

Impact of scan size on foveal avascular zone metrics measured by optical coherence tomography angiography

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Abstract

Background

Although scan size affects optical coherence tomography angiography (OCTA) measurements, it remains unclear whether it similarly affects multiple foveal avascular zone (FAZ)-related parameters across different retinal vascular diseases. Therefore, we aimed to assess the effect of scan size on FAZ and associated metrics.

Methods

This retrospective observational study included 402 eyes, comprising 137 healthy eyes, 85 with diabetic retinopathy (DR), and 180 with branch retinal vein occlusion (BRVO). FAZ area, FAZ perimeter (PER), acircularity index (AI), and foveal density-300 (FD-300) were measured and compared using 3×3 -mm and 6×6 -mm OCTA scans acquired with the RTVue XR Avanti™. Differences between scan sizes were evaluated using the Wilcoxon signed-rank test. Correlation and agreement between the two scan protocols were assessed using Spearman's rank correlation coefficients, intraclass correlation coefficients (ICCs), and Bland–Altman analysis. Differences in scan size-related measurements among disease groups were assessed using the Kruskal–Wallis test with Steel–Dwass post-hoc comparisons.

Results

FAZ area, FAZ PER, AI, and FD-300 exhibited significant differences between the 3×3 -mm and 6×6 -mm scans in all disease groups ($p < 0.0001$). ICCs demonstrated excellent agreement for the FAZ area (0.88–0.98) and PER (0.84–0.92), whereas AI exhibited only moderate agreement (0.34–0.48). Bland–Altman analysis identified systematic bias across all FAZ-related metrics. Scan size-dependent measurement differences significantly differed among disease groups and were more pronounced in DR and BRVO than those in healthy eyes.

Conclusion

FAZ-related metrics measured using 3×3 -mm and 6×6 -mm OCTA scans exhibited systematic measurement differences and were not fully interchangeable. The magnitude of scan size-dependent bias varied according to disease status and was pronounced in retinal vascular diseases. Therefore, standardization of scan size is essential for the reliable interpretation and comparison of OCTA-derived FAZ metrics in both clinical practice and research settings.

BACKGROUND

Optical coherence tomography angiography (OCTA) is a noninvasive imaging method that enables visualization of blood flow in the retinal and choroidal vasculature. Notably, this technique does not require the use of contrast agents, such as fluorescein or indocyanine green, which have been conventionally employed in angiographic examinations [1, 2]. Consequently, compared with conventional fluorescein angiography, OCTA results are not affected by dye leakage and enable clearer and more precise visualization of microvascular structures, including retinal neovascularization, microaneurysms, and capillary nonperfusion or tortuosity [3, 4]. Additionally, owing to its noninvasive nature, OCTA imposes minimal physical burden on patients, allows for repeated examinations, and is characterized by a high level of safety [5]. OCTA also enables quantitative assessment of retinal vascular density and foveal avascular zone (FAZ) morphology, which cannot be readily quantified using fluorescein angiography. Quantitative FAZ parameters, including FAZ area, FAZ perimeter (FAZ PER), acircularity index (AI), and foveal density-300 (FD-300), have attracted considerable attention as biomarkers of macular ischemia, owing to their associations with disease severity and visual function [6–8].

The RTVue XR Avanti™ (Optovue Inc., Fremont, CA, USA) incorporates the AngioAnalytics™ software, which automatically quantifies retinal vessel density and FAZ-related metrics, thereby enabling objective and reproducible quantitative assessment [7, 9]. This software generates the following FAZ-related metrics: FAZ area, FAZ PER, AI, and FD-300. Among these, FAZ area is one of the most widely used biomarkers of macular ischemia and correlates with disease severity and visual function in retinal vascular diseases, including diabetic retinopathy (DR) and retinal vein occlusion [10, 11]. DR severity has been graded according to the modified Davis classification, which classifies diabetic retinopathy into simple, preproliferative, and proliferative stages and is widely used in Japan. The RTVue XR Avanti™ offers multiple scan protocols, including the 3 × 3-mm and 6 × 6-mm scans; the 3 × 3-mm scan provides higher spatial resolution for detailed visualization of the macular microvasculature, whereas the 6 × 6-mm scan captures a wider retinal area, enabling assessment of more extensive retinal vascular changes. However, differences in image resolution associated with scan size may introduce systematic measurement differences, raising concerns regarding the interchangeability of FAZ-related metrics obtained from different scan protocols, even within the same eye. Evidence indicates that scan size influences OCTA-derived FAZ measurements. For example, Tsai et al. [12] reported that FAZ area measured using the 3 × 3-mm scan was systematically larger than that obtained using the 6 × 6-mm scan on the RTVue XR Avanti™. Similarly, Ho et al. [13] revealed that the 3 × 3-mm scan provided more distinct delineation of the FAZ than the 6 × 6-mm scan. Furthermore, Sampson et al. [7] and Lim et al. [14] reported that the 3 × 3-mm scan provides superior performance for the quantification of retinal vessel density and FAZ-related metrics owing to its higher spatial resolution. However, these studies primarily focused on DR or image quality; therefore, it remains unclear whether scan size similarly affects multiple FAZ-related metrics across different retinal vascular regions.

Based on the available evidence, we hypothesized that differences in image resolution between the 3 × 3-mm and 6 × 6-mm scan protocols of the RTVue XR Avanti™ would produce systematic differences both in the FAZ area and other FAZ-related metrics. Therefore, we aimed to evaluate the effects of scan size on FAZ-related measurements by comparing the 3 × 3-mm and 6 × 6-mm scan protocols in healthy eyes,

DR, and branch retinal vein occlusion (BRVO) and determine whether these measurements are interchangeable between scan protocols.

METHODS

Participants

This retrospective observational study enrolled Japanese adults (≥ 18 years) with healthy eyes, DR, or BRVO who visited the Department of Ophthalmology, Aichi Medical University Hospital, between January 2018 and December 2025. To be eligible, participants were required to have undergone both the AngioVue Retina 3×3 -mm scan protocol (3×3 -mm scan) and the HD AngioVue Retina 6×6 -mm scan protocol (6×6 -mm scan) using the RTVue XR Avanti™ (Optovue Inc., Fremont, CA, USA) on the same day. In accordance with the manufacturer's recommendations, only eyes with an image quality index of ≥ 6 on both scan protocols were included [7, 9]. Czako et al. [15] demonstrated that OCTA-derived quantitative parameters are significantly influenced by image quality, even when scans meet the manufacturer's recommended image quality threshold. Therefore, to minimize the confounding effect of image quality and isolate the influence of scan size, only eyes with identical image quality index values on both the 3×3 -mm and 6×6 -mm scan protocols were included in this study. Exclusion criteria were as follows: (1) retinal diseases other than the target conditions; (2) image artifacts likely to affect FAZ segmentation or quantitative measurements, including media opacities such as cataract or vitreous opacity; (3) poor fixation or motion artifacts; (4) segmentation errors resulting in discontinuity of the retinal layers; and (5) marked decentration of the FAZ from the scan center.

