

Multicenter Assessment of OCT and OCT-A Measurement Reproducibility Using AI-Based Analysis in Healthy Eyes

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Abstract

PURPOSE

To assess repeatability and reproducibility of retinal measurements from spectral-domain optical coherence tomography (SD-OCT) and optical coherence tomography-angiography (OCT-A) using artificial intelligence tools Discovery® and CapillaryX® in healthy individuals in two centers.

METHODS

Twenty-three healthy participants underwent OCT and OCT-A examination at two time points in two centers. Retinal nerve fiber layer (RNFL) thickness, ganglion cell layer plus inner plexiform layer (GCL + IPL), and inner nuclear layer plus outer plexiform layer (INL + OPL) were automatically extracted from 3 x 3 mm OCT scans using Discovery® and Cohort Builder software's. Foveal avascular zone (FAZ) in superficial (SCP) and deep capillary plexus (DCP), vessel density (VD) and vascular parameters (bifurcation, diameter and tortuosity in arteries and veins) were derived from OCT-A scans using CapillaryX®. Inter-and intraclass correlation coefficients were calculated.

RESULTS

Among the performed measurements, all retinal layer thicknesses showed good repeatability and reproducibility, except for central RNFL. FAZ area and perimeter in SCP also showed good repeatability and reproducibility. Repeatability and reproducibility of vessel density was moderate to good, bifurcation and tortuosity were moderate, and vessel diameter was poor.

CONCLUSIONS

Most retinal layer thickness measurements with SD-OCT are repeatable and reproducible. OCT-A vascular measurements show variable reproducibility. FAZ metrics in the SCP and VD yield promising results.

Introduction

Diabetic retinopathy is the leading cause of blindness in the working-age population. It affects 34.6% of individuals with diabetes, corresponding to approximately 93 million people worldwide.¹ The disease develops insidiously with few or no symptoms in its early stages.²

On the one hand, diabetes induces vascular changes, characterized by the presence of microaneurysms which are caused by pericyte, endothelial cell, and capillary loss.^{3,4} On the other hand, diabetes induces

neurodegenerative changes in the retina, namely glial activation and neuronal apoptosis, leading to thinning of neuronal layers of the retina.^{5–7}

Neuronal and glial cells that interact with vascular cells in the retina form a neurovascular unit. It is the degradation of this unit that causes diabetic retinopathy.⁸ Currently, the causal relationship between neuronal and vascular degeneration remains unclear.⁶

Optical Coherence Tomography (OCT) provides high-resolution two-dimensional cross-sectional imaging (5–7 μm) to quantify retinal neuronal layer thinning (Fig. 1a).^{9–12} Identification of these histological neuronal changes may help to understand the loss of retinal functions in diabetic patients, such as reduced visual acuity, impaired dark adaptation, color vision loss, and visual field defects.^{13,14} Optical Coherence Tomography – Angiography (OCT-A), a newer imaging technology, uses red blood cell motion as an intrinsic contrast agent (Doppler effect) (Fig. 1b). This allows to visualize superficial and deep capillary plexuses in the retina to quantify vessel density and blood flow, and to follow vascular evolutions.¹⁵ Previous studies have described microvascular impairments in diabetic patients without clinical diabetic retinopathy.¹⁶ The degree of capillary loss in the deep capillary plexus is correlated with the level of visual acuity decline.¹⁷

Therefore, OCT-A could help to detect early vascular changes and identify patients at risk before the appearance of vascular lesions detected by ophthalmoscopy,^{18,19} even in patients with prediabetes.²⁰ OCT-A could also be used to monitor diabetic retinopathy progression.^{21–24}

The combined use of OCT and OCT-A could change diabetic retinopathy management by enabling early detection, risk stratification, and personalized treatment.^{24–27} However, image analysis is operator-dependent, time-consuming, and poorly reproducible. Therefore, the use of autonomous artificial intelligence could increase accuracy and allow large-scale phenotyping.²⁸ Although, few data exist on the reproducibility of OCT and OCT-A measurements.

The aim of the present study is to assess the reproducibility and reliability of retinal neuronal and vascular measurements assessed by OCT and OCT-A using new artificial intelligence tools Discovery®²⁹ and CapillaryX®³⁰ in healthy individuals in two centers. This will allow database integration for cohort studies and improve measurement consistency.

Methods

Study population

In this study, we used retrospective data from the Swiss Ophthalmic Imaging Network (SOIN),³¹ an ongoing prospective multicenter observational study. Briefly, the SOIN aims to build a secure, integrated image and data capture, management and analysis platform for an improved exchange of patient data

between clinicians and researchers. Data were extracted using the Cohort Builder pipeline³² and integrated into the OphtalmoLaus study.³³

Eligible patients consisted of consecutive patients between 18 and 55 years of age, who attended the department of ophthalmology of the University Hospitals of Geneva (HUG) or the Jules-Gonin ophthalmic hospital (HOJG) of Lausanne. Recruitment was performed through poster advertisements in clinic waiting areas.

The study protocol has been approved by the institutional medical ethical committee (BASEC 2022 – 01992). All participants gave written informed consent in accordance with the Declaration of Helsinki.

Optical Coherence Tomography and Optical Coherence Tomography-Angiography

All participants underwent automated refraction. Next, OCT and OCT-A examination was performed with the Topcon DRI OCT Triton 3D system (Topcon, Japan, software version 1.1.9) with the eye tracking function enabled.

For OCT measurements, a macular volume scan (3D horizontal, 512 x 256 sections, 7.0 mm) was performed in both eyes by experienced examiners masked participant characteristics. Regional numeric data of the macular thickness were derived from the Macular Thickness Map for the central and four pericentral subfields as defined by the ETDRS grid.³⁴ Scans were analyzed using ImageNet 6 (software version 1.26.16898). Automated segmentation of macular cross-sections was performed using Discovery® software (RetinAI Medical AG, Bern, Switzerland).^{29,35–37} Thickness measurements of the retinal nerve fiber layer (RNFL), ganglion cell layer plus inner plexiform layer (GCL + IPL), and inner nuclear layer plus outer plexiform layer (INL + OPL) were automatically exported by via the Cohort Builder tool.³²

All macular scans were reviewed for quality and measurement errors by a single grader (AD). Macular grids were recentered on the fovea if needed (Fig. 2). Individuals with an incomplete volume scan, poor image quality (< 35 dB), segmentation errors at the fovea, unsatisfactory real-time tracking, and retinal disorders (i.e. epiretinal membrane, cysts, or previous laser treatment) were excluded.

Next, OCT-A was performed in all participants using the Topcon DRI OCT Triton 3D system (Topcon, Japan) with motion tracking enabled (320 x 320 sections, 4.5 mm). All OCT-A scans were reviewed for quality and measurement errors in a blinded fashion by a single grader (AD). Poor quality images (< 35 dB), and scans with motion or blinking artefacts were excluded.

Boundaries of the superficial and deep capillary plexus were defined by ImageNet software. OCT-A features were selected based on clinical relevance and correlation between the different vascular parameters. Foveal avascular zone (FAZ) in the superficial (SCP) and deep capillary plexus (DCP), vessel

density (VD), and retinal vascular parameters (bifurcation, diameter, and tortuosity in arteries and veins) were automatically extracted using CapillaryX®.³⁰ Values were automatically exported.

All measurements were performed bilaterally and data from both eyes were included.

Study protocol

To assess repeatability of OCT and OCT-A measurements in each center, two observers at HUG and four observers at HOJG performed the measurements independently on the same day using the same device settings and a common standard operating procedure. Observers were masked with respect to the identity and medical history of the participants.

To assess reproducibility, the same observers performed OCT and OCT-A measurements on the same day. Two observers performed the measurements at HUG, while four observers performed the measurements at HOJG.

Statistical analysis

Statistical analysis was performed using Python built-in library version 3.9.0. Mean value and standard deviation were calculated for all outcome variables. Normal distribution of retinal thickness values was verified with the Kolmogorov-Smirnov test and significance of mean value differences was tested with the paired t-test. Statistical significance was defined as $p < 0.05$.

Reproducibility was assessed by the intraclass correlation coefficient (ICC).³⁸ An ICC ≥ 0.70 was considered good, between 0.50 and 0.70 moderate, and < 0.50 poor. Bootstrap resampling was performed to obtain the confidence intervals. ICC was calculated according to the following standard formulas:^{39,40}

$$\begin{aligned} \text{inter} &= \frac{\frac{2}{\text{participants}}}{\frac{2}{\text{participants}} + \frac{2}{\text{technicians}} + \frac{2}{\text{residual}}} \\ \text{intra} &= \frac{\frac{2}{\text{participants}} + \frac{2}{\text{technicians}}}{\frac{2}{\text{participants}} + \frac{2}{\text{technicians}} + \frac{2}{\text{residual}}} \end{aligned}$$

Variance components were calculated using a linear mixed-effects model fitted to each measure:

$$ijtk01ijtkitijtk$$

Where i denotes the participant, t the technician, j the repeated observation, and k the measurement. The fixed effects are β_0 (overall effect) and β_1 effect of laterality (left or right eye). The random effects are the participant-specific random intercept u_i and the technician variance component t . $ijtk$ is the residual error.

