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Intrasession Repeatability of OCT Angiography Parameters in Neurodegenerative Disease

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Purpose: To assess the intrasession repeatability of macular OCT angiography (OCTA) parameters in Alzheimer's disease (AD), mild cognitive impairment (MCI), Parkinson's disease (PD), and normal cognition (NC).

Design: Cross sectional study.

Subjects: Patients with a clinical diagnosis of AD, PD, MCI, or NC were imaged. Images with poor quality and of those with diabetes mellitus, glaucoma, or vitreoretinal disease were excluded from analysis.

Methods, Intervention or Testing: All participants were imaged using the Zeiss Cirrus HD-5000 with AngioPlex (Carl Zeiss Meditec, Software Version 11.0.0.29946) and repeat OCTA images were obtained for both eyes. Perfusion density (PFD), vessel density (VD), and Foveal avascular zone (FAZ) area were measured from 3 × 3 mm and 6 × 6 mm OCTA images centered on the fovea using an ETDRS grid overlay.

Main Outcome Measures: Intraclass correlation coefficients were used to quantify repeatability of PFD, VD, and FAZ area measurements obtained from imaging.

Results: 3 × 3 mm scans of 22 AD, 40 MCI, 21 PD, and 26 NC participants and 6 × 6 mm scans of 29 AD, 44 MCI, 29 PD, and 30 NC participants were analyzed. Repeatability values ranged from 0.64 (0.49−0.82) for 6 × 6 mm PFD in AD participants to 0.87 (0.67−0.92) for 3 × 3 mm PFD in AD participants. No significant differences were observed in repeatability between NC participants and those with neurodegenerative disease.

Conclusions: Overall, similar OCTA repeatability was observed between NC participants and those with neurodegeneration. Regardless of diagnostic group, macular OCTA metrics demonstrated moderate to good repeatability.

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OCT angiography (OCTA) has been established as a rapid, noninvasive method for visualizing the retinal microvasculature[1] and is increasingly being used to investigate retinal microvascular changes in neurodegenerative diseases including Alzheimer's disease (AD),[2,3] mild cognitive impairment (MCI),[4,5] and Parkinson's disease (PD).[6,7] Alzheimer's disease results from neuronal accumulation of amyloid-beta leading to progressive neurocognitive decline.[8] Alzheimer's disease is characterized by memory loss, cognitive impairment, and changes in personality and behavior.[9] Unlike AD, PD is characterized by muscle rigidity, tremor, and retardation of movement due to loss of dopamine-producing neurons in the striatum, although memory impairment and cognitive decline are common features of both diseases.[9] Mild cognitive impairment is defined clinically as a disease where cognitive decline outpaces what one would normally attribute to age-related decline.[10,11] However, in distinction from both AD and PD, MCI does not result in significant impairment of a patient's activities of daily living.[11,12] The cerebral and retinal microvasculature share a common embryonic origin, suggesting that retinal changes may mirror cerebral changes in these disease processes.[2,13] Our group previously demonstrated the utility of OCTA to identify retinal microvascular changes in AD compared to individuals with normal cognition (NC).[14]

While OCTA is a promising technology, reliability of measurements can be affected by various patient factors including ocular media opacity, scan signal strength, imaging artifact, and level of cooperation during the imaging process.[15] The potential for variation in OCTA measurements may affect its widespread clinical adoption.[16] Thus, studies validating the repeatability of OCTA measurements are essential.[17,18]

Although prior studies have evaluated the repeatability of OCTA parameters in individuals with NC as well as patients

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with various ocular diseases,[16,17,19] there have been no prior studies assessing macular OCTA repeatability in patients with neurodegenerative disease. Given the cognitive symptoms associated with neurodegenerative diseases such as AD and MCI, affected patients may have more difficulty following commands and fixating during image acquisition. Additionally, visual disturbances affecting contrast sensitivity, ocular movements, peripheral vision,[20] and head tremor in PD, all of which could possibly lead to difficulty with fixation, might affect OCTA image quality and thus, repeatability. In this study, we report the intrasession repeatability of macular OCTA parameters in patients with AD, MCI, and PD compared to control participants with NC.

Methods

This cross-sectional study (Clinicaltrials.gov identifier NCT03233646) was approved by the Duke Health Institutional Review Board (Pro00082598) and followed the guidelines of the Declaration of Helsinki. All aspects of the study complied with the Health Insurance Portability and Accountability Act of 1996, and written informed consent was obtained from all participants (or their legally authorized representative) before enrollment into the study.

