the binarized image, vessel area density (VAD) was the unitless ratio of the total sum of the white pixels with detected OCTA signal " $A_{(I,j)}$ " divided by total sum area of all pixels in the binarized image " $X_{(I,j)}$," and provided an estimated vessel density of both small-medium vessels and capillaries, excluding large vessels. Flux measured the mean flow intensity in the vessel area, where the sum of all the pixels identified as blood flow in the binarized

vessel map “ $I_{\text{flow}(i)}$ ” was divided by the total of white pixel counts on the binary vessel map (n) multiplied by the full dynamic range of blood flow signal intensity (255), and provided a unitless number of particles passing through a retinal vessel cross-sectional area per unit time. In addition to the custom quantification software, the commercially developed automated quantification software (CIRRUS 11.0; ZEISS) also quantified retinal vascular density and Flux for the global peripapillary regions. As referenced above, this quantification software is commercially available in regions such as the European Union and Japan, but is currently awaiting FDA approval. Perfusion Density (PD) was the total area of perfused capillary vasculature per unit area of the entire 6×6mm RPC en face images. This parameter is most comparable to VAD in the custom software. Flux Index (FI) was the total area of perfused vasculature per unit area, weighted by the brightness of flow signal and corrected for dimmer areas on B-scan, of the entire 6×6mm RPC en face images. This parameter is most comparable to Flux in the custom software.

STATISTICAL ANALYSIS

The statistical analysis was conducted using R (R Core Team, Vienna, Austria), and Microsoft Excel (Redmond, WA), and the data visualization was created using ggplot2 (Wickham H, Springer-Verlag New York) for R markdown.

Bland-Altman plots were used to assess the intra-session and inter-session limits of agreement in repeated OCTA parameter measurements for both non-glaucomatous and glaucomatous eyes. The Bland-Altman plots demonstrate agreement by plotting the difference between paired measurements—with 95% confidence limits—against their mean. Smaller limits indicated better agreement among repeated measurements.⁸

Intra-session repeatability and inter-session reproducibility for each OCTA parameter were quantified by within-eye standard deviation (S_W), within-eye coefficient of repeatability (CR_W), within-eye coefficient of variation (CV_W), and the intraclass correlation coefficients (ICC). Higher agreement corresponds to higher ICC and lower S_W , CR_W and CV_W . These agreement measures were assessed with a linear mixed effects model to account for inter-eye correlation, as both eyes of some subjects were included in the analysis. This model included random effects for subject and eye. S_W represents random measurement error, and was quantified by first calculating the square root of the variance of repeated measurements on each eye, then taking the average of this value across all eyes in the study group.^{7,31} CR_W provides a range of uncertainty for repeated measurements, and was obtained by multiplying the S_W by 1.96 and $\sqrt{2}$.⁷ The difference between any two measurements for a given eye is expected to be less than the CR_W for 95% of pairs of observations.⁷ CV_W is unitless and represents a standardized measure of agreement; it was calculated by dividing the S_W of a given OCTA variable by the variable's mean. CV_W permits direct comparison of agreement estimates among different OCTA parameters.³² ICC is defined as the between-eye variance divided by the sum of the between-eye variance and the within-eye variance.⁷ A higher ICC indicates that a greater proportion of measurement variance is due to actual between-eye differences, and a correspondingly smaller proportion is due to random measurement error. It is important to note that while ICC is useful for comparisons across different OCTA parameters of the same sample, it is not useful to compare between glaucomatous and non-

glaucomatous groups. The glaucoma group is expected to have inherently more between-eye variance due to a wide range of disease severity, which tends to increase ICC for this group compared to the non-glaucoma group.

The potential effect of signal strength on repeatability was evaluated by testing for an association between signal strength and the OCTA parameters using a linear mixed effects model for repeated measurements controlling for inter-eye correlation.