Results

Characteristics of the 23 participants included in the study are shown in Table 1, based on the first measurement and stratified according to the study center. There was a significant difference in sex between the two centers (50% men at HUG and 7% at HOJG).

Table 1

Baseline characteristics of the study population, stratified by study center.

Age (years), mean (SD)	HUG	HOJG
	(N = 10)	(N = 13)
	40.8 (8.5)	57.2 (3.8)
Male sex, n (%)	5 (50)	1 (7)
Spherical equivalent (D), mean (SD)	-0.39 (3.6)	1.3 (1.5)
HUG, University Hospital of Geneva; HOJG, Jules-Gonin Ophthalmic Hospital.		

None of the participants had a refractive error of more than S + 5 or S-8 diopters, any macular disorder, previous laser treatment, or a central field defect preventing fixation during OCT. 26% of the scans from HUG were removed due to image quality issues, whereas all scans acquired at HOJG met or exceeded the manufacturer’s quality threshold and were retained for analysis (Fig. 3). 20 of the OCT macular grids from HUG and 5 of those from HOJG needed to be recentered on the fovea (Fig. 2).

Repeatability and reproducibility of OCT retinal neuronal layer thickness measurements in the central and pericentral area at HUG before recentering and filtering on quality (N = 11 participants; 19 eyes) are presented in Appendix 1.

Table 2 shows repeatability and reproducibility after filtering on quality of OCT measurements of the thickness of the neuronal layers of the retina at the central and pericentral area and recentering at HUG (N = 10 participants, 14 eyes). Repeatability was excellent for all retinal layer thicknesses with intraobserver ICC values ranging from 0.96 [0.89, 1.00] for central RNFL thickness to 1.00 [0.99, 1.00] for pericentral GCL + IPL thickness.

Table 2
Repeatability and reproducibility of retinal layer thickness measurements using OCT at University Hospitals of Geneva

(N = 10 participants, 14 eyes)

Central RNFL thickness (μm)	ICC inter	ICC intra
	0.61 [0.37, 0.76]	0.96 [0.89, 1.0]
Central GCL + IPL thickness (μm)	0.98 [0.94, 0.99]	0.99 [0.98, 1.0]
Central INL + OPL thickness (μm)	0.96 [0.87, 0.99]	0.99 [0.99, 1.0]
Central RNFL + GCL + IPL + INL + OPL thickness (μm)	0.85 [0.62, 0.95]	0.94 [0.83, 1.0]
Pericentral RNFL thickness (μm)	0.87 [0.74, 0.95]	0.97 [0.95, 0.99]
Pericentral GCL + IPL thickness (μm)	0.98 [0.97, 0.99]	1.00 [0.99, 1.0]
Pericentral INL + OPL thickness (μm)	0.89 [0.78, 0.97]	0.99 [0.97, 1.0]
Pericentral RNFL + GCL + IPL + INL + OPL thickness (μm)	0.98 [0.96, 0.99]	0.99 [0.97, 1.0]
RNFL, retinal nerve fiber layer; GCL + IPL, ganglion cell layer and inner plexiform layer; INL + OPL, inner nuclear layer and outer plexiform layer.		
ICC [95% CI]		
ICC < 0.5 = poor repeatability, ICC 0.5–0.7 = moderate repeatability, ICC $\geq$ 0.7 = good repeatability.		

Reproducibility was good for all retinal layer thicknesses with interobserver ICC values between 0.85 [0.62, 0.95] for central RNFL + GCL+IPL + INL+OPL thickness and 0.98 [0.96, 0.99) for pericentral GCL + IPL thickness, except for the central RNFL thickness showing moderate reproducibility (ICC 0.61 [0.37, 0.76]).

Repeatability and reproducibility of OCT retinal neuronal layer thickness measurements in the central and pericentral area at HOJG before recentering (N = 13 participants; 26 eyes) are presented in Appendix 2.

Table 3 shows repeatability and reproducibility after recentering at HOJG (N = 13 participants, 26 eyes). Repeatability was good for all retinal layer thicknesses with intraobserver ICC values between 0.73 [0.41, 0.94] for central RNFL thickness and 0.97 [0.93, 0.99] for pericentral RNFL + GCL+IPL + INL+OPL thickness.

Table 3
Repeatability and reproducibility of retinal layer thickness measurements using OCT at Jules Gonin
Ophthalmic Hospital

(N = 13 participants, 26 eyes)

Central RNFL thickness (μm)	ICC inter	ICC intra
	0.70 [0.30, 0.92]	0.73 [0.41, 0.94]
Central GCL + IPL thickness (μm)	0.80 [0.64, 0.92]	0.85 [0.69, 0.98]
Central INL + OPL thickness (μm)	0.71 [0.45, 0.88]	0.77 [0.50, 0.95]
Central RNFL + GCL + IPL + INL + OPL thickness (μm)	0.77 [0.58, 0.92]	0.84 [0.61, 0.97]
Pericentral RNFL thickness (μm)	0.82 [0.68, 0.94]	0.88 [0.72, 0.97]
Pericentral GCL + IPL thickness (μm)	0.98 [0.96, 0.99]	0.98 [0.96, 0.99]
Pericentral INL + OPL thickness (μm)	0.92 [0.77, 0.97]	0.93 [0.78, 0.98]
Pericentral RNFL + GCL + IPL + INL + OPL thickness (μm)	0.97 [0.93, 0.99]	0.97 [0.93, 0.99]
RNFL, retinal nerve fiber layer; GCL + IPL, ganglion cell layer and inner plexiform layer; INL + OPL, inner nuclear layer and outer plexiform layer.		
ICC [95% CI]		
ICC < 0.5 = poor repeatability, ICC 0.5–0.7 = moderate repeatability, ICC ≥ 0.7 = good repeatability.		

Reproducibility was good with ICC interobserver values between 0.70 [0.30, 0.92] for central RNFL thickness and 0.98 [0.96, 0.99] for pericentral GCL + IPL thickness.

Table 4 shows repeatability and reproducibility of OCT-A measurements of the FAZ in the SCP and DCP, VD and retinal vascular parameters (bifurcation, diameter and tortuosity in arteries and veins) at HUG (N = 10 participants, 20 eyes). Repeatability was good for arterial tortuosity (0.84 [0.68, 0.97]), venous tortuosity (0.74 [0.57, 0.86]), arterial VD (0.84 [0.63, 0.95]), venous VD (0.71 [0.43, 0.94]), arterial bifurcation (0.75 [0.54, 0.88]), FAZ area SCP (0.99 [0.97, 1.00]), FAZ area DCP (0.70 [0.56, 0.84]), and FAZ perimeter SCP (0.94 [0.87, 0.98]); moderate for venous bifurcation (0.59 [0.34, 0.83]), FAZ circularity SCP (0.55 [0.34, 0.78]), FAZ eccentricity SCP (0.52 [0.25, 0.77]), and FAZ perimeter DCP (0.64 [0.43, 0.83]); and poor for arterial median diameter (0.31 [0.04, 0.67]), venous median diameter (0.28 [0.05, 0.57]), FAZ circularity DCP (0.48 [0.26, 0.72]), FAZ eccentricity DCP (0.43 [0.13, 0.71]), FAZ roundness SCP (0.44 [0.13, 0.77]), and FAZ roundness DCP (0.39 [0.07, 0.69]).

Table 4
Repeatability and reproducibility of retinal vascular parameters using OCT-A at University Hospitals of Geneva

(N = 10 participants, 20 eyes)

A median diameter	ICC inter	ICC intra
	0.20 [0.00, 0.56]	0.31 [0.04, 0.67]
V median diameter	0.17 [0.00, 0.43]	0.28 [0.05, 0.57]
A tortuosity	0.73 [0.50, 0.89]	0.84 [0.68, 0.97]
V tortuosity	0.71 [0.50, 0.84]	0.74 [0.57, 0.86]
A vessel density	0.84 [0.63, 0.95]	0.84 [0.63, 0.95]
V vessel density	0.67 [0.32, 0.92]	0.71 [0.43, 0.94]
A bifurcation	0.73 [0.51, 0.87]	0.75 [0.54, 0.88]
V bifurcation	0.54 [0.15, 0.82]	0.59 [0.34, 0.83]
FAZ area SCP	0.99 [0.97, 1.00]	0.99 [0.97, 1.00]
FAZ area DCP	0.65 [0.48, 0.80]	0.70 [0.56, 0.84]
FAZ circularity SCP	0.33 [0.03, 0.59]	0.55 [0.34, 0.78]
FAZ circularity DCP	0.30 [0.05, 0.54]	0.48 [0.26, 0.72]
FAZ eccentricity SCP	0.39 [0.00, 0.70]	0.52 [0.25, 0.77]
FAZ eccentricity DCP	0.16 [0.00, 0.42]	0.43 [0.13, 0.71]
FAZ perimeter SCP	0.93 [0.84, 0.97]	0.94 [0.87, 0.98]
FAZ perimeter DCP	0.54 [0.33, 0.72]	0.64 [0.43, 0.83]
FAZ roundness SCP	0.22 [0.00, 0.49]	0.44 [0.13, 0.77]
FAZ roundness DCP	0.14 [0.00, 0.47]	0.39 [0.07, 0.69]
A, artery; V, vein; FAZ, foveal avascular zone; SCP, superficial capillary plexus; DCP, deep capillary plexus.		
ICC [95% CI]		
ICC < 0.5 = poor repeatability, ICC 0.5–0.7 = moderate repeatability, ICC ≥ 0.7 = good repeatability.		