Study Participants

Patients aged $\geq$ 50 years with a diagnosis of AD, MCI, or PD were enrolled at the Duke Neurological Disorders Clinic and the Eye Multimodal Imaging in Neurodegenerative Disease research group. Clinical diagnosis of AD and MCI followed criteria established by the National Institute on Aging/Alzheimer's Association,[21–23] and of PD, the International Parkinson and Movement Disorder Society clinical criteria.[24,25] Diagnoses of AD, MCI, or PD in all study participants were confirmed using the aforementioned guidelines by experienced neurologists (A.J.L., K.P.L.M.).[23] Positron emission tomography and cerebrospinal fluid sampling were not performed as part of the standard of clinical care, and postmortem histopathological diagnosis confirmation was not possible in this cohort of living patients. Control participants were age 50 or older, did not have a movement disorder or neurodegenerative disease, and had a normal Mini Mental State Examination cognitive score at study enrollment without any documented history of cognitive impairment. Participants were excluded from the study if they had a prior diagnosis of diabetes mellitus, uncontrolled hypertension, glaucoma or glaucoma suspect, vitreoretinal pathology, and/or corrected ETDRS visual acuity worse than 20/40 as measured by study staff at the time of image acquisition. In addition, patients with a history of intraocular surgery (with the exception of cataract surgery) as well as patients with refractive errors (spherical equivalent) $\geq$ +6.0 diopters (D) or −6.0 D were also excluded. Ocular history was obtained using a variety of modalities including initial screening for ophthalmic and systemic conditions by study staff, electronic health record review when available, and assessment using OCT (Cirrus HD-5000, Carl Zeiss Meditec) and ultrawide-field fundus images (California, Optos) to screen for detectable macular and vitreoretinal pathology. Corrected Snellen visual acuity and cognitive function (Mini Mental State Examination) were measured for all study participants on the day of image acquisition. Years of education starting at the first grade were recorded for each patient.

OCTA Image Acquisition

All participants were imaged using the Zeiss Cirrus HD-5000 with AngioPlex (Carl Zeiss Meditec, Software Version 11.0.0.29946) and OCTA images were obtained for both eyes. On average, OCTA imaging was tried about 3 times for each patient, but it varied depending upon patient cooperation and fatigue. Perfusion density (PFD) and vessel density (VD) were measured from 3 × 3 mm and 6 × 6 mm OCTA images centered on the fovea using an ETDRS grid overlay. Zeiss AngioPlex software (Version 11.0.0.29946) was utilized to obtain PFD, VD, and Foveal avascular zone (FAZ) area measurements, as previously described by our group,[14] within regions of interest delineated by the ETDRS circles and rings. For 3 × 3 mm images, the 3 mm ETDRS circle and ring were used as regions of interest; for 6 × 6 mm images, the 6 mm ETDRS circle, inner ring, and outer ring were used as regions of interest. Foveal avascular zone area (mm^2) was calculated for 3 × 3 mm OCTA images using automated segmentation performed by Zeiss AngioPlex software and manually reviewed and corrected (D.S.G.) in instances of segmentation error. Perfusion density was defined as a percentage of area of perfused vasculature per unit area in the region of interest. Vessel density was defined as the total length of perfused vasculature per unit area in the region of interest. Images were manually assessed by experienced study staff (D.A., C.B.R., D.S.G., S.S.) for evidence of image exclusion criteria, which included motion artifact, poor scan quality (< 7/10 signal strength index), segmentation artifact, poor centration, and focal signal loss. Images that failed to pass quality exclusion criteria were excluded from statistical analysis.

Statistical Analysis

All statistical analyses were completed by an experienced statistician (S.S.S.). *P*-values for continuous variables based on Kruskal-Wallis test of difference among medians was employed to assess for significant differences between groups with regards to demographics. Intrasession repeatability was assessed using intraclass correlation (ICC) analysis, which describes the overall correlation between individual intrasession measures. Koo and Li's guidelines for interpreting ICC values were used to report a subjective classification of measurement reliability (< 0.5—poor, $\geq$ 0.5 and < 0.75—moderate, $\geq$ 0.75 and < 0.9—good, $\geq$ 0.9—excellent).[26] Bland-Altman analysis generated limits of agreement for average PFD, VD, and FAZ area measurements.

Computation of Intraclass Correlation

A random effects model, fit with SAS version 9.4 and the General Linear Model procedure, was used to compute the within subject and between subject variability separately for each diagnosis. Each measurement was used as the outcome variable in a model with only subject, which was classified as a random variable.

The model was defined as $y_{ij} = \mu + \alpha_i + e_{ij}, i = 1\ldots k,$ and $j = 1\ldots n_i$ where

$y_{ij} = j^{th}$ replicate for the i^{th} subject.

α_i is a random variable representing between-subject variability

e_{ij} is a random variable representing within-subject variability

The within-subject variance (WIT) which equals the mean square error (MSE) was derived from the error term from the model as the MSE, which was the sum of squares for error divided by the degrees of freedom for error, σ^2.

The sum of squares for the model divided by its degrees of freedom was the mean square between subjects, $\sigma_\alpha{}^2$. Then the between subject variance (BET) was computed as (MSB-MSE)/2,

where 2 is the number of observations (eyes) for each subject (MSB = mean square between subjects).