RESULTS

516 eyes from 263 subjects underwent repeated 6×6mm SD-OCTA peripapillary imaging both in the same session and in different sessions. Of these, 118 glaucomatous eyes and 120 non-glaucomatous eyes met the inclusion/exclusion criteria. 33 eyes had mild glaucoma, 33 eyes had moderate glaucoma, and 52 eyes were severe glaucoma. Out of the 118 glaucomatous eyes, 108 had primary open angle glaucoma (POAG), 6 had mixed mechanism glaucoma, and 1 had uveitic glaucoma. Characteristics of each group are presented in Table 1. The intra-session analysis was comprised of 120 non-glaucomatous eyes (327 images from 76 subjects) and 118 glaucomatous eyes (301 images from 82 subjects). The inter-session cohort consisted of 73 non-glaucomatous eyes (154 images from 48 subjects) and 70 glaucomatous eyes (172 images from 50 subjects).

Bland-Altman plots for each OCTA parameter using intra-session and inter-session measurements are shown in Figure 2 for non-glaucomatous eyes and in Figure 3 for glaucomatous eyes. Narrower confidence limits are indicative of higher agreement. VAD showed the least agreement among all vessel parameters. PD, Flux, and FI measurements have a high degree of agreement, with intra-session confidence limits being narrower than inter-session measurements. This is true for both glaucomatous and non-glaucomatous groups.

Table 2 shows repeatability and reproducibility estimates (S_W , CR_W , CV_W , and ICC) for the OCTA vessel parameters for non-glaucomatous eyes. S_W and CR_W are absolute measures of agreement that do not depend on the observations constituting the sample. Because various OCTA parameters are measured in different units and magnitude of S_W is not independent of those units, the unitless and standardized CV_W is used to compare the agreement of the various OCTA parameters. For intra-session repeatability among non-glaucomatous eyes, estimates of S_W , CR_W , and CV_W for VAD were 0.015, 0.042 and 4.23%, respectively; meanwhile, estimates for PD were lower with values at 0.006, 0.018, and 1.48%. Estimates of S_W and CR_W for Flux and FI were nearly identical. However, the CV_W for FI (2.01%) was lower than Flux (5.25%). Based on comparisons of CV_W , PD and FI were more reliable than VAD and Flux. For inter-session reproducibility among non-glaucomatous eyes, estimates of S_W , CR_W and CV_W for VAD were 0.024, 0.065 and 6.50%, respectively; and estimates for PD were again lower at 0.009, 0.024 and 2.02%. The inter-session reproducibility measures of S_W and CR_W for Flux and FI were also similar but again CV_W for FI (3.16%) was lower than for Flux (8.03%). When comparing the CV_W , PD and FI were again more reliable than VAD and Flux. The intra-session ICCs ranged from 0.928 to 0.945 and were generally higher than inter-session ICCs, which ranged from 0.811 to 0.866.

The intra-session repeatability was greater than the inter-session reproducibility in all the parameters, based on the CV_W .

Table 3 shows the repeatability and reproducibility estimates of the OCTA vessel parameter measurements for glaucomatous eyes. For the intra-session repeatability among glaucomatous eyes, estimates of S_W , CR_W and CV_W for VAD were 0.025, 0.068 and 8.97%, respectively; estimates for PD were lower at 0.007, 0.019, 1.69%. The estimates of S_W , CR_W , and CV_W for Flux (0.010, 0.027, 10.28%) were greater than that for FI (0.007, 0.020, 2.28%). Based on comparisons of CV_W , PD and FI were more reliable than VAD and Flux. For the inter-session repeatability among glaucomatous eyes, estimates of S_W , CR_W and CV_W for VAD were 0.031, 0.087 and 12.09%, respectively; estimates for PD were again lower at 0.009, 0.025 and 2.28%. While the S_W and CR_W appears to be similar for Flux versus FI, the CV_W of Flux (14.21%) was greater than FI (3.51%). PD and FI were again more reliable than their research-oriented counterpart parameters (VAD and Flux) when comparing the CV_W . The intra-session ICCs ranged from 0.904 to 0.972, and inter-session ICCs ranged from 0.855 to 0.955. Based on the CV_W , intra-session repeatability was greater than inter-session reproducibility on all OCTA parameters. A supplemental analysis of the intra-session repeatability data of glaucomatous eyes stratified by disease severity is included in the supplemental material (see online Supplemental Table S1). No significant trend in repeatability was observed with worsening glaucoma stages. The same analysis on the inter-session reproducibility was not produced due to the lack of eyes and uneven distribution of glaucoma stages.