OCTA

OCTA imaging was performed using the RTVue XR Avanti™ system (software version 2017.1.0.155). The 3×3 -mm scan protocol comprised 304 B-scans, each containing 304 A-scans, whereas the 6×6 -mm scan protocol comprised 400 B-scans, each containing 400 A-scans [7, 9]. Retinal microvasculature was visualized using the split-spectrum amplitude-decorrelation angiography algorithm, which detects decorrelation of OCT signal amplitudes between repeated B-scans acquired at the same retinal location to generate angiographic images [17].

After pharmacological pupillary dilation with a combination of 0.5% tropicamide and 0.5% phenylephrine hydrochloride (Mydrin-P®, Santen Pharmaceutical Co., Ltd., Osaka, Japan), OCTA images were consecutively acquired using the RTVue XR Avanti™ system with the 3×3 -mm and 6×6 -mm scan protocols. To ensure optimal image quality, OCTA images were acquired aiming to minimize common imaging artifacts, including poor fixation, motion artifacts, and segmentation errors. All images were reviewed before analysis to confirm that media opacities, such as cataract and vitreous opacity, did not adversely affect the quantitative assessment of FAZ-related metrics.

FAZ area

FAZ area is defined as the area (mm²) of the capillary-free region at the center of the fovea and is a well-established quantitative biomarker of macular ischemia and retinal capillary nonperfusion [10, 17]. In eyes with DR, enlargement of the FAZ area has been consistently associated with disease severity and visual impairment, making it one of the most widely used OCTA-derived biomarkers for assessing retinal ischemia [18, 19].

FAZ PER

FAZ PER is defined as the length (mm) of the FAZ boundary [7, 19]. Unlike the FAZ area, FAZ PER is sensitive to changes in the complexity and irregularity of the FAZ contour. Consequently, it provides complementary information regarding FAZ morphology that cannot be captured by area measurements alone.

AI

The AI is a quantitative measure of the deviation of the FAZ shape from a perfect circle and is calculated as the ratio of the measured FAZ perimeter to the circumference of a circle with an equivalent area, resulting in an AI of 1.0 for a perfectly circular FAZ [7, 20]. Higher AI values indicate greater FAZ boundary irregularity and have been proposed as a sensitive marker of localized capillary remodeling and macular ischemic damage.

FD-300

FD-300 is defined as the vessel density (%) within a 300- μ m-wide annular region surrounding the FAZ boundary (Fig. 1) [7]. Unlike the FAZ area, FD-300 quantifies the perfusion of the perifoveal capillary network and was specifically developed to minimize the influence of FAZ size on vessel density measurements, providing a more robust assessment of perifoveal microvascular perfusion [7].

Statistical analysis

Descriptive statistics were obtained for sex, lens status, age, best-corrected visual acuity, spherical error, cylindrical error, spherical equivalent, and image quality index. Decimal best-corrected visual acuity values were converted to the logarithm of the minimum angle of resolution before statistical analysis. The normality of continuous variables was evaluated using the Shapiro–Wilk test. Given that one or more FAZ-related metrics did not satisfy the assumption of normality, nonparametric statistical methods were consistently used for all comparative analyses throughout the study.

Differences in FAZ-related metrics between the 3 × 3-mm and 6 × 6-mm scan protocols were evaluated using the Wilcoxon signed-rank test. The association between measurements obtained from the two scan protocols was assessed using Spearman's rank correlation coefficient. Measurement reliability and agreement were evaluated by calculating the intraclass correlation coefficient (ICC) using a two-way mixed-effects model for absolute agreement [ICC (3,1)]. Agreement between the two scan protocols was further assessed using Bland–Altman analysis, from which the mean difference, 95% confidence interval

(95% CI), and 95% limits of agreement were calculated. Proportional bias was evaluated using linear regression analysis of the Bland–Altman plots. To determine whether scan size-related measurement differences varied among disease groups, the difference between the 6 × 6-mm and 3 × 3-mm measurements (6 × 6-mm – 3 × 3-mm) was calculated for each FAZ-related metric. Comparisons among disease groups were performed using the Kruskal–Wallis test, followed by the Steel–Dwass test for post-hoc pairwise comparisons when a significant overall difference was detected. All statistical analyses were performed using JMP Pro version 18 (SAS Institute Inc., Cary, NC, USA). Two-sided *p* values < 0.05 were considered statistically significant.

RESULTS

Patient characteristics

A total of 402 eyes met the eligibility criteria, including 137 healthy eyes, 85 with DR, and 180 with BRVO. Based on the modified Davis classification, the DR group comprised 34 eyes with simple DR, 22 eyes with preproliferative DR, and 29 eyes with proliferative DR. The demographic and clinical characteristics of the study population are presented in Table 1. Representative OCTA images obtained using the 3 × 3-mm and 6 × 6-mm scan protocols in healthy eyes, DR, and BRVO are presented in Fig. 2.

Table 1 Patient characteristics

Parameter	Avg	SD	Min	Q1	Med	Q2	Max
Healthy eyes							
Eyes (Right eye : Left Eye)	137(60:77)						
Sex (Male : Female)	74:63						
Lens status (phakic/pseudophakic)	108/29						
Age (year)	61.7	14.3	22	54	63	70.5	87
BCVA (logMAR)	-0.02	0.10	-0.18	-0.08	-0.08	0	0.52
Spherical power (D)	-1.51	3.25	-10	-3.13	-0.5	0.5	5.25
Cylinder power (D)	-0.84	0.61	-3	-1.25	-0.75	-0.5	0
Scan quality indicator ^a	7.4	0.9	6	7	7	8	9
Diabetic retinopathy							
Eyes (Right eye : Left Eye)	85(47:38)						
Sex (Male : Female)	45:40						
Lens status (phakic/pseudophakic)	41/44						
Stage (SDR/NPDR/PDR) ^b	34/22/29						
Age (year)	65.5	10.5	43	58	66	75	82
BCVA (logMAR)	0.12	0.21	-0.18	0	0.05	0.19	0.82
Spherical power (D)	-0.9	1.84	-9	-1.88	-0.5	0.25	1.5
Cylinder power (D)	-0.83	0.47	-1.75	-1.19	-0.75	-0.5	0
Scan quality indicator ^a	7.4	0.8	6	7	7	8	9
Branch retinal vein occlusion							
Eyes (Right eye : Left Eye)	180(90:90)						
Sex (Male : Female)	84:96						
Lens status (phakic/pseudophakic)	127/53						
Age (year)	70.1	10.4	40	62	72	78	88
BCVA (logMAR)	0.15	0.22	-0.18	0	0.10	0.22	1.10
Spherical power (D)	-0.88	2.81	-8.75	-2.5	-0.38	1	5.5
Cylinder power (D)	-1.06	0.73	-3.5	-1.5	-1	-0.5	0

Scan quality indicator ^a	7.3	0.8	6	7	7	8	9
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- a) Scan quality indicator ranges from 1 to 10, with higher values indicating better image quality. Only images with a scan quality indicator ≥ 6 were included in the analysis.
- b) Diabetic retinopathy was graded according to the Davis classification, which is widely used in Japan. Abbreviations: AVE = average; SD = standard deviation; Min = minimum; Q1 = 25% percentile; Med = median; Q2 = 75% percentile; Max = maximum; BCVA = Best-corrected visual acuity; IOL = intraocular lens; SDR = Simple diabetic retinopathy; NPDR = Non-proliferative diabetic retinopathy; PDR = Proliferative diabetic retinopathy.