Finally, ICC was computed as max [BET/(BET + WIT), 0].

In this model, the test statistic F = Between Mean Square/Within Mean Square follows an $F_{k-1,\ N-k}$ distribution and is used to test the hypothesis H_0: $\sigma_\alpha^2 = 0$ vs. H_1: $\sigma_\alpha^2 > 0$.

An approximate 2-sided 100% × (1 − α) confidence interval (CI) for the ICC is given by (c_1, c_2) where

$$c_1 = \max\left\{\frac{F/F_{K-1,N-K,1-\alpha/2} - 1}{n + F/F_{K-1,N-K,1-\alpha/2} - 1}, 0\right\} \text{and}$$

$$c_2 = \max\left\{\frac{F/F_{K-1,N-K,\alpha/2} - 1}{n + F/F_{K-1,N-K,\alpha/2} - 1}, 0\right\}$$

where F is the F statistic from the significance test of the described above and n is the number of replicates.

Results

After image quality exclusion of those who underwent 3 × 3 mm OCTA imaging, 27 eyes of 22 AD participants, 51 eyes of 40 MCI participants, 32 eyes of 21 PD participants, and 44 eyes of 26 NC participants with repeated intrasession image acquisition were analyzed. After image quality exclusion of those who underwent 6 × 6 mm OCTA imaging, 41 eyes of 29 AD participants, 61 eyes of 44 MCI participants, 44 eyes of 29 PD participants, and 46 eyes of 30 participants with NC were analyzed. For 3 × 3 mm imaging, 772 images out of a total of 1080 images were excluded due to our exclusion criteria. This resulted in a total of 308 included images. For 6 × 6 mm imaging, 687 images out of a total of 1071 images were excluded due to our exclusion criteria. This resulted in a total of 384 included images. After exclusion criteria were applied, the majority of included images for each group had a strength signal index (SSI; 0−10 point scale) ≥ 9 (Fig S1). No images with a SSI < 8 were included for analysis. For 3 × 3 mm images, a SSI ≥ 9 was demonstrated in 94.4% of AD participants, 99% of MCI participants, 98.4% of PD participants, 98.8% of NC participants, and 98% of all participants overall (Fig S1). For 6 × 6 mm participants, similar percentage breakdowns were observed with a SSI

Table 1. Demographic Data for Study Participants

Variable	Statistic	Alzheimer's Disease	Mild Cognitive Impairment	Parkinson's Disease	Normal Cognition	*P* Value*
3 × 3 mm Macular OCTA						
Age (Years)	N	22	40	21	26	0.552
	Mean (SD)	70.27 (8.27)	70.89 (7.21)	67.71 (8.96)	68.82 (7.12)	
	Min, Median, Max	53.0, 70.2, 85.0	53.0, 72.0, 89.0	50.0, 68.0, 82.0	53.0, 69.0, 79.0	
Visual Acuity (logMAR)	N	22	40	21	26	**0.024**
	Mean	0.20 (20/32)	0.14 (20/28)	0.15 (20/28)	0.12 (20/26)	
	SD	0.10	0.09	0.10	0.08	
	Min, Median, Max	0.0, 0.2, 0.3	0.0, 0.1, 0.3	0.0, 0.2, 0.3	0.0, 0.1, 0.3	
MMSE	N	22	39	21	25	**< 0.001**
	Mean (SD)	22.86 (4.11)	26.36 (3.92)	29.14 (1.39)	29.16 (1.60)	
	Min, Median, Max	15.0, 24.0, 28.0	8.0, 27.0, 30.0	25.0, 30.0, 30.0	25.0, 30.0, 30.0	
Years of Education	N	22	40	21	26	**< 0.001**
	Mean (SD)	15.95 (2.59)	14.93 (2.39)	16.43 (3.28)	17.50 (2.00)	
	Min, Median, Max	12.0, 16.0, 22.0	12.0, 15.5, 22.0	12.0, 16.0, 23.0	12.0, 18.0, 22.0	
Male Sex	N (%)	8 (36)	18 (45)	9 (43)	10 (38)	0.907
6 × 6 mm Macular OCTA						
Age (Years)	N	29	44	29	30	0.718
	Mean (SD)	71.06 (8.67)	70.62 (7.01)	68.10 (7.85)	69.55 (7.26)	
	Min, Median, Max	53.0, 70.3, 87.0	53.0, 70.9, 89.0	50.0, 72.0, 82.0	53.0, 69.5, 80.0	
Visual Acuity (logMAR)	N	29	44	29	30	0.231
	Mean	0.17 (20/30)	0.15 (20/28)	0.17 (20/30)	0.12 (20/26)	
	SD	0.10	0.11	0.10	0.09	
	Min, Median, Max	0.0, 0.2, 0.3	0.0, 0.1, 0.3	0.0, 0.2, 0.3	0.0, 0.1, 0.3	
MMSE	N	29	44	29	30	**< 0.001**
	Mean (SD)	22.38 (4.08)	26.27 (4.26)	28.21 (2.87)	29.13 (1.48)	
	Min, Median, Max	14.0, 23.0, 28.0	8.0, 27.0, 30.0	18.0, 29.0, 30.0	25.0, 30.0, 30.0	
Years of Education	N	29	44	29	30	**< 0.001**
	Mean (SD)	15.52 (2.64)	14.82 (3.16)	16.48 (2.96)	17.67 (1.94)	
	Min, Median, Max	12.0, 16.0, 21.0	0.0, 16.0, 22.0	12.0, 16.0, 23.0	12.0, 18.0, 22.0	
Male Sex	N (%)	10 (34)	20 (45)	17 (59)	13 (43)	0.336

