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Repeatability and Reproducibility of Foveal Avascular Zone Area Measurement on Normal Eyes by Different Optical Coherence Tomography Angiography Instruments

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Keywords
Optical coherence tomography angiography · Foveal avascular zone area · Superficial retinal capillary plexus · Deep retinal capillary plexus

Abstract
Purpose: To compare the foveal avascular zone (FAZ) area measurements produced by different optical coherence tomography angiography (OCTA). ***Methods:*** Healthy enrolled volunteers underwent OCTA using 2 different devices: Spectralis HRA+OCTA (Heidelberg Engineering, Heidelberg, Germany) and RS-3000 Advance (Nidek, Gamagori, Japan). Two graders measured FAZ in both superficial (SCP) and deep (DCP) retinal capillary plexuses. The SCP and DCP en face images were visualized automatically segmenting 2 separate slabs defined by the arbitrary segmentation lines created by the software of each OCT device. One grader repeated each measure twice. ***Results:*** Fifty-nine eyes were included. The mean FAZ was 0.33 ± 0.09 mm^2 at the SCP and 0.57 ± 0.17 mm^2 at the DCP measured with RS-3000 versus 0.30 ± 0.08 and 0.35 ± 0.08 mm^2, respectively, measured with Spectralis. The measurements of the 2 devices were significantly different ($p < 0.0001$). The intraoperator agreement was excellent at the SCP (intraclass correlation coefficient, ICC: 0.97 with Spectralis and 0.96 with RS-3000). At the DCP, it was good with Spectralis and fair with RS-3000 (ICC: 0.85 and 0.64, respectively). The interoperator agreement was excellent for Spectralis and good for RS-3000 at the SCP (ICC: 0.97 and 0.93, respectively). It was good at the DCP with both devices (ICC: 0.74 with RS-3000 and 0.81 with Spectralis). ***Conclusions:*** FAZ measurements obtained with different OCTA devices differ. These findings should be considered in follow-up studies of patients with retinal vascular diseases.

Introduction

Optical coherence tomography angiography (OCTA) is a newly developed noninvasive imaging technique, allowing en face visualization in vivo and separately of both superficial (SCP) and deep retinal capillary plexuses (DCP) [1, 2]. Moreover, OCTA allows us to detect and measure the foveal avascular zone (FAZ) area, which may

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be affected in several retinal vascular diseases. Therefore, FAZ area measurement must be a reliable and repeatable method. The aim of this study was to compare FAZ area measurements produced by two different optical OCTA devices when imaging normal eyes.

Methods

Study Design and Population

This prospective cross-sectional study, compliant with the tenets of the Declaration of Helsinki, was approved by our institutional review board (IRB), and written informed consent was obtained. Subjects were recruited between September and November 2016 at our institute. Inclusion criteria were healthy volunteers aged 18 years or older with normal visual acuity (best-corrected visual acuity of 20/20 or better) and spherical equivalent less than 3 dpt. Exclusion criteria were any history or clinical evidence of ocular and/or systemic diseases, any previous ocular surgery or laser treatment, and media opacities precluding adequate fundus imaging.

Data Acquisition

All subjects underwent full ophthalmological examination. Both eyes of all subjects underwent OCTA using 2 different devices: Spectralis HRA+OCTA (Heidelberg Engineering, Heidelberg, Germany) and RS-3000 Advance (Nidek, Gamagori, Japan). For each subject, both examinations were performed during the same day in the early afternoon.

Spectralis has an A-scan rate of 70,000 scans/s, using a light source centered on 870 nm, with an axial and transverse resolution of 3.9 and 6 μm in tissue. An OCTA scan pattern of 15 × 5 degrees (44 × 1.5 mm; consisting of 131 B-scans separated by 11 μm) centered on the fovea was acquired. The OCTA image automated real-time mode was settled at 35 (frames averaged per B-scan). The SCP and the DCP en face images were visualized automatically segmenting 2 separate slabs defined by the arbitrary segmentation lines created by the software of the device: a superficial slab extending from the inner limiting membrane (ILM) to the outer border of the inner plexiform layer (IPL) for the SCP, and a deeper one from the outer border of the IPL to the outer border of the outer plexiform layer (OPL) for the DCP.