Comparison between the 3 × 3 mm and 6 × 6 mm scan protocols

The comparisons of FAZ-related metrics between the 3 × 3-mm and 6 × 6-mm scan protocols within each disease group are presented in Table 2. We observed significant differences for all FAZ-related metrics, including FAZ area, FAZ PER, AI, and FD-300, between the two scan protocols in all disease groups ($p < 0.0001$).

Across all disease groups, FAZ area, FAZ PER, and AI were consistently lower when measured using the 6 × 6-mm scan protocol than the 3 × 3-mm scan protocol, whereas FD-300 was consistently higher with the 6 × 6-mm scan protocol. The mean differences (6 × 6-mm scan – 3 × 3-mm scan) in FAZ area were –0.008 mm², –0.034 mm², and –0.022 mm² in the healthy eye, DR, and BRVO groups, respectively. The corresponding mean differences in FAZ PER were –0.101 mm, –0.217 mm, and –0.183 mm, and those in AI were –0.038, –0.054, and –0.056; and those in FD-300 were +2.6, +2.6, and +2.4 percentage points, respectively.

Table 2 Comparison of the FAZ-related metrics between 3×3-mm and 6×6-mm OCTA scans

Parameter		Avg	SD	Min	Q1	Med	Q2	Max	p-value ^a
FAZ area									
Healthy eyes	3×3 mm scans (mm ²)	0.311	0.099	0.108	0.234	0.312	0.353	0.642	<.0001
	6×6 mm scans (mm ²)	0.303	0.098	0.094	0.228	0.309	0.347	0.624	
DR	3×3 mm scans (mm ²)	0.357	0.161	0.048	0.265	0.355	0.424	1.038	<.0001
	6×6 mm scans (mm ²)	0.323	0.140	0.053	0.24	0.332	0.382	0.978	
BRVO	3×3 mm scans (mm ²)	0.341	0.159	0.021	0.226	0.302	0.434	1.002	<.0001
	6×6 mm scans (mm ²)	0.319	0.136	0.015	0.229	0.291	0.397	0.881	
FAZ perimeter									
Healthy eyes	3×3 mm scans (mm)	2.217	0.354	1.251	1.989	2.22	2.397	3.394	<.0001
	6×6 mm scans (mm)	2.116	0.350	1.13	1.866	2.157	2.289	3.119	
DR	3×3 mm scans (mm)	2.452	0.623	0.879	2.189	2.45	2.76	4.502	<.0001
	6×6 mm scans (mm)	2.235	0.542	0.904	1.918	2.296	2.493	4.11	
BRVO	3×3 mm scans (mm)	2.407	0.657	0.551	1.985	2.312	2.783	5.241	<.0001
	6×6 mm scans (mm)	2.224	0.538	0.418	1.886	2.193	2.545	4.401	
Acircularity index ^b									
Healthy eyes	3×3 mm scans	1.136	0.051	1.06	1.1	1.12	1.16	1.38	<.0001
	6×6 mm scans	1.098	0.034	1.04	1.08	1.09	1.11	1.26	
DR	3×3 mm scans	1.189	0.070	1.08	1.135	1.18	1.23	1.43	<.0001

	6×6 mm scans	1.135	0.072	1.05	1.09	1.12	1.15	1.52	
BRVO	3×3 mm scans	1.188	0.080	1.07	1.13	1.17	1.22	1.55	<.0001
	6×6 mm scans	1.132	0.061	0.97	1.09	1.12	1.16	1.4	
Foveal density-300 ^C									
Healthy eyes	3×3 mm scans (%)	48.0	4.03	36.8	46.0	48.1	50.8	60.7	<.0001
	6×6 mm scans (%)	50.6	4.68	37.4	48.0	50.9	53.7	64.7	
DR	3×3 mm scans (%)	43.8	5.65	29.1	40.2	44.2	48.2	56	<.0001
	6×6 mm scans (%)	46.4	6.04	26.7	43.4	47.1	51.0	58.5	
BRVO	3×3 mm scans (%)	45.6	5.05	26.2	42.4	45.8	49.0	58.7	<.0001
	6×6 mm scans (%)	48.0	5.47	31.2	44.8	48.5	51.7	61.4	

a) Wilcoxon signed-rank test

b) ratio between the measured perimeter and the perimeter

of the same size circular area

c) vessel density within 300µm-width-ring surrounding the FAZ.

Data are presented as mean, standard deviation, minimum, 25th percentile, median, 75th percentile, and maximum. The 3 × 3-mm scans provide higher image resolution than the 6 × 6-mm scans because the same number of A-scans are distributed over a smaller retinal area.

Abbreviations: FAZ = foveal avascular zone; DR = diabetic retinopathy; BRVO = Branch retinal vein occlusion; AVE = average; SD = standard deviation; Min = minimum; Q1 = 25% percentile; Med = median; Q2 = 75% percentile; Max = maximum.

Agreement between the 3 × 3-mm and 6 × 6-mm scan protocols

The agreement between the 3 × 3-mm and 6 × 6-mm scan protocols is presented in Figs. 3 and 4 and Tables 3 and 4. ICC analysis demonstrated good agreement for FAZ area and FAZ PER across all disease groups (ICC > 0.70). The ICCs for AI exceeded 0.40, indicating moderate agreement, whereas those for

FD-300 ranged from 0.65 to 0.75, indicating moderate-to-good agreement. Similarly, Spearman's rank correlation analysis demonstrated strong positive correlations for FAZ area and FAZ PER in all disease groups. Although the correlations for AI and FD-300 were relatively weaker, we observed significant positive correlations in all disease groups. Bland–Altman analysis revealed fixed bias for all FAZ-related metrics across all disease groups. Moreover, we noted proportional bias for AI and FD-300 in the healthy eye group; FAZ area, FAZ PER, and AI in the DR group; and FAZ area and FAZ PER in the BRVO group.