logMAR = logarithm of the minimum angle of resolution; MMSE = Mini Mental State Exam; OCTA = OCT angiography; SD = standard deviation. Bolded values indicate *P* values < 0.05 (which was our threshold for statistical significance, and any *P* value lower than 0.05 is statistically significant).
**P* value for continuous variables based on Kruskal-Wallis test of difference among medians. *P* value for sex based on Fisher exact test.

Table 2. Intraclass Correlation for 3 × 3 mm Macular OCTA Parameters

3 × 3 mm OCTA	Variable	N	Intraclass Correlation	Lower Confidence Limit	Upper Confidence Limit
Alzheimer's Disease	FAZ Area	25	0.99	0.99	1.00
	Perfusion Density				
	Circle	27	0.87	0.67	0.92
	Ring	27	0.85	0.63	0.91
	Vessel Density				
	Circle	27	0.82	0.64	0.91
	Ring	27	0.79	0.59	0.90
Mild Cognitive Impairment	FAZ Area	42	0.97	0.94	0.98
	Perfusion Density				
	Circle	51	0.67	0.44	0.78
	Ring	51	0.64	0.38	0.75
	Vessel Density				
	Circle	51	0.70	0.53	0.82
	Ring	51	0.64	0.45	0.78
Parkinson's Disease	FAZ Area	29	0.99	0.98	0.99
	Perfusion Density				
	Circle	32	0.71	0.37	0.80
	Ring	32	0.71	0.39	0.81
	Vessel Density				
	Circle	32	0.68	0.44	0.83
	Ring	32	0.69	0.46	0.84
Normal Cognition	FAZ Area	42	0.98	0.98	1.00
	Perfusion Density				
	Circle	44	0.75	0.61	0.86
	Ring	44	0.75	0.60	0.86
	Vessel Density				
	Circle	44	0.74	0.57	0.85
	Ring	44	0.72	0.54	0.84

FAZ = Foveal avascular zone; OCTA = OCT angiography.
From top to bottom: AD (Alzheimer's disease), MCI (mild cognitive impairment), PD (Parkinson's disease), and normal cognition.

$\geq$ 9 demonstrated in 98.8% of AD participants, 96.7% of MCI participants, 96.6% of PD participants, 98.9% of NC participants, and 97.6% of all participants overall (Fig S1).

Demographic data for all study participants are reported in Table 1. For patients who underwent 3 × 3 mm OCTA imaging, average age in years (± standard deviation) for AD = 70.27 ± 8.27, MCI = 70.89 ± 7.21, PD = 67.71 ± 8.96, and NC = 68.82 ± 7.12. For patients that underwent 6 × 6 mm OCTA imaging, average age in years (± standard deviation) for AD patients = 71.06 ± 8.67, MCI = 70.62 ± 7.01, PD = 68.10 ± 7.85, and NC = 69.55 ± 7.26. No significant differences in mean age or sex were observed among or between groups ($P > 0.05$). However, a significant difference in visual acuity ($P = 0.024$) was observed between groups (mean visual acuity AD = 20/32, MCI = 20/28, PD = 20/28, and NC = 20/26) in participants who underwent 3 × 3 mm imaging with worse visual acuity noted in individuals with AD compared with other groups.

For 3 × 3 mm scans, ICC values for PFD, VD, and FAZ area are reported in Table 2. Repeatability of PFD and VD ranged from moderate (ICC of 3 mm ring PFD and VD = 0.64, MCI) to good (ICC of 3 mm circle PFD = 0.87, AD) repeatability, regardless of diagnostic group. FAZ area for all groups demonstrated excellent repeatability (ICC range 0.97−0.99). No significant differences in ICC values were observed among or between the majority of groups. There was a statistically significant difference in ICC values in FAZ area between AD (ICC 0.99; CI: 0.99−1.00) and MCI (ICC 0.97; 0.94−0.98) participants due to nonoverlapping CIs. However, both demonstrated excellent repeatability (ICC > 0.90).