The RS-3000 Advance has an A-scan rate of 53,000 scans/s, using a light source centered on 880 nm, with an axial and transverse resolution of 7 and 20 μm in tissue. The Angioscan 8 OCTA software was used for acquisition of a 3 × 3 mm macular map, consisting of 256 B-scans separated by 11 μm. The bright intensity score of the image, which may range from –10 to +10, was settled at 0, as usually automatically provided by the device itself. The SCP and the DCP en face images were visualized automatically segmenting 2 separate slabs defined by the arbitrary segmentation lines created by the software of the device: a slab extending from the ILM to 8 μm below the inner boundary of the inner nuclear layer (INL) for the SCP, and a deeper one from 12 μm below the inner boundary of the INL to a segmenting line localized 86 μm below for the DCP.

All images from the 2 devices were reviewed to confirm consistent segmentation by the automated instrument software. In all images automated segmentation was correct and no manual resegmentation was necessary. Poor-quality images (quality index lower than 35 dB for Spectralis and signal strength index lower than 7 for RS-3000 Advance) were excluded. The enface images of the SCP and DCP were automatically generated by the inbuilt software of both devices (Fig. 1).

Data Analysis and Study Setting

Two well-trained graders (A.C. and E.D.D) independently measured the FAZ area, defined as the avascular area in the center of the fovea, in a random sequence. The inbuilt automatic area measurement editor of both devices was used (Fig. 1) One of the two graders repeated each measure twice at 1 week apart.

Statistical Analysis

Intra- and interoperator agreement were graded considering the absolute value of the intraclass correlation coefficient (ICC) and the confidence interval. For the assessment of reproducibility, data of grader A.C. and first measurement data of grader E.D.D. were used. A Bland-Altman plot was constructed by plotting each test-retest difference against its mean.

Mann-Whitney and Friedman nonparametric tests were used for comparison between 2 groups and among more than 2 groups, respectively. Linear regression analysis evaluated the linear association between the test-retest score difference and the X value. Statistical analyses were performed using SAS version 9.3 software (SAS Institute, Cary, NC, USA).

Results

A total of 33 subjects were enrolled (17 males, 16 females; mean age: 26 ± 3 years). OCTA en face images of 59 eyes were included. Seven eyes (10.6%) were excluded because of poor OCT image quality. The mean FAZ area was 0.33 ± 0.09 mm^2 at the SCP and 0.57 ± 0.17 mm^2 at the DCP measured with RS-3000 versus 0.30 ± 0.08 and 0.35 ± 0.08 mm^2, respectively, measured with Spectralis. The measurements of the 2 devices were significantly different both at the SCP and at the DCP (both $p < 0.0001$).

The intraoperator agreement was excellent with both devices at the SCP: ICC = 0.96 (95% CI: 0.93–0.98) with RS-3000 and 0.97 (95% CI: 0.93–0.98) with Spectralis. At the DCP, the intraoperator agreement was good with Spectralis: ICC = 0.85 (95% CI: 0.62–0.93), and fair with RS-3000: ICC = 0.64 (95% CI: 0.33–0.81). The intraoperator agreement was corroborated by Bland-Altman analysis (Fig. 2).

The interoperator agreement was excellent for Spectralis: ICC = 0.97 (95% CI: 0.93–0.98), and good for RS-3000: ICC = 0.93 (95% CI: 0.57–0.98) at the SCP. It was good at the DCP with both devices: ICC = 0.74 (95% CI: 0.52–0.87) with RS-3000 and 0.81 (95% CI: 0.63–0.91) with Spectralis. The interoperator agreement was corroborated by Bland-Altman analysis (Fig. 3).