Table 3 Agreement and correlation between 3×3-mm and 6×6-mm OCTA scans

Parameter	ICCs ^a	Spearman's r^b	<i>p</i> -value
Healthy eyes			
FAZ area	0.9798	0.9681	<.0001
FAZ perimeter	0.9155	0.9418	<.0001
Acircularity index	0.3356	0.6798	<.0001
Foveal density-300	0.6519	0.7900	<.0001
Diabetic retinopathy			
FAZ area	0.8837	0.8967	<.0001
FAZ perimeter	0.8488	0.9034	<.0001
Acircularity index	0.4213	0.7338	<.0001
Foveal density-300	0.7552	0.8269	<.0001
Branch retinal vein occlusion			
FAZ area	0.8949	0.9252	<.0001
FAZ perimeter	0.8384	0.8994	<.0001
Acircularity index	0.4769	0.6369	<.0001
Foveal density-300	0.6733	0.7234	<.0001

^a) Agreement between the 3×3-mm and 6×6-mm scans was evaluated using intraclass correlation coefficients derived from a two-way mixed-effects model for absolute agreement

^b) Spearman's rank correlation coefficient.

Abbreviations: FAZ = foveal avascular zone; ICCs = Intraclass correlation coefficient.

Table 4 Bland–Altman plots for FAZ-related metrics

Parameter	Mean Difference ^a	SD	SE	Upper 95% CI	Lower 95% CI	Upper LoA ^b	Lower LoA ^b
Healthy eyes							
FAZ area	-0.0082	0.0183	0.0015	-0.0051	-0.0113	0.0275	-0.0443
	y = -0.0207x - 0.0018 (p=0.1926) ^d						
FAZ PER	-0.1013	0.1141	0.0090	-0.0834	-0.1191	0.1225	-0.3250
	y = -0.0118x – 0.0756 (p=0.6513) ^d						
AI	-0.0382	0.0436	0.0034	-0.0316	-0.0449	0.0471	-0.1237
	y = -0.4721x + 0.4890 (p<.0001) ^d						
FD-300 ^c	2.6434	2.7436	0.2348	3.1078	2.1790	8.0209	-2.7341
	y = 0.1668x - 5.5683 (p=0.0031) ^d						
Diabetic retinopathy							
FAZ area	-0.0340	0.0655	0.0071	-0.0199	-0.0482	0.0943	-0.1624
	y = -0.1506x + 0.0172 (p=0.0015) ^d						
FAZ PER	-0.2170	0.2456	0.0266	-0.1640	-0.2699	0.2643	-0.6982
	y = -0.1446x + 0.1220 (p=0.0017) ^d						
AI	-0.0545	0.0619	0.0067	-0.0411	-0.0678	0.0668	-0.1758
	y = 0.0261x - 0.0848 (p=0.0017) ^d						
FD-300 ^c	2.5992	3.3040	0.3584	3.3120	1.8866	9.0752	-3.8766
	y = 0.0733x - 0.7061 (p=0.2568) ^d						
Branch retinal vein occlusion							
FAZ area	-0.0212	0.0647	0.0048	-0.0117	-0.0307	0.1055	-0.1480
	y = -0.1641x + 0.0329 (p<.0001) ^d						
FAZ PER	-0.1823	0.2938	0.0219	-0.1391	-0.2255	0.3935	-0.7581
	y = -0.2093x + 0.3027 (p<.0001) ^d						
AI	-0.0556	0.0557	0.0042	-0.0474	-0.0637	0.0536	-0.1647
	y = -0.2965x + 0.2885 (p=0.2568) ^d						

FD-300 ^c	2.4002	3.6531	0.2723	2.9376	1.8630	9.5605	-4.7600
$y = 0.0892x - 1.7726$ ($p=0.2568$) ^d							

- a) Mean difference was calculated as 6×6-mm scan minus 3×3-mm scan;
- b) Upper and lower limits of agreement were defined as mean difference $\pm$ 1.96 standard deviations;
- c) Differences in FD-300 are expressed as percentage points;
- d) Linear regression analysis was performed to assess proportional bias and one-way analysis of variance.

Abbreviations: FAZ = foveal avascular zone; SD = standard deviation; SE = Standard Error; CI = Confidence Interval; LoA = Limit of Agreement

Comparison of scan size-related differences in FAZ-related metrics among disease groups

Comparisons of scan size-related differences in FAZ-related metrics (6 × 6-mm scan – 3 × 3-mm scan) among disease groups are presented in Table 5. The Kruskal–Wallis test demonstrated significant differences among the three disease groups in the FAZ area, FAZ PER, and AI ($p < 0.0001$). Post-hoc pairwise comparisons using the Steel–Dwass test revealed that the difference in FAZ area was significantly greater in the DR group (–0.034 mm²) than in the healthy eye group (–0.008 mm²). For FAZ PER, we observed significant differences between the healthy eye (–0.101 mm) and both the DR (–0.217 mm) and BRVO (–0.182 mm) groups. Similarly, the difference in AI was significantly greater in the DR (–0.054) and BRVO (–0.056) than that in the healthy eye (–0.038) groups.

Table 5 Comparison of scan size differences in FAZ-related metrics among disease groups

Parameter	Healthy eyes (n=137)	DR (n=85)	BRVO (n=180)	p-value
FAZ area	-0.008 $\pm$ 0.005 ^a	-0.034 $\pm$ 0.006 ^a	-0.021 $\pm$ 0.004	<.0001
FAZ PER	-0.101 $\pm$ 0.020 ^{ab}	-0.217 $\pm$ 0.025 ^a	-0.182 $\pm$ 0.018 ^b	<.0001
AI	-0.038 $\pm$ 0.004 ^{ab}	-0.054 $\pm$ 0.006 ^a	-0.056 $\pm$ 0.004 ^b	<.0001
FD-300	2.643 $\pm$ 0.282	2.599 $\pm$ 0.357	2.400 $\pm$ 0.246	.8576

Values represent the differences between the 6 × 6-mm and 3 × 3-mm scan measurements (6 × 6-mm scan – 3 × 3-mm scan). FD-300 is expressed in percentage points. Data are presented as mean $\pm$ standard deviation for descriptive purposes. Statistical comparisons among groups were performed

using the Kruskal–Wallis test, followed by the Steel–Dwass test for pairwise comparisons. Values with different superscript letters (a, b) indicate statistically significant differences ($p < 0.05$).

DISCUSSION

In the present study, we compared FAZ-related metrics obtained using the 3×3 -mm and 6×6 -mm OCTA scan protocols in healthy eyes, eyes with DR, and eyes with BRVO. We identified significant differences between the two scan protocols for all FAZ-related metrics, including FAZ area, FAZ PER, AI, and foveal FD-300. Although FAZ area and FAZ PER demonstrated strong correlations and good agreement, Bland–Altman analysis revealed systematic measurement bias, indicating that measurements obtained from the two scan protocols are not fully interchangeable. Moreover, except for FD-300, scan size-related measurement differences were significantly greater in eyes with retinal vascular diseases than those in healthy eyes, suggesting that the effect of scan size on FAZ-related metrics varies according to the underlying disease.