Reproducibility was good for arterial tortuosity (0.73 [0.50, 0.89]), venous tortuosity (0.71 [0.50, 0.84]), arterial VD (0.84 [0.63, 0.95]), arterial bifurcation (0.73 [0.51, 0.87]), FAZ area SCP (0.99 [0.97, 1.00]), and FAZ perimeter SCP (0.93 [0.84, 0.97]); moderate for venous VD (0.67 [0.32, 0.92]), venous bifurcation (0.54 [0.15, 0.82]), FAZ area DCP (0.65 [0.48, 0.80]), and FAZ perimeter DCP (0.54 [0.33, 0.72]); and poor

for arterial median diameter (0.20 [0.00, 0.56]), venous median diameter (0.17 [0.00, 0.43]), FAZ circularity SCP (0.33 [0.03, 0.59]), FAZ circularity DCP (0.30 [0.05, 0.54]), FAZ eccentricity SCP (0.39 [0.00, 0.70]), FAZ eccentricity DCP (0.16 [0.00, 0.42]), FAZ roundness SCP (0.22 [0.00, 0.49]) and FAZ roundness DCP (0.14 [0.00, 0.47]).

Table 5 shows the repeatability and reproducibility of OCT-A measurements of the FAZ in the SCP and DCP, VD, and vascular parameters (bifurcation, diameter and tortuosity in arteries and veins) at HOJG (N = 13 participants, 26 eyes). Repeatability was good for venous median diameter (0.75 [0.52, 0.91]), arterial tortuosity (0.83 [0.72, 0.93]), arterial VD (0.70 [0.49, 0.89], venous VD (0.78 [0.63, 0.90]), arterial bifurcation (0.70 [0.45, 0.84]), FAZ area SCP (0.95 [0.90, 0.99]) and FAZ perimeter SCP; moderate for arterial median diameter (0.60 [0.27, 0.89]), venous tortuosity (0.68 [0.42, 0.89]), venous bifurcation (0.69 [0.51, 0.87]), FAZ eccentricity SCP (0.61 [0.30, 0.88]), FAZ eccentricity DCP (0.58 [0.37, 0.74]), FAZ roundness SCP (0.55 [0.25, 0.86]), and FAZ roundness DCP (0.51 [0.26, 0.74]); and poor for FAZ area DCP (0.40 [0.11, 0.76]), FAZ circularity SCP (0.49 [0.23, 0.75]), FAZ circularity DCP (0.31 [0.08, 0.56]), and FAZ perimeter DCP (0.34 [0.11, 0.66]).

Table 5
Repeatability and reproducibility of retinal vascular parameters using OCT-A at Jules Gonin Ophthalmic Hospital

(N = 13 participants, 26 eyes)

A median diameter	ICC inter	ICC intra
	0.47 [0.01, 0.75]	0.60 [0.27, 0.89]
V median diameter	0.47 [0.07, 0.77]	0.75 [0.52, 0.91]
A tortuosity	0.74 [0.60, 0.84]	0.83 [0.72, 0.93]
V tortuosity	0.61 [0.27, 0.86]	0.68 [0.42, 0.89]
A vessel density	0.61 [0.34, 0.81]	0.70 [0.49, 0.89]
V vessel density	0.63 [0.41, 0.81]	0.78 [0.63, 0.90]
A bifurcation	0.67 [0.35, 0.82]	0.70 [0.45, 0.84]
V bifurcation	0.48 [0.21, 0.69]	0.69 [0.51, 0.87]
FAZ area SCP	0.95 [0.90, 0.98]	0.95 [0.90, 0.99]
FAZ area DCP	0.31 [0.00, 0.73]	0.40 [0.11, 0.76]
FAZ circularity SCP	0.39 [0.07, 0.65]	0.49 [0.23, 0.75]
FAZ circularity DCP	0.16 [0.00, 0.39]	0.31 [0.08, 0.56]
FAZ eccentricity SCP	0.49 [0.18, 0.74]	0.61 [0.30, 0.88]
FAZ eccentricity DCP	0.41 [0.20, 0.55]	0.58 [0.37, 0.74]
FAZ perimeter SCP	0.88 [0.76, 0.95]	0.89 [0.79, 0.97]
FAZ perimeter DCP	0.21 [0.00, 0.59]	0.34 [0.11, 0.66]
FAZ roundness SCP	0.42 [0.03, 0.76]	0.55 [0.25, 0.86]
FAZ roundness DCP	0.17 [0.00, 0.44]	0.51 [0.26, 0.74]
A, artery; V, vein; FAZ, foveal avascular zone; SCP, superficial capillary plexus; DCP, deep capillary plexus.		
ICC [95% CI]		
ICC < 0.5 = poor repeatability, ICC 0.5–0.7 = moderate repeatability, ICC ≥ 0.7 = good repeatability.		

Reproducibility was good for arterial tortuosity (0.74 [0.60, 0.84]), FAZ area SCP (0.95 [0.90, 0.98]), and FAZ perimeter SCP (0.88 [0.76, 0.95]); moderate for venous tortuosity (0.61 [0.27, 0.86]), arterial VD (0.61 [0.34, 0.81]), venous VD (0.63 [0.41, 0.81]), and arterial bifurcation (0.67 [0.35, 0.82]); and poor for arterial median diameter (0.47 [0.01, 0.75]), venous median diameter (0.47 [0.07, 0.77]), venous bifurcation (0.48

[0.21, 0.69]), FAZ area DCP (0.31 [0.00, 0.73]), FAZ circularity SCP (0.39 [0.07, 0.65]), FAZ circularity DCP (0.16 [0.00, 0.39]), FAZ eccentricity SCP (0.49 [0.18, 0.74]), FAZ eccentricity DCP (0.41 [0.20, 0.55]), FAZ perimeter DCP (0.21 [0.00, 0.59]), FAZ roundness SCP (0.42 [0.03, 0.76]) and FAZ roundness DCP (0.17 [0.00, 0.44]).

Discussion

To allow the integration of clinical imaging databases from different Swiss investigation centers for future cohort studies, we evaluated the repeatability and reproducibility of OCT and OCT-A measurements using two artificial intelligence tools: Discovery®²⁹ and CapillaryX®.³⁰ In this multicenter study, we have assessed the reliability of OCT and OCT-A scans to characterize retinal neuronal and vascular layers in healthy individuals.

For OCT-measurements, we found excellent repeatability and reproducibility for all retinal layers in both centers, except for the central RNFL showing moderate agreement.

For OCT-A measurements, we showed good repeatability and reproducibility for the FAZ area and the FAZ perimeter in the SCP in both centers. Vessel density showed moderate to good reliability, whereas bifurcation and tortuosity showed moderate reliability. In contrast, vessel diameter showed poor reliability in both centers, which could be explained by limitations in image resolution and segmentation algorithms for small vessels.

This is the first study describing the methodology and reproducibility of OCT and OCT-A measurements performed within the Swiss Ophthalmic Imaging Network. The infrastructure of this imaging network includes “Swiss Eye Expertise”, a tele-expertise bridge between clinicians and hospitals; “EyeConsent”, dynamic management of consent from 30'000 patients, and “MI DataLab”, advanced IT resources for imaging research. The establishment of precise measurement and analysis protocols allows to collect quality clinical data and to define the relevant neuronal and vascular parameters for the detection of diabetic retinopathy. By the use of autonomous artificial intelligence tools, SOIN aims to reduce variability, improve reproducibility, and facilitate the integration of clinical imaging databases from different centers. This infrastructure enables large-scale phenotyping and supports progress toward predictive, preventive, and personalized approaches in ophthalmic research.

Repeatability, often referred to as intra-observer reliability, of OCT measurements was first assessed at the HOJG based on retrospective clinical data from the OphthmoLaus database.³³ The analysis showed good ICC values for all variables ensuring the reliability of the measurement protocol. The same protocol was used by the HUG showing ICC values that were in line with the results of the HOJG and confirming the robustness of the measurement procedure.