For 6 × 6 mm images, ICC values for PFD and VD are reported in Table 3. Similar to 3 × 3 mm imaging, the majority of 6 × 6 mm OCTA variables ranged from moderate (ICC of 6 mm outer ring PFD and inner ring VD = 0.56, NC) to good (ICC of 6 mm circle PFD = 0.85, MCI) repeatability, regardless of diagnostic group. No significant differences in repeatability were observed among or between groups. Box plots of ICC values for all groups for both 3 × 3 mm and 6 × 6 mm OCTA scan types are displayed in Figure 2.

In order to assess potential confounding by patient age in our analysis, ICC values were also calculated in subgroups stratified by age (< 70 and $\geq$ 70 years) for each diagnosis. Intraclass correlation values for 3 × 3 mm OCTA scans are reported in Table 4, and 6 × 6 mm OCTA scans are reported in Table 5. The repeatability of FAZ area in 3 × 3 mm scans of MCI participants was found to be significantly higher in younger (< 70 years) than older patients ($\geq$ 70 years); however, both age groups showed excellent repeatability (ICC = 0.99 < 70 years; ICC = 0.93 $\geq$ 70 years).

Table 3. Intraclass Correlation for 6 × 6 mm Macular OCTA Parameters

6 × 6 mm OCTA	Variable	N	Intraclass Correlation	Lower Confidence Limit	Upper Confidence Limit
Alzheimer's Disease	Perfusion Density				
	Circle	41	0.64	0.49	0.82
	Inner Ring	41	0.75	0.57	0.85
	Outer Ring	41	0.64	0.43	0.79
	Vessel Density				
	Circle	41	0.73	0.55	0.85
	Inner Ring	41	0.75	0.58	0.86
	Outer Ring	41	0.69	0.49	0.82
Mild Cognitive Impairment	Perfusion Density				
	Circle	61	0.85	0.67	0.87
	Inner Ring	61	0.70	0.55	0.81
	Outer Ring	61	0.83	0.67	0.86
	Vessel Density				
	Circle	61	0.82	0.71	0.89
	Inner Ring	61	0.70	0.55	0.81
	Outer Ring	61	0.82	0.71	0.89
Parkinson's Disease	Perfusion Density				
	Circle	44	0.78	0.59	0.86
	Inner Ring	44	0.75	0.65	0.88
	Outer Ring	44	0.78	0.50	0.82
	Vessel Density				
	Circle	44	0.73	0.55	0.84
	Inner Ring	44	0.77	0.61	0.87
	Outer Ring	44	0.67	0.47	0.80
Normal Cognition	Perfusion Density				
	Circle	46	0.71	0.45	0.79
	Inner Ring	46	0.60	0.33	0.73
	Outer Ring	46	0.56	0.42	0.77
	Vessel Density				
	Circle	46	0.69	0.51	0.82
	Inner Ring	46	0.56	0.32	0.73
	Outer Ring	46	0.68	0.49	0.81

OCTA = OCT angioraphy. From top to bottom: AD (Alzheimer's disease), MCI (mild cognitive impairment), PD (Parkinson's disease), and normal cognition.

Overall, all diagnostic groups demonstrated excellent repeatability of FAZ area measurements (ICC $>$ 0.90) (Table 4). No significant differences were observed between or among older and younger individuals in all diagnostic groups with regard to PFD or VD (Tables 4 and 5).

Bland-Altman analysis was used to assess limits of agreement for each OCTA parameter (Figs S3 and S4). Generally, mean differences between scan pairs for both 3 × 3 mm and 6 × 6 mm OCTA images were small, with few measurement pairs lying outside the limits of agreement (Figs S3 and S4).

Discussion

Several OCTA repeatability studies have investigated the repeatability of peripapillary OCTA parameters,[27–29] and this study is one of the first to assess macular OCTA repeatability across a variety of neurodegenerative diseases. In this study, we found that individuals with neurodegenerative disease and individuals with NC had similar repeatability metrics for the majority of OCTA parameters. However, repeatability was generally higher for FAZ area compared to PFD and VD across all diagnostic groups. In addition, repeatability was generally similar for younger (age $<$ 70) and older patients (age $\geq$ 70) across all diagnostic groups among the majority of OCTA parameters. Prior studies have shown that individuals with neurodegenerative disease often have decreased microvascular density compared to those with NC;[4,14] however, until now, the repeatability of these measurements had not been assessed in neurodegenerative diseases and compared among diagnostic groups. In this study, we conclude that the overall repeatability of macular OCTA was moderate to good and, notably, repeatability was not worse in individuals with neurodegenerative disease compared to NC across all imaging parameters. Our results of the cohort with NC are similar to the findings of another study by Yang et al[30] investigating macular OCTA repeatability in patients with NC. Yang et al[30] reported moderate and good repeatability of VD in 3 × 3 mm scans and 6 × 6 mm scans, respectively, using the Zeiss Cirrus HD-5000.

Overall, moderate to good intrasession repeatability was observed for most macular OCTA parameters regardless of diagnostic group or scan type (3 × 3 mm versus 6 × 6 mm).