The influence of scan size on OCTA-derived quantitative parameters has been previously reported: the 3×3 -mm scan protocol provides higher spatial resolution than the 6×6 -mm scan, whereas the 6×6 -mm scan protocol enables a wider field of view at the expense of lower image sampling density, which may reduce the visualization of fine retinal capillary structures [1, 5, 7, 13]. Spaide et al. [1] also reported that quantitative OCTA measurements are influenced by image quality and acquisition conditions and suggested that differences in scan protocols may contribute to measurement variability. Consistent with these previous findings, our results confirmed the presence of systematic measurement differences between the 3×3 -mm and 6×6 -mm scan protocols. More importantly, we demonstrated that the magnitude of these differences differed among FAZ-related metrics and was more pronounced in eyes with retinal vascular diseases than in healthy eyes. Collectively, these findings suggest that the effect of scan size on quantitative FAZ measurements depends both on image resolution and the underlying retinal vascular pathology.

In the present study, the FAZ area and FAZ PER demonstrated strong correlations and high ICCs, whereas the ICCs for AI and FD-300 were relatively lower. The FAZ area reflects the overall size of the foveal avascular zone and is less susceptible to the influence of small local segmentation errors, as it primarily represents a global structural characteristic of the FAZ [6]. Similarly, although we observed systematic differences between the two scan protocols, FAZ PER, which quantifies the overall length of the FAZ boundary, maintained good agreement between scan protocols. Conversely, AI is designed to quantify irregularities in the FAZ contour, making it highly sensitive to subtle changes in boundary morphology. Therefore, reductions in image resolution and minor differences in boundary segmentation associated with scan size are likely to have a greater impact on AI than on FAZ area or FAZ PER, which may explain the relatively low agreement observed for AI in our study. Previous studies have suggested that AI is more sensitive than FAZ area to subtle alterations in the morphology of the FAZ boundary, as it reflects contour irregularity rather than overall lesion size [7, 11]. In contrast, FD-300 quantifies vessel density within a $300\text{-}\mu\text{m}$ -wide annular region surrounding the FAZ; therefore, it depends not only on the FAZ itself

but also on the visualization of the surrounding perifoveal capillary network. Although FD-300 was developed to minimize the influence of FAZ size on vessel density measurements, its accuracy remains dependent on image resolution and capillary detectability [7, 20]. Accordingly, the differences in FD-300 observed in the present study may reflect scan size-dependent differences in the visualization of the retinal microvasculature, particularly the perifoveal capillary network.

The scan size-related differences in FAZ area, FAZ PER, and AI were significantly greater in the DR and BRVO groups than in the healthy eye group in our study. These findings suggest that retinal microvascular abnormalities associated with retinal vascular diseases may amplify the influence of scan size on quantitative FAZ measurements. In both DR and BRVO, capillary nonperfusion and reduced retinal perfusion enlarge the FAZ and increase the irregularity of the FAZ boundary [6, 10, 18]. These pathological alterations may produce a heterogeneous perifoveal capillary network with focal capillary disruption and localized areas of nonperfusion along the FAZ margin. This would enhance the morphological complexity of the FAZ boundary, rendering it more susceptible to differences in spatial resolution and image sampling density between scan protocols compared with healthy eyes. This mechanism may explain why scan size-related measurement differences were substantially greater in diseased eyes than in healthy eyes in the present study. In particular, the 6 × 6-mm scan protocol provides lower spatial resolution than the 3 × 3-mm scan protocol, which may reduce the visualization of fine capillary structures along the FAZ boundary. As the FAZ contour is generally smooth in healthy eyes, the influence of reduced spatial resolution is likely to be limited. In contrast, eyes with DR and BRVO exhibit enhanced FAZ boundary irregularity, making quantitative FAZ measurements more susceptible to subtle differences in capillary visualization. Therefore, scan size-related differences are more likely to affect FAZ area, FAZ PER, and particularly AI. This interpretation is consistent with our finding that scan size-related differences in AI were significantly greater in eyes with DR and BRVO than in healthy eyes. Collectively, these findings highlight the importance of standardizing imaging protocols when evaluating FAZ-related metrics in retinal vascular diseases and suggest that systematic measurement bias associated with scan size should be considered when interpreting OCTA-derived FAZ measurements and when comparing findings across clinical studies.

In the present study, the FAZ area and FAZ PER demonstrated strong correlations and high ICCs; however, Bland–Altman analysis revealed fixed bias for all FAZ-related metrics. These findings indicate that a high correlation or ICC does not necessarily imply measurement interchangeability. Although the 3 × 3-mm and 6 × 6-mm scan protocols evaluate the same retinal microvascular structures, the quantitative measurements derived from these scan protocols cannot be considered statistically or clinically equivalent. Therefore, measurements obtained using different scan protocols should not be used interchangeably in clinical practice or research without careful consideration of the systematic bias associated with scan size. In this context, recent OCTA studies have increasingly explored FAZ-related metrics as biomarkers of disease activity and therapeutic response [7, 12]. However, when imaging protocols are not standardized, part of the observed differences in FAZ-related metrics may reflect methodological variation rather than true pathological changes. In the present study, scan size-related measurement differences were significantly greater in eyes with DR and BRVO than in healthy eyes,

suggesting that the influence of scan size may be further increased in retinal vascular diseases. Therefore, when FAZ-related metrics are used as research endpoints or clinical biomarkers, direct comparisons between measurements obtained using different scan protocols should be cautiously interpreted. Moreover, standardization of scan protocols is essential to ensure the reliability and comparability of quantitative OCTA measurements across clinical studies and routine clinical practice.

This study has some limitations. First, this was a retrospective, single-center observational study, and selection bias cannot be completely excluded. Furthermore, as only eyes with adequate image quality were included, the present findings may be primarily applicable to eyes with relatively high-quality OCTA images. Second, all measurements were obtained using a single OCTA device; therefore, whether similar scan size-related differences exist in other OCTA platforms remains uncertain. Third, although the present study included eyes with DR and BRVO, it remains unclear whether similar scan size-dependent measurement differences are present in other retinal vascular disorders or macular diseases. Future multicenter studies including multiple OCTA platforms and a broader spectrum of retinal diseases are warranted to validate and extend the present findings. Despite these limitations, the study also has notable strengths. It included a relatively large cohort of more than 400 eyes, and all paired scans had identical image quality indices, minimizing the potential confounding effect of image quality and allowing scan size-related measurement differences to be evaluated more accurately.

CONCLUSION

The present study revealed systematic differences in all FAZ-related metrics, including FAZ area, FAZ PER, AI, and FD-300, between the 3 × 3-mm and 6 × 6-mm OCTA scan protocols. Although measurements obtained from the two scan protocols demonstrated strong correlations, they were not fully interchangeable, indicating that scan size is an important determinant of quantitative FAZ measurements. Furthermore, scan size-related measurement differences were more pronounced in eyes with DR and BRVO than in healthy eyes. Overall, these findings highlight the importance of standardizing scan protocols and accounting for scan size when using FAZ-related metrics for clinical evaluation, longitudinal follow-up, or as quantitative endpoints in clinical research.