We showed a good correlation for measurements of neuronal and vascular intraretinal layers, both within and between examiners. This likely reflects the high image quality of the scans, the use of standardized acquisition protocols, and the distinct reflectivity profiles that facilitate accurate layer segmentation.

However, foveal retinal neuronal layer thicknesses showed a slightly lower correlation because the foveal neuronal layers are extremely thin and therefore difficult to segment (Fig. 4). Our results are in line with the findings of previous studies, reporting good repeatability for OCT measurement of retinal neuronal layer thickness.^{41–44}

Nevertheless, OCT-A vascular parameters showed variable ICC values ranging from good to poor. These results are in contrast with previous studies.^{45–51} The most reliable variables in both centers were arterial tortuosity, arterial and venous vessel density, arterial bifurcation, superficial capillary plexus foveal avascular zone area and superficial capillary plexus foveal avascular perimeter. According to previous papers, superficial capillary plexus foveal avascular zone circularity is one of the less repeatable OCT-A parameters, as shown by our results on the repeatability of the deep capillary plexus avascular zone circularity, eccentricity and roundness. These parameters seem to be more affected by noise and quality issues. In contrast, the FAZ perimeter and FAZ area are not that subjective to noise and quality issues, which explains their superior reliability.

Repeatability for arterial and venous diameter was moderate to good and vessel tortuosity and density showed good repeatability, in contrast with the bifurcation of the veins which showed lower repeatability values mainly due to some outliers. Only the FAZ perimeter SCP showed good repeatability. Lower repeatability of the deep vascular parameters compared with the superficial parameters could be explained by the fact that our AI model was primarily trained on SCP data. Additional training of our AI model for segmentation of DCP data is needed.

Overall, our findings confirm the high precision of the Topcon OCT device and the good repeatability of the Discovery® software.³² However, previous studies showed that results cannot be interchanged when using different scan protocols and devices.⁵² Further investigation is needed to improve the repeatability of OCT-A measurements, in particular for DCP parameters, and to validate these findings in larger cohorts.

ICC values derived from repeatability studies allow to assess the reproducibility of measurement methods, often referred to as inter-observer reliability. As expected, the results for reproducibility were slightly lower than the results for repeatability due to the variability induced by multiple observers. Our results are in line with previously published studies and confirm the suitability of OCT and OCT-A measures for multicenter studies.^{53,54}

In both centers, reproducibility was good for all intraretinal layer thicknesses, with a slightly lower reproducibility for the central RNFL thicknesses. Reproducibility of the OCT-A measures ranged from moderate to good for most variables, except for arterial and venous median diameter, venous bifurcation, circularity, eccentricity, and roundness of the foveal avascular zone in the SCP and DCP, which showed poor reproducibility. These findings suggest that certain vascular parameters are sensitive to image quality, variability in segmentation, and systematic differences between observers. The poor reproducibility of vein bifurcation measurements could be explained by systematic differences

between observers. This highlights the importance of standardized training and strict application of the standardized operating procedure to reduce observer bias.

When we compare both study centers, reliability of OCT measurements was slightly higher for HUG compared to HOJG. The difference between both centers could be explained by (1) higher resolution of the scans for HUG due to a smaller field of view, a greater number of sections, and (2) more frequent recentering of the scans at the post-processing stage. These differences should be interpreted with caution, as the acquisition involved a different number of technicians.

Reliability of OCT-A was also higher for HUG compared to HOJG. The younger mean age of the included participants at HUG could result in better fixation, although eye was enabled in both centers. Finally, the skills of the technicians could differ between both centers. Therefore, an additional quality control of the AV segmentation and FAZ annotation was necessary. After re-running the analysis, intra-observer reliability significantly increased after manual annotation of the FAZ, but no additional increase of the reliability was observed after manual AV segmentation.

Image quality of OCT-A measurements also differed between both centers. This is due to the fact that a predefined threshold for OCT-A quality was set at HOJG, which excluded scans below 35 dB as part of a larger dataset (OphtalmoLaus).³³ Additional corrections for deep features were performed. In contrast, the HUG dataset better reflects real-life hospital conditions. Variations in pixel density could be solved by advanced software. However, in patients with maculopathy, adjustments of the algorithm should not overrule anatomical accuracy. OCT-A images were assessed by a deep learning technique. For methodological reasons, manual annotation of the FAZ was needed for the deep capillary plexus, since the model was trained for the superficial plexus. Overall, deep learning FAZ annotation performed well.

Differences between both centers could also be explained by differences in the baseline characteristics of the study population. At HOJG, participants were significantly older and predominantly female (7% male). Age progression is known to substantially decrease macular thickness and vessel density, which explains the lower OCT and OCT-A values at HOJG.^{55,56} However, until now no studies have shown a significant effect of sex on retinal or vascular layer measurements. Increasing axial length or spherical equivalent is known to decrease retinal thickness and increasing astigmatism is known to significantly reduce FAZ, parafoveal vessel density and perfusion density.^{12,57} We did not find any significant difference in astigmatism or spherical equivalent between both groups.

Our findings suggest that international standardized methods and acceptance criteria for image analysis, experienced observers, and accurate automated segmentation software are needed to reduce discrepancy and improve reproducibility of OCT-A measurements. In contrast, OCT-measurements showed good reliability, suggesting that the current standardized operating procedures are appropriate and that human factors influencing the results are limited.

Our findings also show the accuracy of the RetinAI Discovery and CapillaryX® software.^{29,30,35–37} Previously, an ARVO poster reported the impact of acquisition device and quality control on test-retest

reliability of thickness measurements derived from the RetinAI OCT segmentation model.²⁹ To our knowledge, this is the first paper assessing repeatability and reproducibility of retinal layer thickness and vascular parameters using these automated software after repeated measurements by different observers. This allows a better understanding of the design, efficacy, safety, and reliability of both artificial intelligence tools, which is an essential step through the development and validation process of Artificial Intelligence for clinical applications.⁵⁸

Some methodological issues deserve discussion. A 3 x 3 mm size of OCT-A scan was chosen, which is known to have a higher interrater reliability compared to larger scans.^{59,60} Vessel density was measured in pixels/mm² rather than as a count per mm², which is more frequently used in recent literature. However, the use of a measurement in pixels/mm² has the advantage to be more precise. Measurements were performed by two observers at HUG and four observers at HOJG, which required the use of appropriate statistical formulas for calculating the ICC and account for interobserver variability. Furthermore, the OCT-A scans at HUG were taken one year after the OCT scans due to technical issues.

Some quality issues were identified. First, in certain cases, recentering of the OCT scans was necessary. Therefore, both the results before and after recentering of the OCT scans were presented in this paper, showing a significant increase in repeatability and reproducibility following this adjustment. However, recentering of the scans often resulted in an incomplete scan area that made the ETDRS outer ring region not fully measured. Therefore, peripheral retinal thickness measurements were not included in this paper.

Second, certain OCT-A scans showed artefacts due to eye movement and superposition of segmentation levels. These scans were excluded to avoid erroneous analysis of blood vessel density, AV segmentations, FAZ area, and FAZ circularity. However, small segmentation errors located away from the center of the deep FAZ were automatically excluded by the software (Fig. 5). Furthermore, we did not observe artefacts due to reflections of hard exudates since we only included healthy participants. No reflections due to vitreous opacities were observed.

Our results confirm that OCT measurements are more reliable than OCT-A measurements, which is consistent with previous studies. OCT is known to be a very reliable measurement thanks to its laser technique and the use of good segmentation software, which reduce variability. In contrast, OCT-A is more susceptible to fixation-related artefacts, which become more important in patients with maculopathy. Although our study population only included healthy eyes, limiting the impact of bad fixation, many participants presented errors due to superposition of segmentation levels. This reduces reliability because measurements may not correspond to the same anatomical plane.

Future work should focus on developing three-dimensional assessments of vessel density and the foveal avascular zone to minimize artefacts inherent to two-dimensional analyses.

This high precision is particularly relevant to define quantitative cut-off values for diagnosis or biomarkers for disease progression. At present, OCT-A is mainly used as a qualitative tool, allowing visualization of neovascularization and its subtype classification.

This study highlights the need of evaluating the repeatability and reproducibility of OCT and OCT-A measurements, not only for research studies, but also to support evidence-based clinical decision-making. Measurements demonstrating poor repeatability should be excluded from clinical analyses to prevent potential misinterpretation. Tools such as Bland-Altman and box plot graphics are useful to identify outliers and can constitute an efficient tool for quality control in all centers. Furthermore, intercenter collaboration is important to make measurements reproducible by aligning the acquisition protocols and analysis procedures. Further collaboration between OCT and OCT-A manufacturers may help reduce variability introduced by proprietary post-processing algorithms.