While not the main purpose of this study, it is useful to make some comparisons between the non-glaucomatous and glaucomatous groups. When comparing intra-session repeatability for the non-glaucomatous versus glaucomatous groups, the glaucomatous group is less repeatable, with CV_W approximately twice the value of that in the non-glaucomatous group for the two custom, research-oriented OCTA parameters (VAD CV_W of 4.23% vs 8.97% and Flux CV_W of 5.25% vs 10.28% in non-glaucomatous and glaucomatous groups, respectively). In contrast, the intra-session CV_W for the two commercially developed, clinic oriented OCTA parameters are very similar between the two groups (PD CV_W of 1.48% vs 1.69% and FI CV_W of 2.01% vs 2.28% in non-glaucomatous vs glaucomatous groups respectively). 6 months inter-session reproducibility data for the non-glaucomatous group and 12 months inter-session reproducibility data for the glaucomatous group are included in the supplemental material (online Supplementary Tables S2 and S3, respectively <https://bjo.bmj.com/>). When comparing the inter-session agreement measures of each OCTA vessel parameter between the non-glaucomatous versus the glaucomatous group, our results show that PD had the highest reproducibility and were similar between groups, with CV_W ranging from 2.02% to 2.06% in the non-glaucomatous group and 2.21% to 2.28% in the glaucomatous group. This was followed by FI with CV_W ranging from 3.16% to 3.88% and 3.44% to 3.51% for non-glaucomatous and glaucomatous eyes, respectively. The two custom research-oriented OCTA parameters, VAD and Flux, had greater differences in CV_W between the non-glaucomatous and glaucomatous groups, with greater reproducibility for the non-glaucomatous group (VAD CV_W ranging from 6.50% to 7.90% in non-glaucomatous group vs 12.09% to 13.29% in glaucomatous group; Flux

CV_W ranging from 8.03% to 9.66% in non-glaucomatous group vs 14.21% to 16.01% in glaucomatous group).

Table 4 shows the linear mixed effects model used to assess whether signal strength is associated with the reliability of OCTA measurements. Signal strength had a significantly positive association with OCTA vessel parameters for both non-glaucomatous and glaucomatous groups with a *p*-value of <0.0001.

Discussion

In the current study, we compared the intra-session repeatability versus inter-session reproducibility of OCTA measurements of the peripapillary region for both non-glaucomatous eyes and glaucomatous eyes. Furthermore, we compared the agreement measures of vessel parameters from two different quantification software programs (custom research oriented and commercially developed, clinic oriented) and found that OCTA vessel parameters were highly repeatable and reproducible for both non-glaucomatous and glaucomatous groups. However, measurements were even more reliable with the commercially-developed quantification software currently available outside the United States.

By studying repeatability and reproducibility of a given device and software, we can then determine whether a difference in OCTA parameter measurements on a given patient is clinically significant or within the established and accepted “noise” of the technology and patients’ daily physiologic variation. The inter-session CR_W is the best measure for this assessment, as it represents the estimated range within which the difference between any two repeated measurements for a given eye will fall 95% of the time. For example, among the non-glaucomatous eyes, difference in the commercially-developed PD for any two measurements greater than the inter-session CR_W of 0.024 could start to be considered clinically meaningful. Among the glaucomatous eyes, PD differences greater than the inter-session CR_W of 0.025 could start to be considered clinically meaningful.