Abbreviations

FAZ Foveal avascular zone

DR Diabetic retinopathy

BRVO Branch retinal vein occlusion

PER FAZ perimeter

AI Acircularity index

FD-300 Foveal density-300

OCTA Optical coherence tomography angiography

ICCs intraclass correlation coefficients

Declarations

Ethics approval and consent to participate

This study was a retrospective investigation and was approved by the Ethics Committee of Aichi Medical University School of Medicine (Approval No. 2024-045). The study adhered to the principles of the Declaration of Helsinki as well as the Ethical Guidelines for Medical and Health Research Involving Human Subjects issued by the Ministry of Health, Labour and Welfare of Japan. Every effort was made to ensure respect for human dignity, protection of human rights, and safeguarding of participants' personal information. Data obtained during routine clinical practice were retrospectively collected for this study, and no additional invasive procedures or interventions were performed for research purposes. The requirement for informed consent to participate was waived by the Ethics Committee of Aichi Medical University School of Medicine because of the retrospective nature of the study. Instead, an opt-out approach was adopted. Sufficient information regarding the study objectives, methods, and handling of personal information was publicly disclosed, and participants were given the opportunity to decline participation in the study at any time.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request. The data are not publicly available because they contain information that could compromise the privacy of the research participants.

Competing interests

The authors report no conflicts of interest in this work.

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Authors' contributions

KM contributed to the conception and design of the study, data acquisition, analysis, drafting of the manuscript, and final approval. MK contributed to data interpretation, critical revision of the manuscript, and final approval.

Both authors agree to be accountable for all aspects of the work.

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Figures

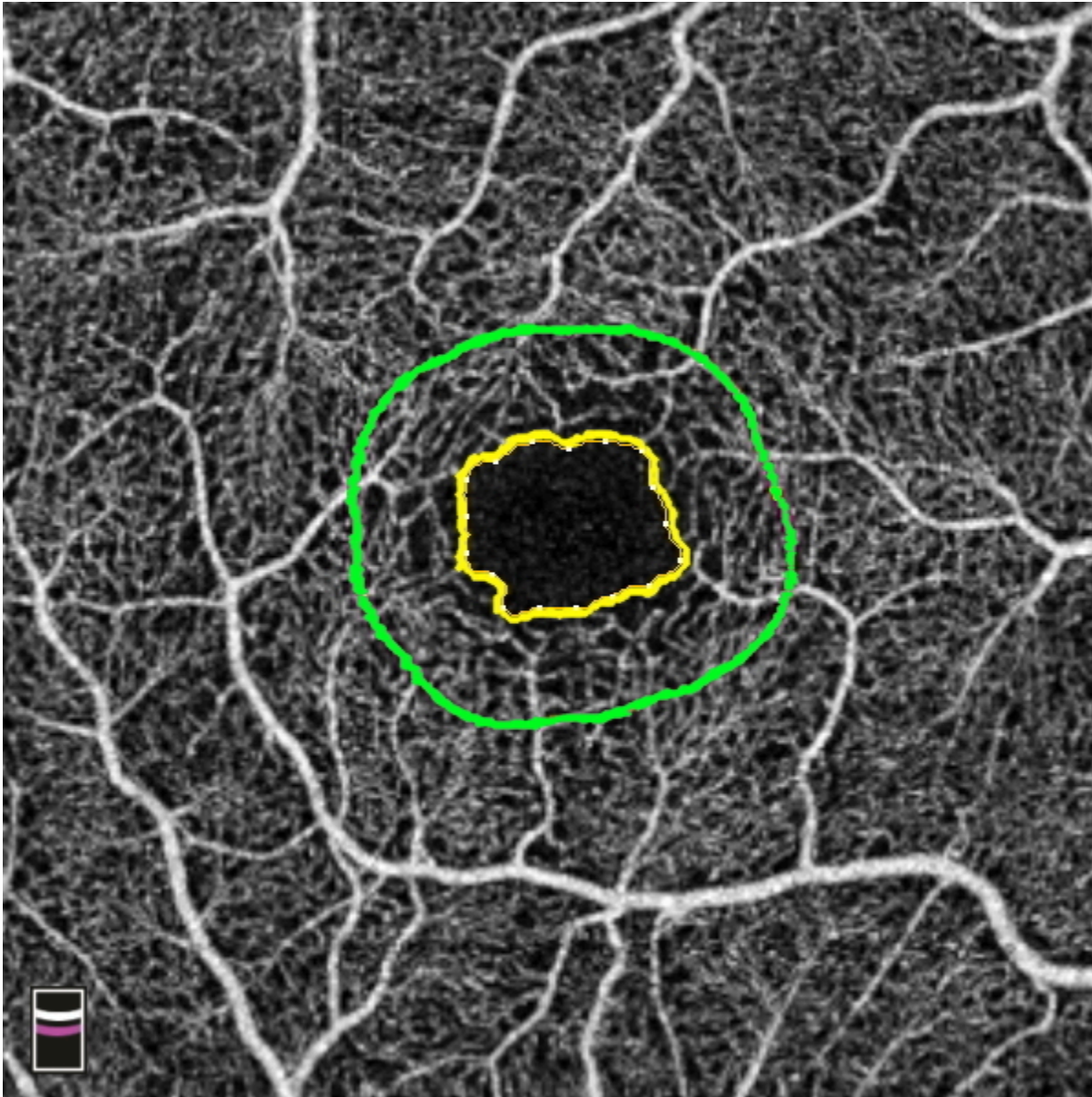


Figure 1

Definition of FAZ boundary and FD-300 measurement region on OCTA.

Representative 3 × 3-mm OCTA image depicting the foveal avascular zone (FAZ) analysis in the healthy eye of a man in his 5th decade of age. The yellow outline indicates FAZ boundary, and the green ring indicates the 300-μm-wide annulus surrounding the FAZ used for calculating foveal vessel density-300. All FAZ-related metrics were automatically quantified using the AngioAnalytics™ software integrated into the RTVue XR Avanti™ system without manual correction of the FAZ boundary. FAZ, foveal avascular zone; OCTA, optical coherence tomography angiography; FD-300, foveal vessel density-300