Further research is needed to standardize OCT and OCT-A imaging biomarkers in diabetic retinal disease.^{61,62} The use of standard protocols for image acquisition and analysis is crucial to explain the pathophysiology of development and evolution of diabetic retinopathy within the neurovascular unit. This will support early diagnosis and personalized treatment; including vascular (angiotensin 1 and 2 receptor antagonists) and neuroprotective (statins, ginkgo biloba, palmitoylethanolamide or topical administration of somatostatin and brimonidine) treatment of diabetic retinopathy,⁶³ in order to avoid or to delay laser and surgical treatments, which are risky and expensive. Improved management of this chronic degenerative condition may help slow disease progression and preserve visual function, ultimately enhancing patients' quality of life. However, before incorporation of OCT and OCT-A to routine screening programs for diabetic retinopathy, their incremental benefit needs to be determined in addition to conventional clinical investigations.

To improve the model of artificial intelligence software, a database containing segmentations validated by specialists is needed. Next, ophthalmologists should define morphological phenotypes relevant to the pathogenesis of the disease by comparing data from healthy individuals and patients at intermediate or advanced stages of the disease. Finally, understanding the role of these phenotypes in disease progression could support the development of a kinetic model of diabetic retinopathy and other retinal diseases, allowing predictive and personalized care.

In conclusion, most retinal layer thickness measurements with SD-OCT are repeatable and reproducible. OCT-A vascular measurements show variable reproducibility. FAZ metrics in the SCP and VD yield promising results. These findings highlight the importance of validating OCT and OCT-A measurements reliability, not only for research purposes, but also to support evidence-based clinical decision making. To improve reproducibility, international accepted criteria for image acquisition and analysis, experienced observers, and accurate automated segmentation software are needed.

Declarations

Conflicts of interest

The authors have no conflicts of interest to declare.

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References

1. Yau JW, Rogers SL, Kawasaki R, et al. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care* 2012; 35(3): 556–64.
2. Dyck PJ, Norell JE, Tritschler H, et al. Challenges in design of multicenter trials: end points assessed longitudinally for change and monotonicity. *Diabetes Care* 2007; 30(10): 2619–25.
3. Friedenwald JS. Diabetic retinopathy. *Am J Ophthalmol* 1950; 33(8): 1187–99.
4. Cogan DG, Toussaint D, Kuwabara T. Retinal vascular patterns. IV. Diabetic retinopathy. *Arch Ophthalmol* 1961; 66: 366–78.
5. Barber AJ, Lieth E, Khin SA, Antonetti DA, Buchanan AG, Gardner TW. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. *J Clin Invest* 1998; 102(4): 783–91.
6. Simo R, Hernandez C, European Consortium for the Early Treatment of Diabetic R. Neurodegeneration in the diabetic eye: new insights and therapeutic perspectives. *Trends Endocrinol Metab* 2014; 25(1): 23–33.
7. van Dijk HW, Verbraak FD, Kok PH, et al. Early neurodegeneration in the retina of type 2 diabetic patients. *Invest Ophthalmol Vis Sci* 2012; 53(6): 2715–9.
8. Antonetti DA, Klein R, Gardner TW. Diabetic retinopathy. *N Engl J Med* 2012; 366(13): 1227–39.
9. Fischer MD, Huber G, Beck SC, et al. Noninvasive, in vivo assessment of mouse retinal structure using optical coherence tomography. *PLoS One* 2009; 4(10): e7507.

10. van Dijk HW, Verbraak FD, Stehouwer M, et al. Association of visual function and ganglion cell layer thickness in patients with diabetes mellitus type 1 and no or minimal diabetic retinopathy. *Vision Res* 2011; 51(2): 224–8.
11. De Clerck EE, Schouten JS, Berendschot TT, et al. New ophthalmologic imaging techniques for detection and monitoring of neurodegenerative changes in diabetes: a systematic review. *Lancet Diabetes Endocrinol* 2015; 3(8): 653–63.
12. De Clerck EEB, Schouten J, Berendschot T, et al. Macular thinning in prediabetes or type 2 diabetes without diabetic retinopathy: the Maastricht Study. *Acta Ophthalmol* 2018; 96(2): 174–82.
13. Han Y, Bearse MA, Jr., Schneck ME, Barez S, Jacobsen CH, Adams AJ. Multifocal electroretinogram delays predict sites of subsequent diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2004; 45(3): 948–54.
14. Abcouwer SF, Gardner TW. Diabetic retinopathy: loss of neuroretinal adaptation to the diabetic metabolic environment. *Ann N Y Acad Sci* 2014; 1311: 174–90.
15. Pappelis K, Jansonius NM. Quantification and Repeatability of Vessel Density and Flux as Assessed by Optical Coherence Tomography Angiography. *Transl Vis Sci Technol* 2019; 8(3): 3.
16. Zhang B, Chou Y, Zhao X, Yang J, Chen Y. Early Detection of Microvascular Impairments With Optical Coherence Tomography Angiography in Diabetic Patients Without Clinical Retinopathy: A Meta-analysis. *Am J Ophthalmol* 2021; 222: 226–37.
17. Dupas B, Minvielle W, Bonnin S, et al. Association Between Vessel Density and Visual Acuity in Patients With Diabetic Retinopathy and Poorly Controlled Type 1 Diabetes. *JAMA Ophthalmol* 2018; 136(7): 721–8.
18. Johannesen SK, Viken JN, Vergmann AS, Grauslund J. Optical coherence tomography angiography and microvascular changes in diabetic retinopathy: a systematic review. *Acta Ophthalmol* 2019; 97(1): 7–14.
19. Kannenkeril D, Nolde JM, Kiuchi MG, et al. Retinal Capillary Damage Is Already Evident in Patients With Hypertension and Prediabetes and Associated With HbA1c Levels in the Nondiabetic Range. *Diabetes Care* 2022; 45(6): 1472–5.
20. Li Rudvan AL, Can ME, Efe FK, Keskin M, Beyan E. Evaluation of retinal microvascular changes in patients with prediabetes. *Niger J Clin Pract* 2021; 24(6): 911–8.
21. Aschauer J, Pollreisz A, Karst S, et al. Longitudinal analysis of microvascular perfusion and neurodegenerative changes in early type 2 diabetic retinal disease. *Br J Ophthalmol* 2022; 106(4): 528–33.
22. Kim K, Kim ES, Yu SY. Optical coherence tomography angiography analysis of foveal microvascular changes and inner retinal layer thinning in patients with diabetes. *Br J Ophthalmol* 2018; 102(9): 1226–31.
23. Kim K, Kim ES, Kim DG, Yu SY. Progressive retinal neurodegeneration and microvascular change in diabetic retinopathy: longitudinal study using OCT angiography. *Acta Diabetol* 2019; 56(12): 1275–82.

24. Kim K, Kim ES, Yu SY. Prediction of Diabetic Retinopathy Severity Using a Combination of Retinal Neurodegeneration and Capillary Nonperfusion on Optical Coherence Tomography Angiography. *Retina* 2023; 43(8): 1291–300.
25. Kirthi V, Reed KI, Alattar K, et al. Multimodal testing reveals subclinical neurovascular dysfunction in prediabetes, challenging the diagnostic threshold of diabetes. *Diabet Med* 2023; 40(3): e14952.
26. Sun Z, Tang F, Wong R, et al. OCT Angiography Metrics Predict Progression of Diabetic Retinopathy and Development of Diabetic Macular Edema: A Prospective Study. *Ophthalmology* 2019; 126(12): 1675–84.
27. Jampol LM, Glassman AR, Sun J. Evaluation and Care of Patients with Diabetic Retinopathy. *N Engl J Med* 2020; 382(17): 1629–37.
28. Ortin Vela S, Beyeler MJ, Trofimova O, et al. Phenotypic and genetic characteristics of retinal vascular parameters and their association with diseases. *Nat Commun* 2024; 15(1): 9593.
29. Romero-Bascones DB, J.; Lasagni Vital, R.M.; et al. Impact of acquisition device and quality control on test-retest repeatability of thickness measurements derived from OCT segmentation models. *ARVO* 2025; 2025.
30. Elwakil AM, T.; Quiros, J.V.; Liefers, B.; Kropp, M.; Duriez, A.; De Clerck, E.E.B.; Klaver, C.C.W.; Schlingemann, R.; Bergin, C.; Meloni, I.; Tomasoni, M. CapillaryX: A Fine-Tunable Pipeline for OCTA Segmentation and Feature Extraction. Preprint 2025.
31. Bergin C, Mantel I, Schlingemann RO, Tomasoni M, Wolfensberger TJ. SOIN - MI Data Lab: Personalized Ophthalmology Through Collaborative Data Collection and Dynamic Patient Consent. *Stud Health Technol Inform* 2022; 294: 281–2.
32. Mousavi SG, A.; Elwakil, A.; Brock, L.; Dherse, A.; Forestier, E.; Palaz, M.; Seiler, E.; Duriez, A.; Martin, T.; Wolfensburger, T.; Schlingemann, R.; Meloni, I.; Tomasoni, M. Cohort Builder: A Software Pipeline for Generating Patient Cohorts with Predetermined Baseline Characteristics from Medical Records and Raw Ophthalmic Imaging Data. 2024/03/27 ed; 2024. p. 1151–5.
33. Meloni IE, A.; Aurélie, G.; Flavie, T.; Navarro, A.; Bagatella, L.; Racodon, F.; Jeunet, F.; Wolfensberger, T.J.; Konstantinidis, L.; Schlingemann, R.; Bergin, C.; Tomasoni, M.. Cohort profile: OphtalmoLaus, an extension of the CoLausIPsyCoLaus cohort to investigate the relationships between ocular, cardiovascular, and cognitive parameters. Preprint 2025.
34. Massin P, Erginay A, Haouchine B, Mehidi AB, Paques M, Gaudric A. Retinal thickness in healthy and diabetic subjects measured using optical coherence tomography mapping software. *Eur J Ophthalmol* 2002; 12(2): 102–8.
35. Mantel I, Mosinska A, Bergin C, et al. Automated Quantification of Pathological Fluids in Neovascular Age-Related Macular Degeneration, and Its Repeatability Using Deep Learning. *Transl Vis Sci Technol* 2021; 10(4): 17.
36. Habra O, Gallardo M, Meyer Zu Westram T, et al. Evaluation of an Artificial Intelligence-Based Detector of Sub- and Intraretinal Fluid on a Large Set of Optical Coherence Tomography Volumes in