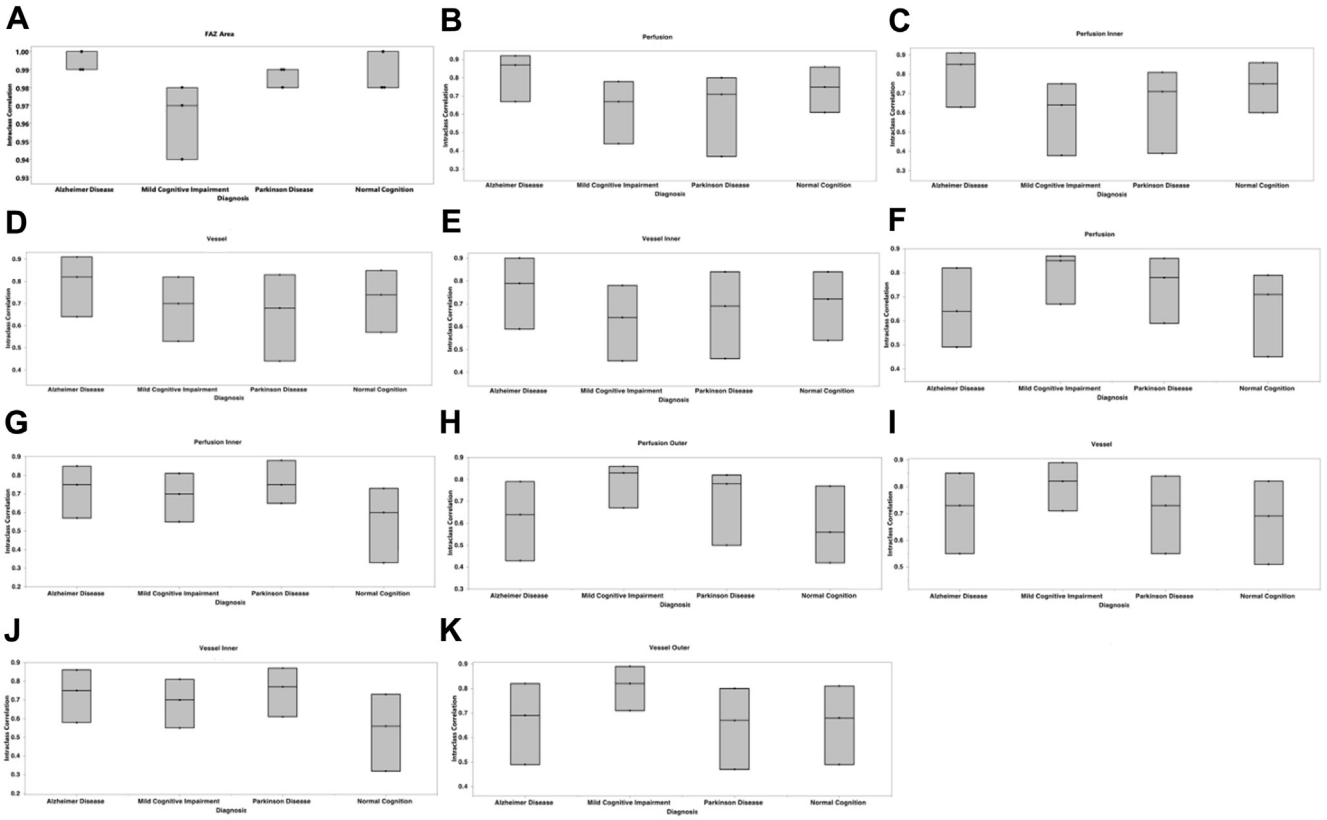

Figure 2. Box plot of intraclass correlation for 3 × 3 mm and 6 × 6 mm OCT angiography parameters; panels (**A**–**E**) (3 × 3 mm): foveal avascular zone (FAZ), perfusion density circle, perfusion density ring, vessel density circle, vessel density ring; panels (**F**–**K**) (6 × 6 mm): perfusion density circle, perfusion density inner ring, perfusion density outer ring, vessel density circle, vessel density inner ring, vessel density outer ring.

The only exception was for FAZ area which had excellent repeatability across all patient subgroups. One could hypothesize that individuals with neurodegenerative disease would have decreased intrasession repeatability of OCTA imaging given that cognitive and motor symptoms may interfere with image acquisition.[15] However, individuals with NC in our study showed ICC values similar to those observed in patients with neurodegenerative disease. These results support the further development of OCTA in identification of earlier stages of neurodegenerative disease which may not precede any overt cognitive or motor deficits. Scan quality significantly impacts repeatability,

Table 4. ICC and CIs for 3 × 3 mm OCT Angiography Parameters (FAZ, Perfusion Density Circle, Perfusion Density Ring, Vessel Density Circle, Vessel Density Ring) Stratified by Age