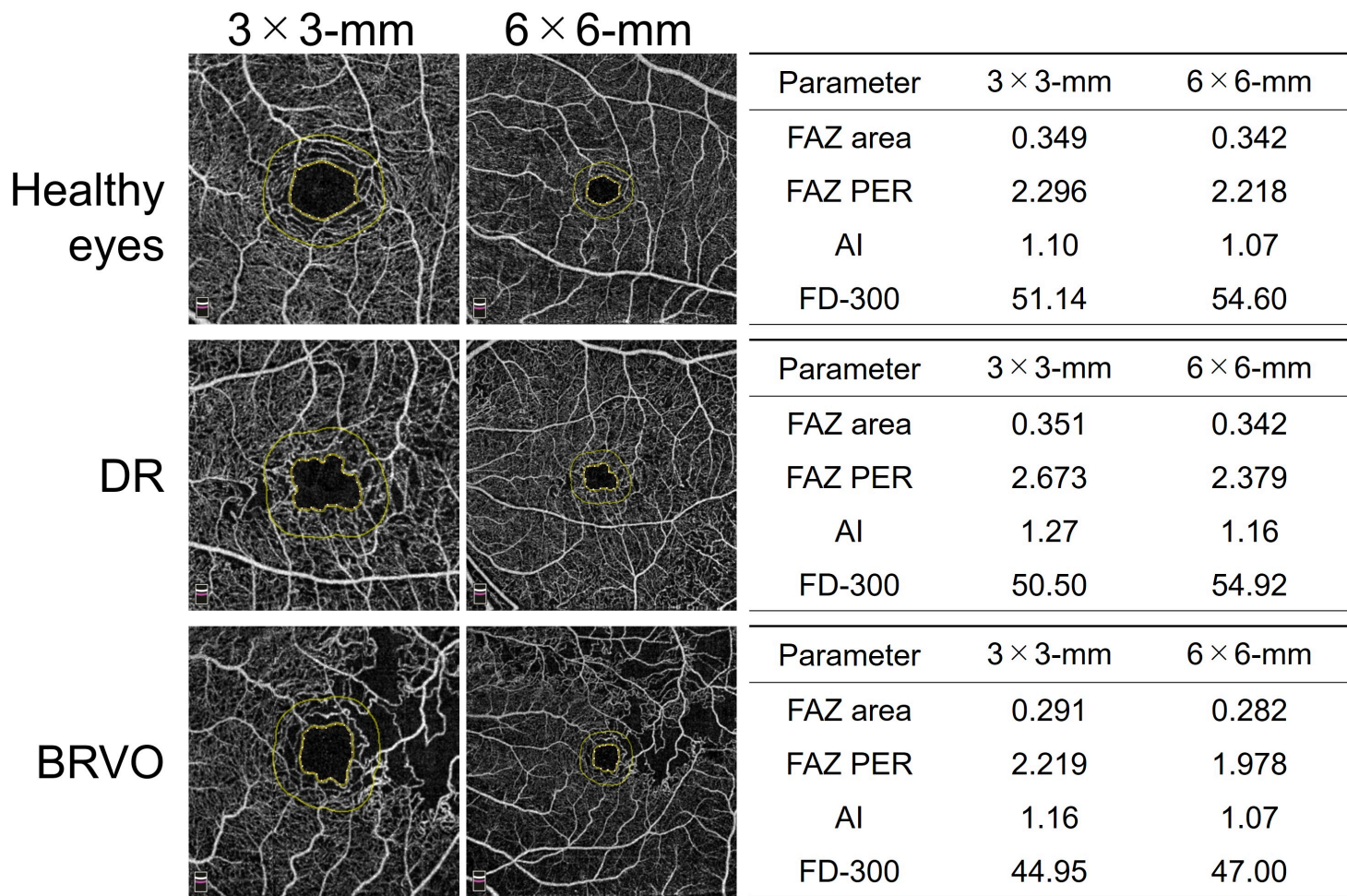


Figure 2

Representative OCTA images and corresponding FAZ-related measurements obtained using scan protocols.

OCTA images from healthy eyes, an eye with diabetic retinopathy (DR), and an eye with BRVO are presented. Representative cases were selected from each disease group based on the smallest difference in the FAZ area between the two scan protocols. The inner yellow outline indicates the automatically segmented boundary of FAZ, whereas the outer yellow ring delineates the 300-μm-wide annulus surrounding the FAZ used for calculating FD-300. The corresponding quantitative measurements of FAZ area, FAZ PER, AI, and FD-300 are displayed for each scan. OCTA, optical coherence tomography angiography; FAZ, foveal avascular zone; DR, diabetic retinopathy; BRVO, branch retinal vein occlusion; PER, perimeter; AI, acircularity index; FD-300, foveal vessel density-300