- Age-Related Macular Degeneration and Diabetic Macular Edema. *Ophthalmologica* 2022; 245(6): 516–27.
37. Seiler E, Delachaux L, Cattaneo J, et al. Importance of OCT-derived biomarkers for the recurrence of central serous chorioretinopathy using statistics and predictive modelling. *Sci Rep* 2024; 14(1): 23940.
 38. Bland JM, Altman DG. A note on the use of the intraclass correlation coefficient in the evaluation of agreement between two methods of measurement. *Comput Biol Med* 1990; 20(5): 337–40.
 39. Harvey ND. A Simple Guide to inter-rater, intra-rater and test-retest reliability for animal behaviour studies; 2021.
 40. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. *Psychol Bull* 1979; 86(2): 420–8.
 41. Xu X, Guo X, Xiao H, Mi L, Chen X, Liu X. Reproducibility of macular ganglion cell-inner plexiform layer thickness in normal eyes determined by two different OCT scanning protocols. *BMC Ophthalmol* 2017; 17(1): 37.
 42. Kim JS, Ishikawa H, Sung KR, et al. Retinal nerve fibre layer thickness measurement reproducibility improved with spectral domain optical coherence tomography. *Br J Ophthalmol* 2009; 93(8): 1057–63.
 43. Paunescu LA, Schuman JS, Price LL, et al. Reproducibility of nerve fiber thickness, macular thickness, and optic nerve head measurements using StratusOCT. *Invest Ophthalmol Vis Sci* 2004; 45(6): 1716–24.
 44. Schuman JS, Pedut-Kloizman T, Hertzmark E, et al. Reproducibility of nerve fiber layer thickness measurements using optical coherence tomography. *Ophthalmology* 1996; 103(11): 1889–98.
 45. Al-Sheikh M, Tepelus TC, Nazikyan T, Sadda SR. Repeatability of automated vessel density measurements using optical coherence tomography angiography. *Br J Ophthalmol* 2017; 101(4): 449–52.
 46. You Q, Freeman WR, Weinreb RN, et al. Reproducibility of Vessel Density Measurement with Optical Coherence Tomography Angiography in Eyes with and without Retinopathy. *Retina* 2017; 37(8): 1475–82.
 47. Carpineto P, Mastropasqua R, Marchini G, Toto L, Di Nicola M, Di Antonio L. Reproducibility and repeatability of foveal avascular zone measurements in healthy subjects by optical coherence tomography angiography. *Br J Ophthalmol* 2016; 100(5): 671–6.
 48. Mastropasqua R, Toto L, Mattei PA, et al. Reproducibility and repeatability of foveal avascular zone area measurements using swept-source optical coherence tomography angiography in healthy subjects. *Eur J Ophthalmol* 2017; 27(3): 336–41.
 49. Guo J, She X, Liu X, Sun X. Repeatability and Reproducibility of Foveal Avascular Zone Area Measurements Using AngioPlex Spectral Domain Optical Coherence Tomography Angiography in Healthy Subjects. *Ophthalmologica* 2017; 237(1): 21–8.

50. La Spina C, Carnevali A, Marchese A, Querques G, Bandello F. Reproducibility and Reliability of Optical Coherence Tomography Angiography for Foveal Avascular Zone Evaluation and Measurement in Different Settings. *Retina* 2017; 37(9): 1636–41.
51. Shahlaee A, Pefkianaki M, Hsu J, Ho AC. Measurement of Foveal Avascular Zone Dimensions and its Reliability in Healthy Eyes Using Optical Coherence Tomography Angiography. *Am J Ophthalmol* 2016; 161: 50–5 e1.
52. Vujosevic S, Toma C, Villani E, et al. Early Detection of Microvascular Changes in Patients with Diabetes Mellitus without and with Diabetic Retinopathy: Comparison between Different Swept-Source OCT-A Instruments. *J Diabetes Res* 2019; 2019: 2547216.
53. Shoji T, Yoshikawa Y, Kanno J, et al. Reproducibility of Macular Vessel Density Calculations Via Imaging With Two Different Swept-Source Optical Coherence Tomography Angiography Systems. *Transl Vis Sci Technol* 2018; 7(6): 31.
54. Corvi F, Pellegrini M, Erba S, Cozzi M, Staurenghi G, Giani A. Reproducibility of Vessel Density, Fractal Dimension, and Foveal Avascular Zone Using 7 Different Optical Coherence Tomography Angiography Devices. *Am J Ophthalmol* 2018; 186: 25–31.
55. Subhi Y, Forshaw T, Sorensen TL. Macular thickness and volume in the elderly: A systematic review. *Ageing Res Rev* 2016; 29: 42–9.
56. Jo YH, Sung KR, Shin JW. Effects of Age on Peripapillary and Macular Vessel Density Determined Using Optical Coherence Tomography Angiography in Healthy Eyes. *Invest Ophthalmol Vis Sci* 2019; 60(10): 3492–8.
57. Jung JJ, Soh YQ, Sha P, Yu S, Durbin MK, Hoang QV. Effects of Induced Astigmatism on Spectral Domain-OCT Angiography Quantitative Metrics. *Am J Ophthalmol* 2020; 219: 49–58.
58. Abramoff MD, Tobey D, Char DS. Lessons Learned About Autonomous AI: Finding a Safe, Efficacious, and Ethical Path Through the Development Process. *Am J Ophthalmol* 2020; 214: 134–42.
59. Rabiolo A, Gelormini F, Marchese A, et al. Macular Perfusion Parameters in Different Angiocube Sizes: Does The Size Matter in Quantitative Optical Coherence Tomography Angiography? *Invest Ophthalmol Vis Sci* 2018; 59(1): 231–7.
60. Ho J, Dans K, You Q, Nudleman ED, Freeman WR. COMPARISON OF 3 MM x 3 MM VERSUS 6 MM x 6 MM OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY SCAN SIZES IN THE EVALUATION OF NON-PROLIFERATIVE DIABETIC RETINOPATHY. *Retina* 2019; 39(2): 259–64.
61. Vujosevic S, Cunha-Vaz J, Figueira J, et al. Standardization of Optical Coherence Tomography Angiography Imaging Biomarkers in Diabetic Retinal Disease. *Ophthalmic Res* 2021; 64(6): 871–87.
62. Kalra G, Kar SS, Sevgi DD, Madabhushi A, Srivastava SK, Ehlers JP. Quantitative Imaging Biomarkers in Age-Related Macular Degeneration and Diabetic Eye Disease: A Step Closer to Precision Medicine. *J Pers Med* 2021; 11(11).
63. Simo R, Hernandez C, European Consortium for the Early Treatment of Diabetic R. Neurodegeneration is an early event in diabetic retinopathy: therapeutic implications. *Br J*