	Alzheimer's Disease		Mild Cognitive Impairment		Parkinson's Disease		Normal Cognition	
	ICC	*95% CI*	*ICC*	*95% CI*	*ICC*	*95% CI*	*ICC*	*95% CI*
Age < 70								
FAZ area	1.00	0.98–1.00	0.99	0.98–1.00	1.00	0.98–1.00	0.99	0.98–1.00
Perfusion Circle	0.20	0.00–0.76	0.60	0.28–0.83	0.67	0.45–0.91	0.60	0.46–0.86
Perfusion Ring	0.20	0.00–0.74	0.50	0.17–0.78	0.71	0.44–0.91	0.67	0.55–0.89
Vessel Circle	0.33	0.00–0.76	0.70	0.39–0.86	0.72	0.37–0.89	0.60	0.29–0.80
Vessel Ring	0.19	0.00–0.69	0.60	0.25–0.82	0.69	0.32–0.88	0.64	0.33–0.82
Age ≥ 70								
FAZ area	0.99	0.98–1.00	0.93	0.85–0.97	0.98	0.96–0.99	1.00	0.97–1.00
Perfusion Circle	0.89	0.72–0.96	0.69	0.35–0.80	0.43	0.01–0.78	0.82	0.91–0.88
Perfusion Ring	0.88	0.67–0.95	0.64	0.27–0.77	0.50	0.06–0.80	0.78	0.43–0.88
Vessel Circle	0.84	0.62–0.94	0.68	0.44–0.84	0.56	0.12–0.82	0.81	0.57–0.92
Vessel Ring	0.81	0.54–0.93	0.62	0.34–0.80	0.62	0.21–0.85	0.75	0.47–0.89

CI = confidence interval; FAZ = foveal avascular zone; ICC = intraclass correlation.

Table 5. ICC and CIs for 6 × 6 mm OCT Angiography Parameters (Perfusion Density Circle, Perfusion Density Inner Ring, Perfusion Density Outer Ring, Vessel Density Circle, Vessel Density Inner Ring, Vessel Density Outer Ring) Stratified by Age

	Alzheimer's Disease		Mild Cognitive Impairment		Parkinson's Disease		Normal Cognition	
	ICC	*95% CI*	*ICC*	*95% CI*	*ICC*	*95% CI*	*ICC*	*95% CI*
Age < 70								
Perfusion Circle	0.75	0.31–0.86	0.60	0.36–0.83	0.71	0.31–0.83	0.20	0.00–0.63
Perfusion Inner	0.82	0.59–0.93	0.67	0.28–0.81	0.64	0.35–0.84	0.33	0.00–0.60
Perfusion Outer	0.60	0.19–0.82	0.62	0.30–0.81	0.67	0.22–0.80	0.33	0.00–0.66
Vessel Circle	0.77	0.50–0.91	0.69	0.42–0.85	0.52	0.14–0.76	0.48	0.13–0.73
Vessel Inner	0.82	0.59–0.93	0.62	0.31–0.82	0.66	0.34–0.84	0.32	0.00–0.63
Vessel Outer	0.72	0.40–0.88	0.67	0.38–0.84	0.43	0.02–0.71	0.51	0.16–0.74
Age ≥ 70								
Perfusion Circle	0.69	0.31–0.86	0.87	0.71–0.91	0.80	0.59–0.91	0.80	0.61–0.93
Perfusion Inner	0.70	0.59–0.93	0.87	0.67–0.90	0.89	0.68–0.94	0.57	0.27–0.83
Perfusion Outer	0.67	0.19–0.82	0.78	0.62–0.89	0.78	0.45–0.87	0.80	0.54–0.91
Vessel Circle	0.68	0.50–0.91	0.86	0.74–0.92	0.79	0.56–0.91	0.81	0.59–0.92
Vessel Inner	0.71	0.59–0.93	0.77	0.60–0.88	0.81	0.59–0.91	0.59	0.21–0.81
Vessel Outer	0.65	0.40–0.88	0.84	0.71–0.92	0.72	0.45–0.87	0.79	0.56–0.91

CI = confidence interval; ICC = intraclass correlation.

and rigorous scan quality criteria with exclusion of poor-quality images is important to minimize the impact of scan quality on our repeatability data.

When stratifying patients by age, we observed significantly higher repeatability of FAZ area measurements in younger (< 70) than in older (≥ 70) individuals, specifically in individuals with MCI. This may be partly attributed to more advanced cognitive decline in older individuals with cognition-impairing conditions like MCI. But it is important to note that despite significantly higher repeatability metrics in younger MCI participants when compared to older participants, both groups still demonstrated excellent repeatability (ICC > 0.9). We did not observe similar differences in PFD or VD measurements (3 × 3 mm or 6 × 6 mm) between younger and older individuals.