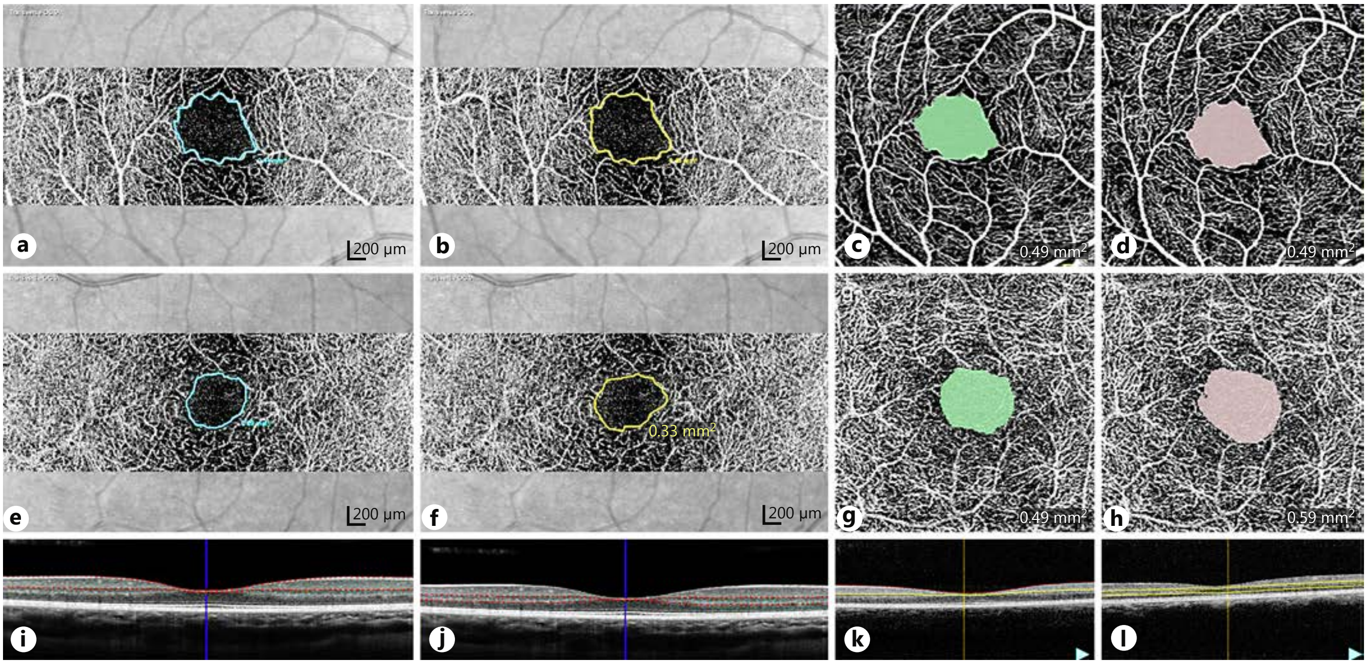

Fig. 1. En face images of the superficial (SCP) (**a–d**) and deep capillary plexus (DCP) (**e–h**) from Spectralis (**a**, **b**, **e**, **f**) and RS-3000 (**c**, **d**, **g**, **h**). The SCP image was visualized automatically segmenting a superficial slab extending from the inner limiting membrane (ILM) to the outer border of the inner plexiform layer (IPL) for the Spectralis (**i**) and from the ILM to 8 μm below the inner boundary of the inner nuclear layer (INL) for the RS-3000 (**k**). The DCP image was visualized automatically segmenting a deeper slab extending from the IPL outer border to the outer plexiform layer (OPL) outer border for the Spectralis (**j**) and from 12 μm below the inner boundary of the INL to a segmenting line localized 86 μm below for the RS-3000 (**l**). The FAZ area was automatically measured and visualized with different colors for the 2 graders (blue and yellow for Spectralis and green and rose for RS-3000).