Figures

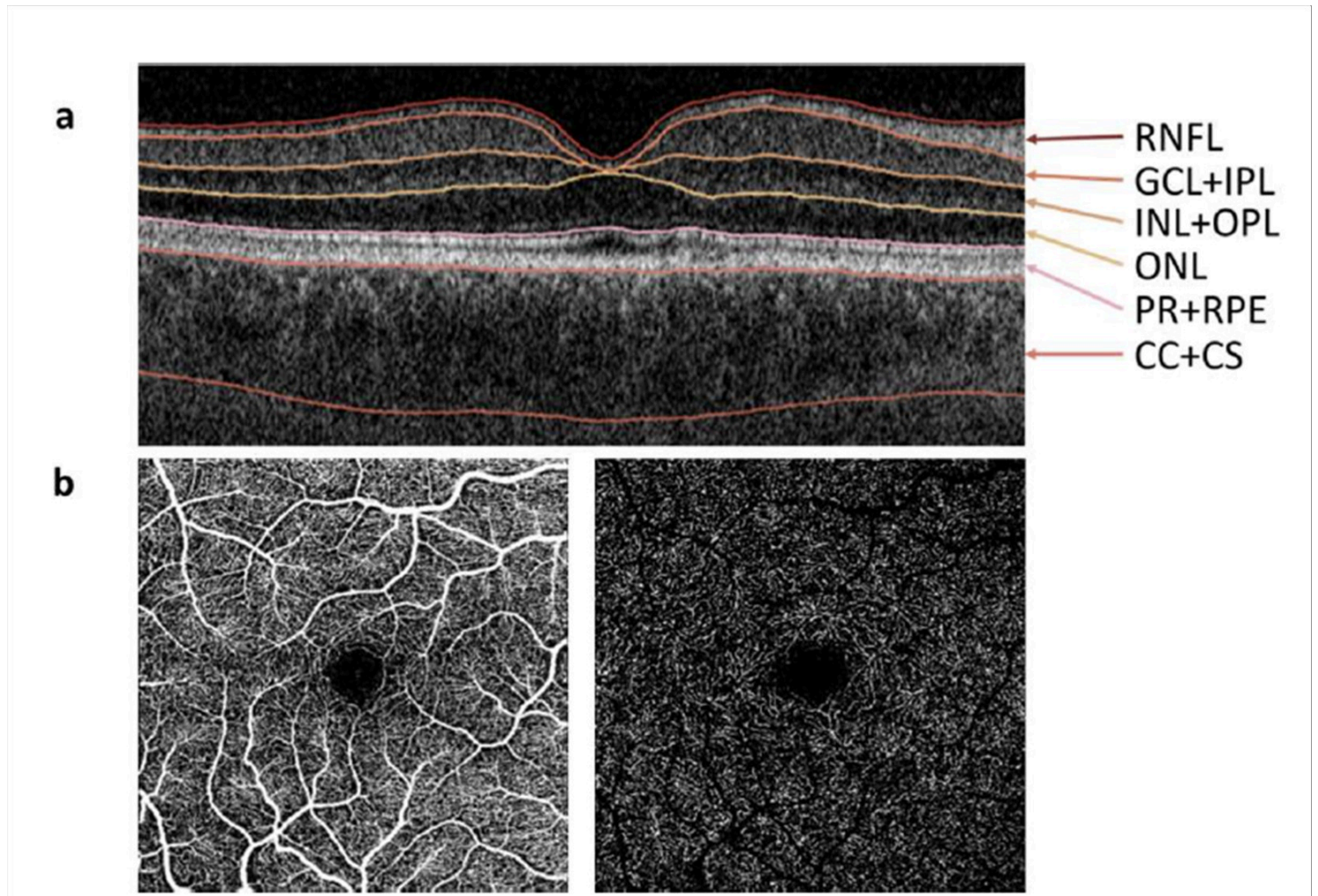


Figure 1

Structural and vascular retinal imaging using spectral-domain OCT and OCT-A.

a. Cross-sectional OCT B-scan of the macula illustrating automated segmentation of the main retinal layers: retinal nerve fibre layer (RNFL), ganglion cell layer plus inner plexiform layer (GCL+IPL), inner nuclear layer plus outer plexiform layer (INL+OPL), outer nuclear layer (ONL), photoreceptors plus retinal pigment epithelium complex (PR+RPE), and choriocapillaris plus choroidal stroma (CC+CS). **b.** En-face OCT-A images showing the retinal microvasculature in the superficial (left) and deep (right) capillary plexuses. These layers are used to quantify neuronal and vascular parameters analyzed in this study.

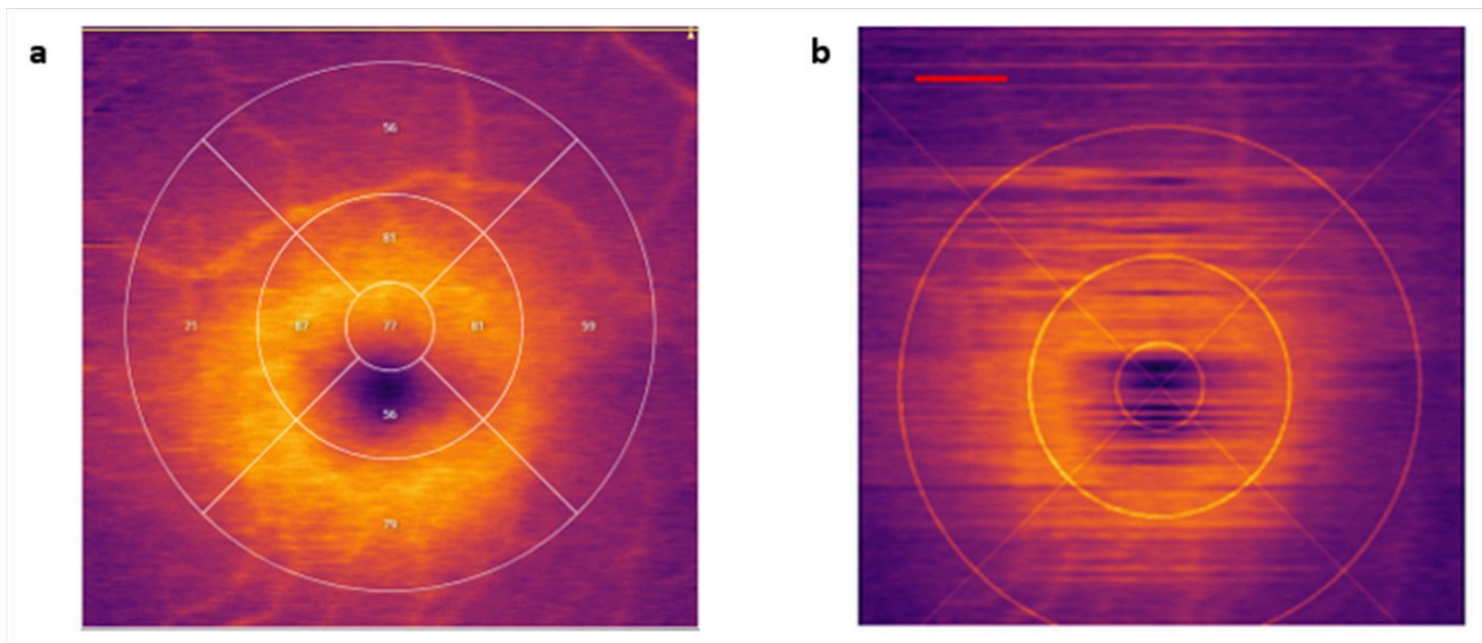


Figure 2

Recentering of macular OCT maps to improve segmentation accuracy.

a. En-face reconstruction of the ganglion cell plus inner plexiform layers (GCL+IPL) with the Early Treatment Diabetic Retinopathy Study (ETDRS) sector map automatically generated by the Discovery® software before recentering. **b.** The same map after manual recentering on the foveal pit, showing improved foveal alignment due to cropping of the outer scan area.

Accurate centering enhances layer thickness repeatability and ensures more consistent measurements across sections.

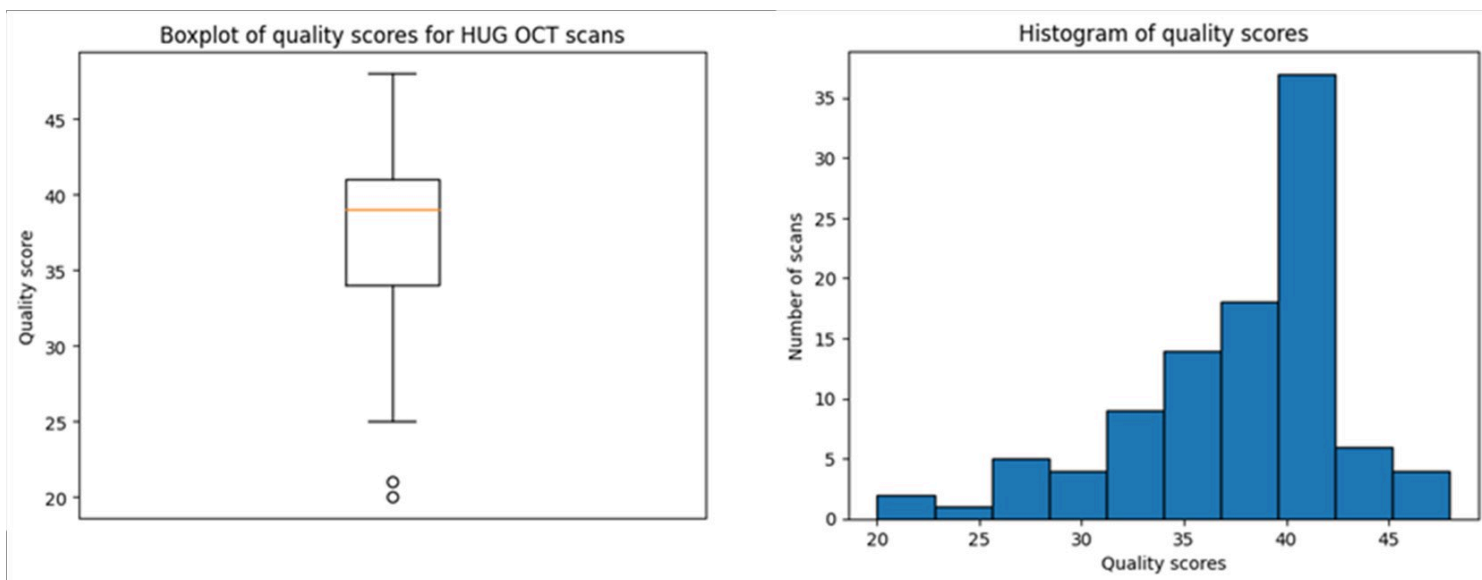


Figure 3

OCT scan quality across centers.