Hong et al[31] investigated intrasession repeatability in elderly patients with NC using swept-source 3 × 3 mm OCTA centered on the fovea and found good to excellent repeatability for PFD and VD (ICC > 0.79; CI 0.58–0.95). Given our large CIs and findings of moderate to good repeatability for most OCTA metrics, their results do not significantly differ from ours herein. However, in their study, swept-source OCTA (PLEX Elite 9000 SS-OCTA) was utilized, with a different scan protocol in contrast to our study which utilized spectral-domain OCTA[31]. These differences in imaging device and scan protocols may contribute to the differences in the observed repeatability between the 2 studies. Indeed, a systematic review conducted by Rifai et al[32] found that, in general, heterogeneity in the different types of OCTA machines and scan algorithms serves as an obstacle limiting comparison among different OCTA studies. As a result, greater effort needs to be made toward standardization of scan type, scan metrics, and OCTA processing algorithms to allow for more accurate comparisons across studies.[32–34]

The variation among available studies aligns with the observations of Rifai et al[32] and further supports the understanding that OCTA repeatability may be dependent on instrumentation variables including image device, scan protocol, and segmentation algorithm. Future studies assessing the repeatability of these metrics in patients with neurodegenerative disease across various commercially available software programs, segmentation algorithms, and OCTA devices would be beneficial to determine which factors may help to further optimize repeatability of macular OCTA.

Our study does have some limitations. First, we did not employ a severity scale in our classification of patients with PD; however, our study population was not large enough for analysis once categorized. However, patients with more advanced neurodegenerative disease may not be able to cooperate or sit for high-quality OCTA scans that would meet inclusion criteria for analysis, especially with the increased prevalence of debilitating motor and cognitive dysfunction in patients with advanced neurodegenerative disease. In addition, once neurodegenerative disease is more advanced, multimodal retinal imaging as a diagnostic tool is less likely to be necessary. It is in the earlier stages of disease, when the neurodegenerative diagnosis may be less clear, that retinal imaging may be a potential adjunctive diagnostic tool and knowing the repeatability of macular OCTA may be most pertinent. In our study, we were particularly interested in understanding the macular OCTA repeatability in the earlier stages of neurodegenerative disease. Another limitation of our study was that we did not assess axial length. Axial length does have the ability to impact OCTA measurements in myopic patients;[35,36] however, in a separate study conducted by Sampson et al[35] in which eyes ranging from −8.0 D to +5.0 D were analyzed, they found that axial lengths falling between 23.29 mm and 24.44 mm were predicted to result in a < 5% change in OCTA measurements. In other words, the impact of axial length on OCTA efficacy can be minimized by excluding relatively short and long eyes.

Thus, as part of our exclusion criteria, patients with refractive errors (spherical equivalent) $\geq$ +6.0 D or −6.0 D were removed from analysis. One more limitation of the study was that a higher proportion of female participants were enrolled, which may reflect our patient population as well as their engagement with research volunteerism. This may have introduced bias in our results, although there is not a definite mechanism by which female participants would have different repeatability when compared with male participants. It is also important to highlight that there were no significant differences in the distribution of male and female participants between the subgroups (AD, MCI, PD, and NC). A statistical limitation of our study was that we interpreted significance and nonsignificance in ICC values between groups by comparing overlap or nonoverlap of 95% CIs. Although this provides a reasonable estimation of significance, there are uncommon scenarios where 2 groups can have overlapping CI yet are found to have significantly different means.[37] It is possible that given our reliance on CI, significant differences between groups exist within our study and that we missed them and subsequently biased our results toward the null hypothesis; however, we feel that using 95% CI as an estimate of significance for the purposes of this study is sufficient and we accept this as a limitation of our analysis. Finally, our relatively small sample size may have also served as a limitation in our study. This might partly explain the larger CI in ICC values for certain OCTA parameters.

As this work assessing macular OCTA repeatability in neurodegenerative diseases represents one of the first of its kind, it will be important to continue investigating the repeatability of OCTA variables across the spectrum of patients with neurodegenerative disease and cognitive impairment. Although we found generally moderate to good OCTA repeatability in these patient populations, future work with larger sample sizes and across varying OCTA image devices could add further perspective to the findings of our study and be incorporated into further investigations on the role of OCTA in neurodegenerative diseases.

Footnotes and Disclosures

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Author Contributions:

Conception and design: Akrobetu, Robbins, Ma, Quist, Stinnett, Grewal, Fekrat

Analysis and interpretation: Akrobetu, Robbins, Stinnett, Grewal, Fekrat

Data collection: Akrobetu, Robbins, Ma, Soundararajan, Quist, Stinnett, Moore, Johnson, Liu, Grewal, Fekrat

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Abbreviations and Acronyms:
AD = Alzheimer's disease; **CI** = confidence interval; **D** = diopters; **FAZ** = Foveal avascular zone; **ICC** = intraclass correlation; **MCI** = mild cognitive impairment; **MSE** = mean square error; **NC** = normal cognition; **OCTA** = OCT angiography; **PD** = Parkinson's disease; **PFD** = Perfusion density; **SSI** = strength signal index; **VD** = vessel density.

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