Discussion

The advent of OCTA has appraised the interest in configuration and quantification of the FAZ area [1–8]. The FAZ enlargement rate, identified using fluorescein angiography, is an indicator of the progression of macular ischemia [9, 10]. When comparing fluorescein angiography and OCTA in the classification of diabetic retinopathy, OCTA allows better discrimination of FAZ disruption and capillary dropout in the superficial retinal layers [11]. Moreover, OCTA can separately visualize the SCP and the DCP; therefore, the possible different involvement of FAZ in these 2 plexuses is under investigation [6]. In agreement with the findings of Carpineto et al. [12] and Mastropasqua et al. [13], we detected excellent repeatability and reproducibility in FAZ area measurement when measuring the FAZ at the SCP [12, 13]. On the contrary, repeatability and reproducibility were lower when measuring the FAZ area at the DCP, in agreement with the finding of Ghasemi Falavarjani et al. [6]. FAZ limits can be clearly identified at the more superficial layer, whereas in the deeper retina the edge points along the centerline of the vessels are more difficult to delineate. Campbell et al. [16], using a novel OCTA algorithm, visualized that around the FAZ the retinal plexuses converge to form a single parafoveal capillary loop and collectively define the FAZ borders, in agreement with early histological studies in primates [14, 15]. Therefore, the authors recommend that the FAZ size be measured using an en face projection that includes all retinal plexuses [16].

In the present study we included only healthy eyes. Therefore, automated segmentation was correct in all images and no manual resegmentation was necessary. We may expect that in nonhealthy eyes, when intraretinal pathological changes are present, automatic segmentation may result in compromising measurement repeatability.

In the present study we compared FAZ measurements obtained by 2 different OCTA instruments, detecting that the measurements of the 2 devices were significantly different at both plexuses. We used the OCTA en face