The results of our study were supported by past studies investigating the repeatability and reproducibility of OCTA parameters of the peripapillary region. Past studies reported an intra-session CV_W for peripapillary vessel density ranging from 1.3 to 6.8 % among non-glaucomatous eyes and 1.8 to 6.6 % among glaucomatous eyes.^{9–19} Although, these studies were predominantly on small samples, our intra-session CV_W estimates are comparable with 4.3% for VAD and 1.5% for PD in the non-glaucomatous group, and 9.0% for VAD and 1.7% for PD in the glaucomatous group. A recent study by Mastropasqua et al. reported similar intra-session repeatability measurements of healthy eyes compared to our non-glaucomatous group, despite a much younger population (the inter-session reproducibility was not compared due to different statistical analysis methods used). Prior studies on inter-session reproducibility had imaging sessions that occurred within 3 months, which is 2 to 4 times sooner than in our study: 6 months for glaucomatous eyes and 1 year for non-glaucomatous eyes. The larger time frame in our study is more representative of the time frame that we would be repeating imaging on patients in a real clinic setting, but it may explain the lower reproducibility compared to some prior studies. For peripapillary vessel

density, these studies reported inter-session CV_W of 0.9 to 4.3% among non-glaucomatous eyes and 3.0 to 6.9 % for glaucomatous eyes.^{9–13} Our inter-session CV_W estimates in the non-glaucomatous group were 6.5% for VAD and 2.0% for PD, and 12.1% for VAD and 2.3% for PD in the glaucomatous group. Additionally, in the supplemental analysis, the repeatability and reproducibility were compared between both the glaucomatous group and the non-glaucomatous groups by providing equal inter-session time intervals. However, this data should be interpreted with caution because: (a) an interval of 1 year may be too long not to expect subclinical progression in the glaucoma group,²⁹ and (b) this analysis yields a smaller group for the 6-month interval for the non-glaucoma group. A longer timeframe between sessions may allow for greater physiologic variation in a given patient's vascular status, but this may also be more realistic since we aim to follow glaucoma patients with OCTA longitudinally for years. Of note, the glaucoma patients in our study were deemed stable by the treating glaucoma specialist without evidence of progression between the visits.

The higher variation of vessel parameters in the inter-session repeatability measurements compared to that for intra-session could result from variation in physiological parameters that we did not measure and thus could not control for. Studies have reported that acute intake of caffeine decreases macular flow and vessel density,²⁰ and acute intake of nicotine decreases macular microcirculation.^{21–22} Other studies reported that exercise acutely decreases peripapillary and parafoveal flow density²³ but has the long-term effect of increasing peripapillary flow density,²⁴ and induced hypertension decreases parafoveal and peripapillary flow density.^{25–26} In comparison, the structural measure of RNFL thickness has been shown to have very high reproducibility^{33–35} even in glaucomatous eyes, with a CV_W of 3.1% reported in a study with 18 months or longer between session in clinically stable glaucoma.³⁴ Our study is consistent with the possibility that inter-session reproducibility of OCTA parameters may be susceptible to variations in an individual's physiologic state. Alternatively, longitudinal studies performed over the course of years will be helpful in determining whether there are stepwise reductions in OCTA parameter values predictive of true clinically meaningful disease progression.

Another agreement estimate measured in this study was the ICC. ICC is perhaps the most commonly used estimate of agreement, and it represents the proportion of total variation that is due to the measurement technique, independent of differences within the study population. ICC is by definition a relative measure, such that for two groups with the same random measurement error but different variances, the group with the greater variability will have a higher ICC. Thus, it is important to note that ICC is expected to be higher in the glaucomatous group, because there is high inter-eye variation due to the wide spectrum of disease, which ranges from mild to severe glaucomatous damage. Therefore, comparing the ICCs between the non-glaucomatous and glaucomatous groups may not be meaningful. When comparing intra-session to inter-session ICCs, however, the inter-session ICCs were generally lower for both glaucomatous and non-glaucomatous groups, signifying more variability in the OCTA images between sessions compared to within the same session. Since this difference was observed in both disease and non-diseased eyes, it suggests differences are due to variations over time that are independent of changes in pathology.