Distribution of OCT image quality scores obtained at the University Hospitals of Geneva (HUG), showing that scans below the manufacturer's minimal quality threshold (< 35 dB) were excluded from further analysis. At the Jules-Gonin Ophthalmic Hospital (HOJG), all scans met or exceeded the manufacturer's quality requirements, indicating consistently high image quality and no need for additional quality filtering.

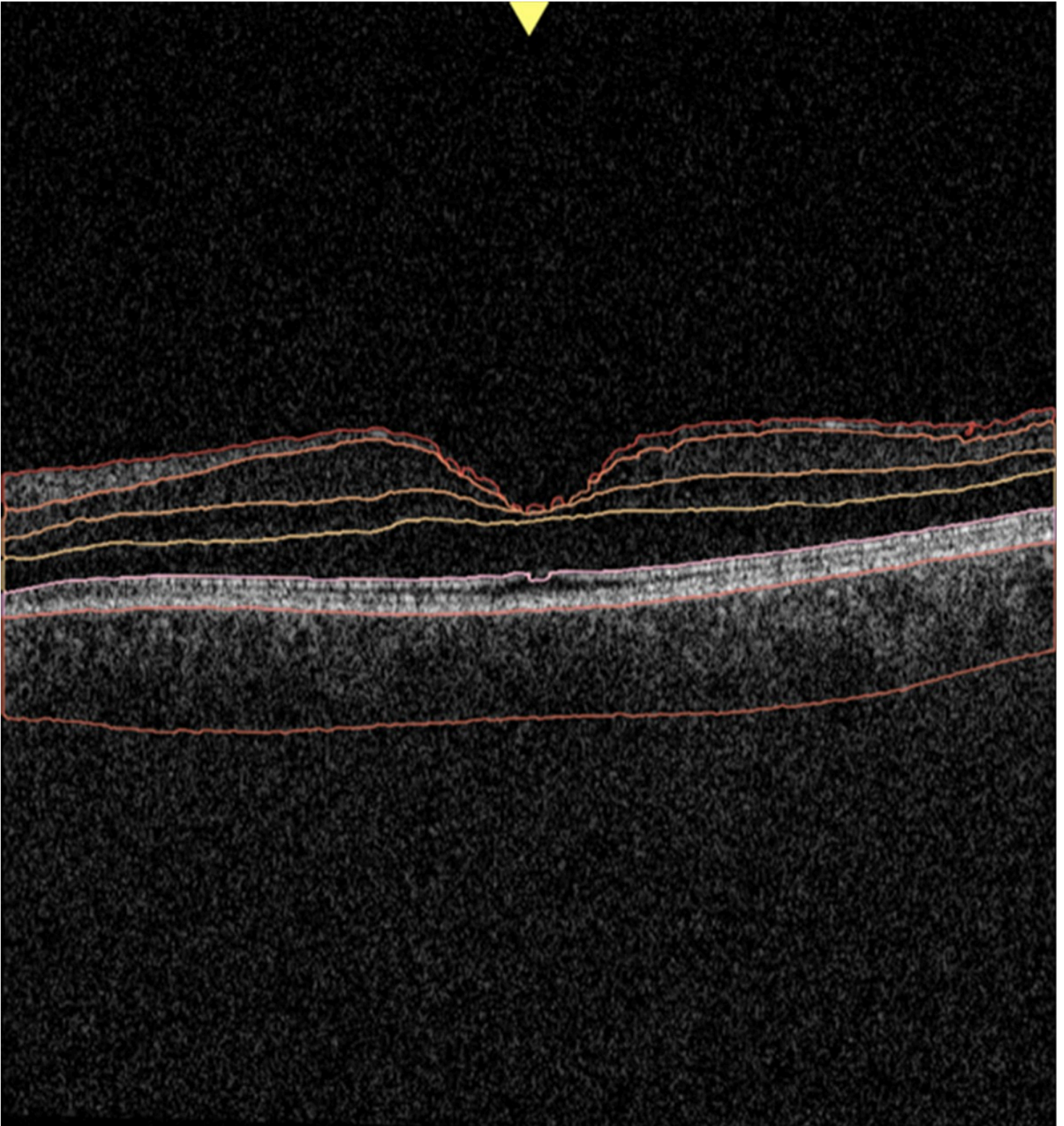


Figure 4

Example of foveal segmentation errors in low-quality OCT images.

Automated layer segmentation occasionally failed at the fovea in scans with reduced signal strength, leading to discontinuities or misalignment of the retinal boundaries (shown here on the ganglion cell and inner plexiform layers). Such artifacts contributed to lower repeatability of central retinal thickness measurements and were excluded from quantitative analysis.

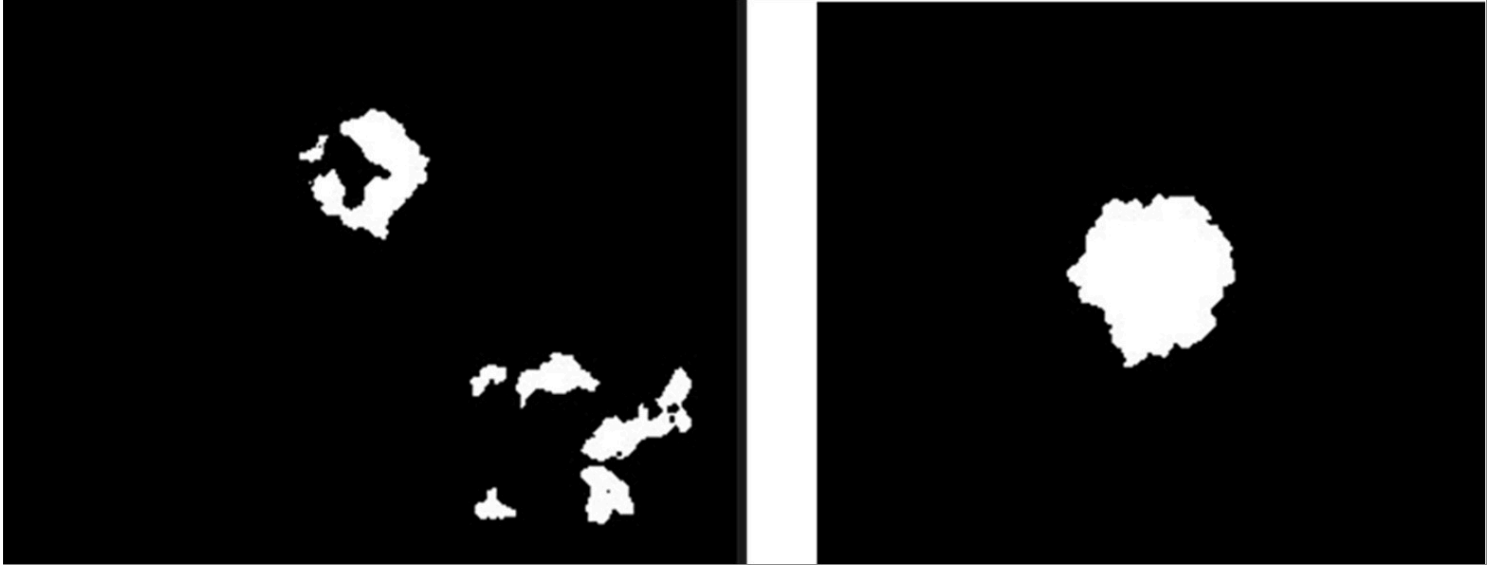


Figure 5

Example of segmentation errors in the deep foveal avascular zone (FAZ).

Left: Automated segmentation of the FAZ in the deep capillary plexus (DCP) showing fragmentation and misidentification of vascular boundaries. Right: Automated segmentation of the FAZ in the DCP of the same eye on a different scan, illustrating accurate delineation of the FAZ.