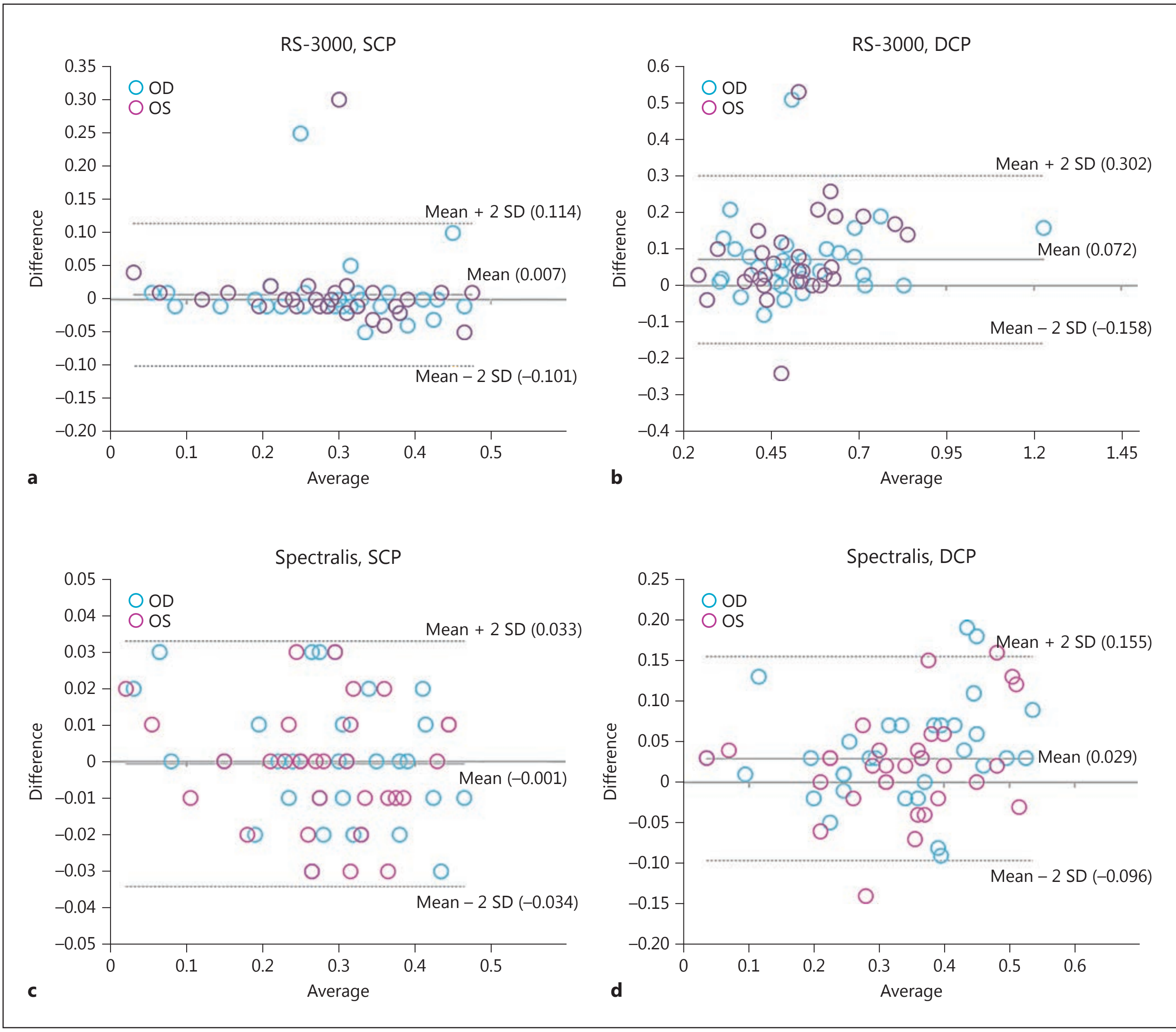

Fig. 2. Bland-Altman plot analysis of intraoperator repeatability. A nonsignificant bias resulted for the superficial capillary plexus (SCP) both for RS-3000 (**a**) and Spectralis (**c**) (mean: 0.007 and –0.001, respectively). A bigger but always limited bias was shown for the deep capillary plexus (DCP) for RS-3000 (**b**) and Spectralis (**d**) (mean: 0.072 and 0.029, respectively). No significant trend was evident. The narrowest agreement ranges were always shown for Spectralis compared to RS-3000 for both SCP and DCP (0.067 for Spectralis and 0.215 for RS-3000 at the SCP; 0.251 for Spectralis and 0.46 for RS-3000 at the DCP). In every plot 95% of the measured differences were within the range; in particular, they were all within the range when evaluating SCP with Spectralis. OD, oculus dextrus (right eye); OS, oculus sinister (left eye).

images of the SCP and DCP, automatically generated by the devices, for the measurement. However, the instruments use different extending slabs to identify them. To image the SCP, the RS-3000 sets the outer border of the slab deeper than that adopted by the Spectralis (Fig. 1). Campbell et al. [16] were able to confirm in vivo the presence of 3 vascular plexuses previously described in primate histological studies: 1 SCP and 2 additional capillary plexuses, 1 on either side of the INL (the intermediate capillary plexus [ICP] and the DCP) [14, 16]. A deeper superficial slab, including more packed vessels than both the SCP and the ICP, could explain the major