We also evaluated the effect of signal strength of the OCTA scans and found a significant increase of OCTA vessel parameters with an increase in signal strength of the repeated scans for both non-glaucomatous and glaucomatous groups. This finding is consistent with previous studies that reported vessel density was highly associated with signal strength.^{16–17,36–38} Therefore, differences in signal strength should be considered when comparing OCTA parameter measurements longitudinally for a given patient.

Furthermore, we compared the repeatability and reproducibility measurements between a custom research-oriented quantification software and a commercially-developed clinic-oriented quantification software. We found that the commercially-developed quantification software appears to be more repeatable and reproducible, with less difference between the glaucoma and non-glaucoma groups. It is possible that the commercially-developed quantification software could have additional processes that improved data smoothing and noise reduction, with the goal of being more repeatable. Chlebiej *et al.* explained that noise affects the visualization of vessels in OCTA images, and data filtering for noise suppression improved visualization of the retinal vascular networks.³⁹

One of the limitations of the current study is the lack of healthy eyes with no suspicion of glaucoma. Our subject population consisted of patients who visited an academic glaucoma practice and the non-glaucomatous group consisted of many “glaucoma suspects”, including those with unilateral glaucoma in the other eye, in contrast to previous studies in which glaucoma suspects and pre-perimetric glaucoma were included in glaucomatous group.^{9,15} In addition, the study selected images from different sessions within 1 year for non-glaucomatous eyes and 6 months for glaucomatous eyes. It is possible that subclinical glaucomatous changes may have developed in the “non-glaucomatous” group. However, this study was not intended to compare non-glaucomatous and glaucomatous eyes directly, but rather to understand intra-session versus inter-session reliability for each group. Additionally, the selected timeframes are representative of imaging intervals for a true clinical setting, and a supplemental table provides data with the same intersession criteria for the reader’s interest. Another limitation is the glaucoma subtypes and severity were not considered. Out of the 118 glaucomatous eyes, 108 eyes were primary open angle glaucoma. Further studies evaluating the effects of various glaucoma treatments and other physiological changes could further inform our interpretation of OCTA reproducibility over time. In addition, a sub-analysis considering the different glaucoma types and disease severity could provide more insight in using OCTA in clinical settings.

In conclusion, peripapillary OCTA vessel parameter measurements are highly repeatable and reproducible for both non-glaucomatous eyes and glaucomatous eyes. In general, we found intra-session measurements to have greater agreement than inter-session measurements. This may be related to variations in physiological state in both glaucomatous and non-glaucomatous groups, but additional research is needed to investigate this. As previously reported in other studies, signal strength had a significant, independent association with OCTA parameters. Furthermore, the clinically oriented automated quantification software, which is currently only commercially available outside the US, demonstrated a higher degree of repeatability and reproducibility compared to the custom quantification software. Establishing the degree of agreement will allow us to understand the inherent variability in

repeated OCTA measurements. Moving forward, it is critical to consider the repeatability and reproducibility of OCTA quantification software in future OCTA studies that study glaucoma longitudinally and studies that measure the effect of therapeutic interventions on the retinal microvasculature.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1:

Two sample peripapillary optical coherence tomography angiography en face images of the superficial retinal layer from the same patient demonstrating (A) presence of acceptable amount of motion artefact versus (B) significant motion artefact requiring exclusion.

Figure 2: Bland-Altman plots for non-glaucomatous eyes showing the agreement of optical coherence tomography angiography vessel parameters between the repeated images. The mean difference is denoted by the blue line (centre), and the 95% limit of agreement for prediction of a mean difference is denoted by the two red lines (upper and lower).