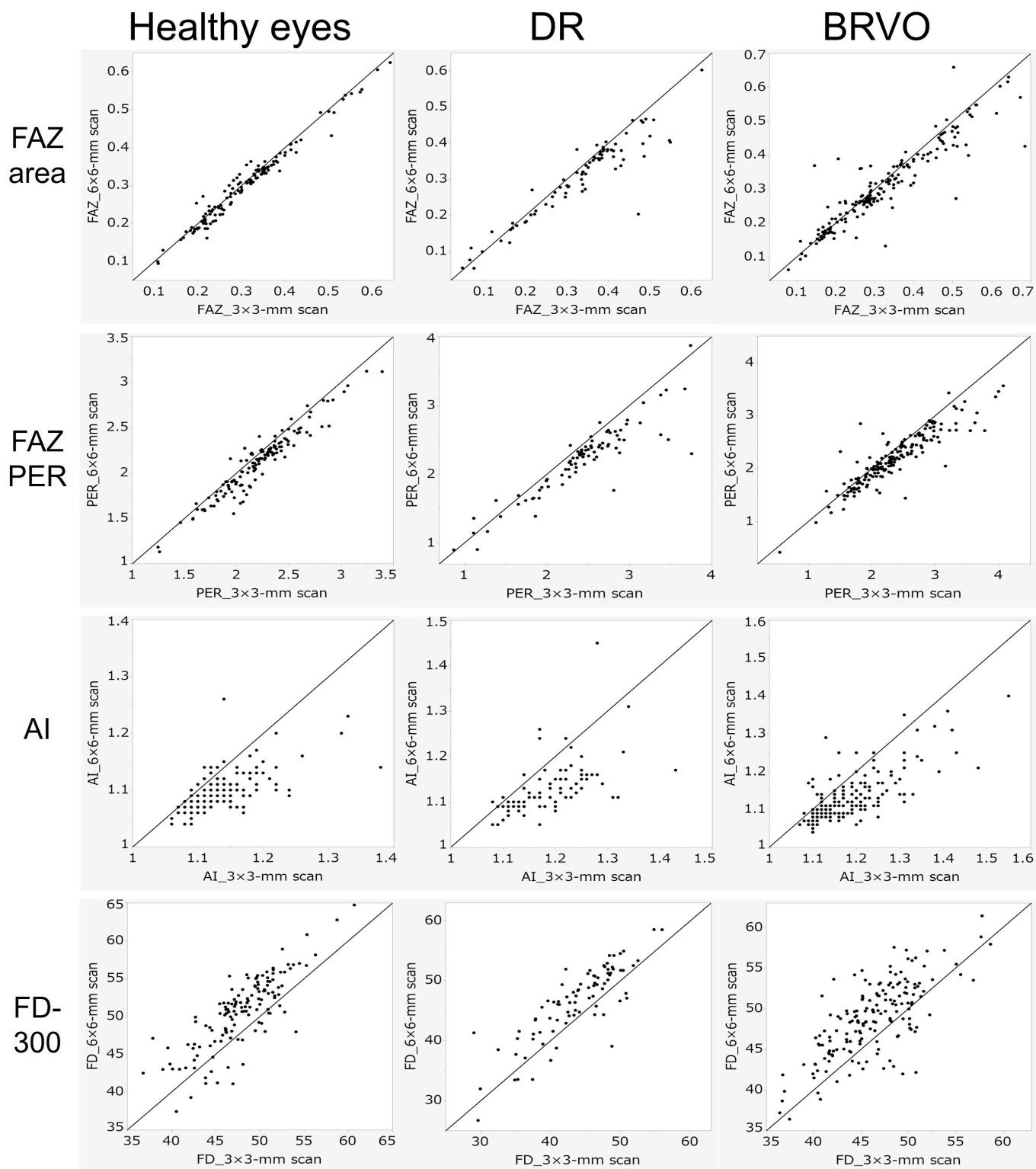


Figure 3

Scatter plots comparing FAZ-related metrics measured using the OCTA scans.

Scatter plots comparing the measurements of the FAZ area, FAZ PER, AI, and FD-300 obtained from 3 × 3-mm and 6 × 6-mm OCTA scans in healthy eyes, eyes with DR, and eyes with BRVO. The solid diagonal line represents the line of equality (45° line, $y = x$). Data points below the line indicate lower values

obtained with the 6 × 6-mm scan, whereas points above the line indicate higher values obtained with the 6 × 6-mm scan. OCTA, optical coherence tomography angiography; FAZ, foveal avascular zone; DR, diabetic retinopathy; BRVO, branch retinal vein occlusion; PER, perimeter; AI, acircularity index; FD-300, foveal vessel density-300

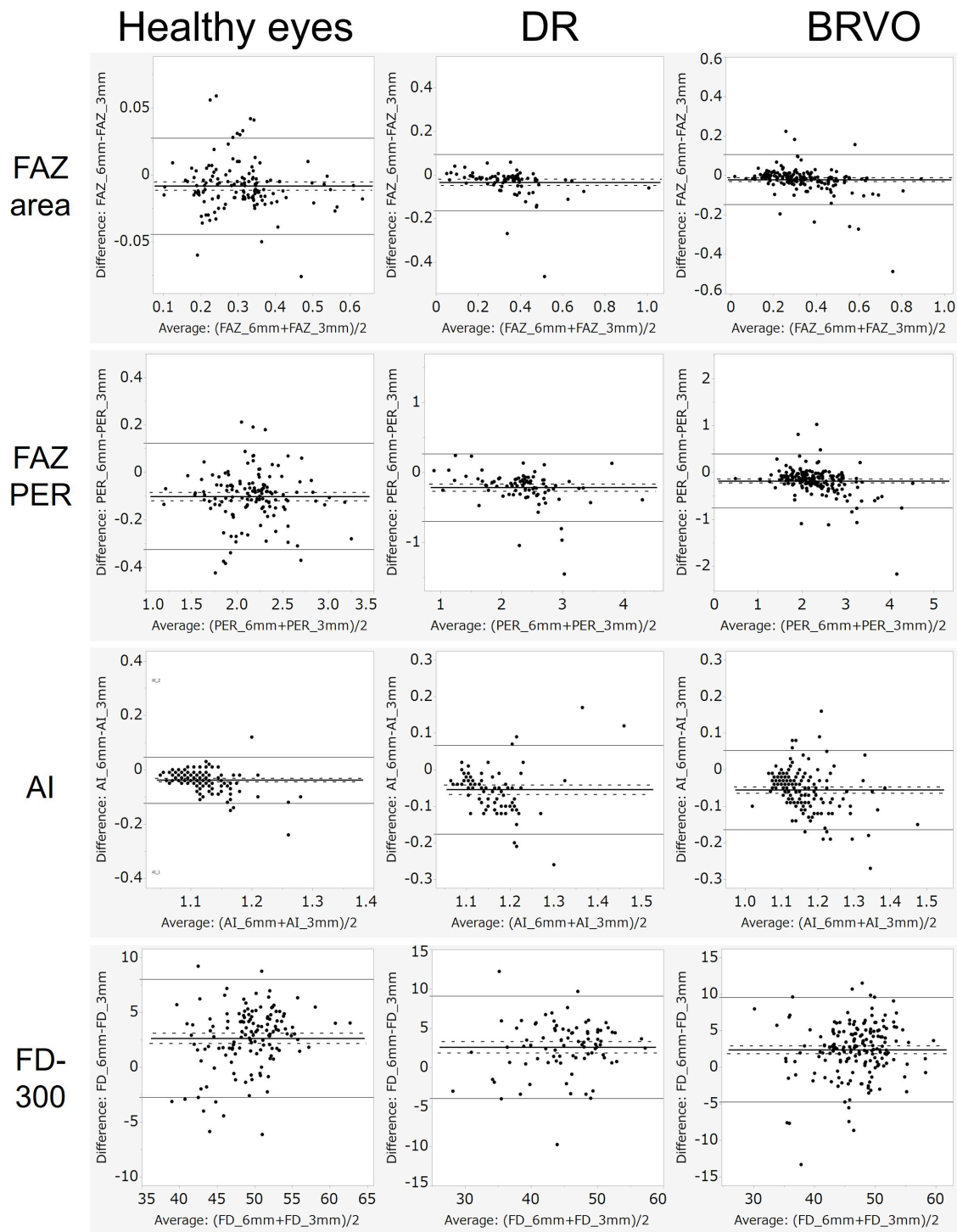


Figure 4

Bland–Altman analysis of FAZ-related metrics.

Bland–Altman plots depicting the agreement between FAZ area, FAZ PER, AI, and FD-300 measured using 3 × 3-mm and 6 × 6-mm OCTA scans in healthy eyes, eyes with DR, and eyes with BRVO. Differences were calculated by subtracting the measurements obtained from the 3 × 3-mm scan from those obtained from the 6 × 6-mm scan, and the averages were calculated as the mean of the two measurements. The solid central line represents the mean difference (systematic bias), the dashed lines represent the 95% confidence interval of the mean difference, and the upper and lower solid lines represent the 95% limits of agreement (mean difference $\pm$ 1.96 standard deviations). These plots illustrate the magnitude and direction of measurement bias associated with scan size, as well as the agreement between the two scan protocols across all disease groups OCTA, optical coherence tomography angiography; FAZ, foveal avascular zone; DR, diabetic retinopathy; BRVO, branch retinal vein occlusion; PER, perimeter; AI, acircularity index; FD-300, foveal vessel density-300