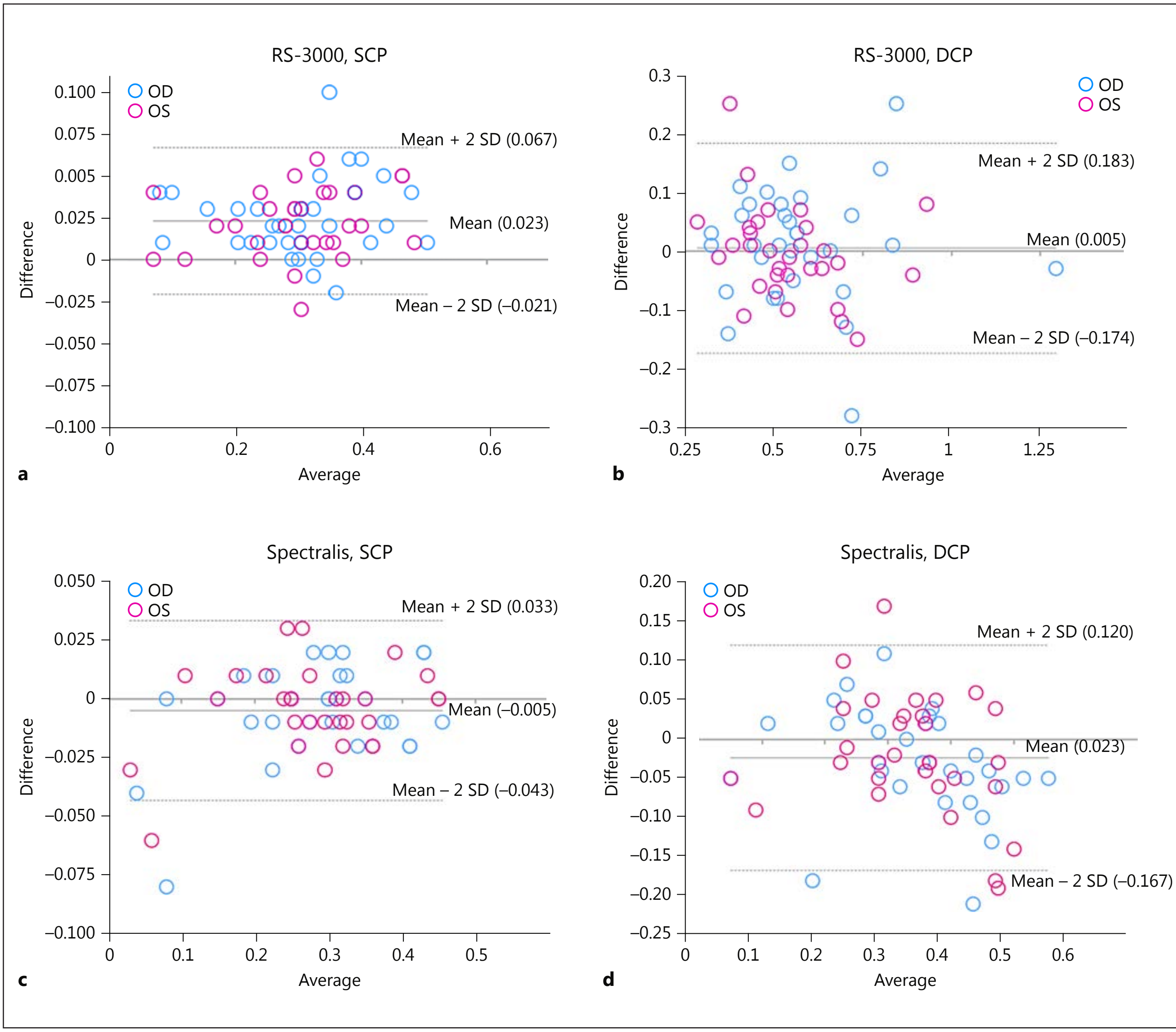

Fig. 3. Bland-Altman plot analysis of interoperator agreement. A nonsignificant bias resulted both for RS-3000 (**a**, **b**) and Spectralis (**c**, **d**), analyzing both the superficial capillary plexus (SCP) (mean: 0.023 and –0.005, respectively) and the deep capillary plexus (DCP) (mean: 0.005 and –0.023, respectively). No significant trend was evident. The narrowest agreement ranges were shown for the SCP (0.076 for Spectralis and 0.088 for RS-3000). For the DCP, the agreement range was slightly narrower for Spectralis compared to RS-3000 (0.287 for Spectralis and 0.357 for RS-3000). In every analysis, 95% of differences were within the range at SCP, while they were at the limit when analyzing the foveal avascular zone at the DCP with RS-3000 (5%) and slightly outside with Spectralis (8%). OD, oculus dextrus (right eye); OS, oculus sinister (left eye).

discrepancy between the RS-3000 and the Spectralis measurements we detected at the SCP and DCP. The main limitation of the present study is the small sample size.

In conclusion, FAZ measurements obtained with different OCTA devices are different. These differences appear to be primarily attributable to the different analysis algorithms used to set the SCP and DCP. These findings should be taken into consideration when separately studying the FAZ area in different retinal layers or when planning a follow-up study.

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Disclosure Statement

The authors have no conflict of interest to disclose for the submitted paper.

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