Figure 3:

Bland-Altman plots for glaucomatous eyes showing the agreement of optical coherence tomography angiography vessel parameters between the repeated images. The mean difference is denoted by the blue line (centre), and the 95% limit of agreement for prediction of a mean difference is denoted by the two red lines (upper and lower).

Table 1.

Participants demographics with OCTA vessel parameters

Variables	Non-glaucomatous		Glaucomatous	
	Intra-session	Inter-session < 365 days	Intra-session	Inter-session < 180 days
	120 eyes from 76 subjects	73 eyes from 48 subjects	118 eyes from 82 subjects	70 eyes from 50 subjects
Sociodemographic variables				
Age (years)	51.97 ± 16.86	51.89 ± 15.80	60.82 ± 13.44	59.91 ± 14.56
Male Gender	33 (43.4)	20 (41.7)	42 (51.2)	25 (50.0)
Systemic variables				
Hypertension	16 (21.1)	9 (18.8)	30(36.6)	16 (32.0)
Diabetes Mellitus	3 (3.9)	1 (2.1)	14 (17.1)	9 (18.0)
Ophthalmic variables				
Glaucoma diagnosis, binary	0	0	82 (100)	50 (100)
Glaucoma, bilateral	0	0	62(75.6)	39 (78.0)
Glaucoma, unilateral	17 (22.4) *	15 (31.2) *	20(24.4)	11 (22.0)
Non-glaucoma, bilateral	59(77.6)	33 (68.8)	0	0
Mild-moderate diabetic retinopathy	0	0	4 (4.9)	3 (6.0)
OCTA Variable				
Vessel Area Density ^a	0.361	0.362	0.275	0.258
Perfusion Density ^b	0.430	0.430	0.397	0.392
Flux ^a	0.129	0.130	0.095	0.090
Flux Index ^b	0.358	0.359	0.319	0.313

All values represented as mean ± standard deviation or frequency (percent)

* Non-glaucomatous eye was used in this analysis

^a Custom segmentation software parameter^b Commercially available segmentation algorithm parameter

Table 2.

Repeatability and reproducibility estimates of peripapillary vessel parameters for NON-GLAUCOMATOUS eyes

OCTA Variable	S _W		CR _W		CV _W *%		ICC	
	Intra-session N= 120 eyes (76 subjects)	Inter-session < 365 days N=73 eyes (48 subjects)	Intra-session N= 120 eyes (76 subjects)	Inter-session < 365 days N=73 eyes (48 subjects)	Intra-session N= 120 eyes (76 subjects)	Inter-session < 365 days N=73 eyes (48 subjects)	Intra-session N= 120 eyes (76 subjects)	Inter-session < 365 days N=73 eyes (48 subjects)
Vessel Area Density ^a	0.015 (0.014, 0.017)	0.024 (0.020, 0.027)	0.042 (0.038, 0.046)	0.065 (0.055, 0.075)	4.23 (3.82, 4.61)	6.50 (5.52, 7.54)	0.931 (0.903, 0.951)	0.811 (0.701, 0.880)
Perfusion Density ^b	0.006 (0.006, 0.007)	0.009 (0.007, 0.010)	0.018 (0.016, 0.019)	0.024 (0.021, 0.028)	1.48 (1.34, 1.62)	2.02 (1.71, 2.31)	0.937 (0.912, 0.955)	0.852 (0.759, 0.904)
Flux ^a	0.007 (0.006, 0.007)	0.010 (0.009, 0.012)	0.019 (0.017, 0.021)	0.029 (0.025, 0.033)	5.25 (4.77, 5.76)	8.03 (6.83, 9.11)	0.928 (0.896, 0.948)	0.816 (0.722, 0.883)
Flux Index ^b	0.007 (0.006, 0.008)	0.011 (0.010, 0.013)	0.020 (0.018, 0.022)	0.031 (0.027, 0.036)	2.01 (1.83, 2.19)	3.16 (2.74, 3.59)	0.945 (0.920, 0.962)	0.866 (0.789, 0.917)

Estimates taken from annular OCTA parameter controlling for inter-eye correlation.

Figures inside parentheses represent 95% confidence limits.

* Estimate represented in %.

^a Custom segmentation software parameter.

^b Commercially developed segmentation algorithm parameter.

ICC, interclass correlation coefficient; S_W, within-eye standard deviation; CR_W, within-eye coefficient of repeatability; CV_W, within-eye coefficient of variation.

Table 3.

Repeatability and reproducibility estimates of peripapillary vessel parameters for GLAUCOMATOUS eyes

OCTA Variable	S_W		CR_W		$CV_W^{* \%}$		ICC	
	Intra-session N= 118 eyes (82 subjects)	Inter-session < 180 days N=70 eyes (50 subjects)	Intra-session N= 118 eyes (82 subjects)	Inter-session < 180 days N=70 eyes (50 subjects)	Intra-session N= 118 eyes (82 subjects)	Inter-session < 180 days N=70 eyes (50 subjects)	Intra-session N= 118 eyes (82 subjects)	Inter-session < 180 days N=70 eyes (50 subjects)
Vessel Area Density^a	0.025 (0.022, 0.027)	0.031 (0.027, 0.035)	0.068 (0.061, 0.076)	0.087 (0.075, 0.098)	8.97 (8.02, 9.86)	12.09 (10.47, 13.68)	0.909 (0.876, 0.936)	0.874 (0.805, 0.920)
Perfusion Density^b	0.007 (0.006, 0.007)	0.009 (0.008, 0.010)	0.019 (0.017, 0.020)	0.025 (0.022, 0.028)	1.69 (1.53, 1.85)	2.28 (1.98, 2.61)	0.972 (0.959, 0.980)	0.955 (0.926, 0.971)
Flux^a	0.010 (0.009, 0.011)	0.013 (0.011, 0.014)	0.027 (0.024, 0.030)	0.035 (0.031, 0.040)	10.28 (9.20, 11.36)	14.21 (12.24, 16.21)	0.904 (0.868, 0.930)	0.855 (0.771, 0.904)
Flux Index^b	0.007 (0.007, 0.008)	0.011 (0.009, 0.012)	0.020 (0.018, 0.022)	0.030 (0.026, 0.034)	2.28 (2.05, 2.51)	3.51 (3.01, 4.01)	0.959 (0.942, 0.970)	0.922 (0.879, 0.950)

Estimates taken from annular OCTA parameter controlling for inter-eye correlation.

Figures inside parentheses represent 95% confidence limits.

* Estimate represented in %.

^a Custom segmentation software parameter.^b Commercially developed segmentation algorithm parameter.ICC, interclass correlation coefficient; S_W , within-eye standard deviation; CR_W , within-eye coefficient of repeatability; CV_W , within-eye coefficient of variation.

Table 4.

Signal Strength association with OCTA parameters

OCTA Variable	Non-Glaucomatous eyes			Glaucomatous eyes		
	Beta (%)	SE*	p-Value	Beta (%)	SE*	p-Value
Vessel Area Density ^a	0.013 (0.009, 0.016)	0.002	0.000	0.020 (0.014, 0.026)	0.003	0.000
Perfusion Density ^b	0.004 (0.003, 0.006)	0.001	0.000	0.005 (0.003, 0.007)	0.001	0.000
Flux ^a	0.006 (0.004, 0.008)	0.001	0.000	0.007 (0.005, 0.010)	0.001	0.000
Flux Index ^b	0.007 (0.006, 0.009)	0.001	0.000	0.008 (0.006, 0.010)	0.001	0.000

Linear mixed effects model of signal strength on OCTA vessel parameters controlling for inter-eye correlation.

Figures inside parentheses represent 95% confidence limits.

*
Standard error

^aCustom segmentation software parameter.

^bCommercially available developed segmentation algorithm parameter.