Quantitative Analysis of Three Distinct Retinal Capillary Plexuses in Healthy Eyes Using Optical Coherence Tomography Angiography

Sean T. Garrity,1 Nicholas A. Iafe,1 Nopasak Phasukkijwatana,1,2 Xuejing Chen,3 and David Sarraf1,4

1Stein Eye Institute, David Geffen School of Medicine at UCLA, University of California Los Angeles, Los Angeles, California, United States
2Department of Ophthalmology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
3New England Eye Center Tufts University School of Medicine, Boston, Massachusetts, United States
4Greater Los Angeles VA Healthcare Center, Los Angeles, California, United States

PURPOSE. To identify and quantify the three distinct retinal capillaryplexuses and the foveal avascular zone (FAZ) in healthy subjects according to age using optical coherence tomography angiography (OCTA) with novel projection artifact removal (PAR) software and improved segmentation.

METHODS. All eyes in this cross-sectional study underwent OCTA imaging using RTVue XR Avanti with novel PAR AngioVue software. OCTA scans were analyzed and the three main parafoveal retinal capillaryplexuses were segmented and vessel density and FAZ area were calculated.

RESULTS. A total of 152 normal eyes from 95 subjects (39 males, 56 females, mean age 42 ± 25 years) were included. The mean vessel density was 15.48 ± 2.04 mm⁻¹ in the superficial retinal capillaryplexus (SCP), 15.28 ± 1.82 mm⁻¹ in the intermediate retinal capillaryplexus (ICP), and 16.33 ± 2.32 mm⁻¹ in the deep retinal capillaryplexus (DCP) for 3 × 3-mm OCTA images. Analysis of 3 × 3-mm scans yielded a mean FAZ area of 0.270 ± 0.101 mm². The average reduction in vessel density per year of age with 3 × 3-mm OCTA scans was 0.04 mm⁻¹ (0.22%) in the SCP, 0.05 mm⁻¹ (0.27%) in the ICP, and 0.06 mm⁻¹ (0.30%) in the DCP. The average increase in FAZ area per year of age was 0.0015 mm² (0.72%).

CONCLUSIONS. Novel PAR software may provide improved visualization of all three major parafoveal retinal capillaryplexuses including the ICP. Using this technology, SCP, ICP, and DCP vessel density decreased with increasing age while FAZ area increased with age.

Keywords: optical coherence tomography angiography, intermediate capillaryplexus, retinal capillaryplexus

Optical coherence tomography angiography (OCTA) is an advanced imaging technology that allows for direct evaluation of the retinal microvasculature with depth-resolved capability.1 Fluorescein angiography has traditionally been the gold standard for the vascular assessment of retinal and choroidal disorders but does not provide adequate resolution of the intermediate retinal capillaryplexus (ICP) or deep retinal capillaryplexus (DCP) and requires the injection of a dye.2 OCTA is a noninvasive method that uses amplitude or phase decorrelation technology with high frequency and dense volumetric scanning to detect blood flow and to visualize blood vessels at various depth-resolved levels of the retina and choroid without the injection of a dye. With OCTA, a retinal region is repeatedly scanned and changes in reflectivity at each voxel (portmanteau of “volume” and “pixel”) above a threshold are considered to have flow, resulting in an image of the values of each voxel. The three-dimensional capability of OCTA allows for more refined visualization of the trilaminar retinal capillaryplexus.

An emerging body of literature has evaluated the retinal microvasculature in both healthy and pathologic eyes using OCTA. As the technology of OCTA is still developing, these pioneering studies have been limited by various factors, including projection artifacts.1 Projection artifacts result in superficial vessels projecting shadows onto deeper layers that are incorrectly detected as flow with OCTA, limiting visualization of underlying vasculature. Most published studies using OCTA have separated the retinal capillary system into two majorplexuses, the superficial retinal capillaryplexus (SCP) and the DCP largely due to projection artifact but morphological studies in humans and animal models have consistently demonstrated the presence of three distinct triplanar retinal capillaryplexuses in the macula: the SCP and DCP as well as the ICP.3–6 The ability to adequately visualize the ICP in addition to the SCP and DCP with OCTA could significantly enrich our understanding of various ischemic retinal disorders, including acute macular neuroretinopathy (AMN), paracentral acute middle maculopathy (PAMM), diabetic retinopathy, and macular telangiectasia type 2.1,7–10
With recent technological advancements to reduce image artifacts, it has now become possible to identify these three plexuses. The few published reports studying the three retinal capillary plexuses with OCTA have assessed healthy and/or diabetic eyes with relatively small numbers of healthy eyes. To validate OCTA findings in abnormal eyes, it is necessary to have reliable normative OCTA data from healthy eyes. Previously we reported quantitative analysis of a large group of healthy eyes using available OCTA technology, which segmented the retinal microvasculature into the SCP and DCP. We found that SCP and DCP vessel density decreased with age whereas foveal avascular zone (FAZ) area increased with age.

Due to recent enhancements in OCTA software, including a novel algorithm for projection artifact removal (PAR) and improved segmentation, we are now able to report normative data of the three distinct retinal capillary plexuses from the same group of previously reported healthy eyes from 70 patients and from 25 additional healthy patients. In the present study, OCTA vessel density and FAZ area analysis was performed on the SCP, ICP, and DCP in normal eyes across nearly all decades of life.

**METHODS**

This retrospective study was approved by the University of California Los Angeles Institutional Review Board and adhered to the tenets of the Declaration of Helsinki and the Health Insurance Portability and Accountability Act regulations. Informed consent was obtained from each subject before OCTA imaging. A total of 100 patients were recruited for this study; and a total of 167 normal eyes were evaluated. The patients and OCTA scans included in this study were the same as was used in our previous study; an additional group of 25 healthy patients was also evaluated. The inclusion criteria were volunteer patients from the Stein Eye Institute with no evidence of ocular media opacity, retinal disease, or significant refractive error (myopia of 6 diopters [D] or more or hyperopia of 3 D or more) in the study eye as evaluated by dilated fundus examination. Exclusion criteria included poor-quality images with a signal strength index (SSI) less than 40, significant motion artifact, inaccurate or incorrect segmentation at the level of the SCP, ICP, or DCP, or subject’s inability to abstain from blinking or movement during image acquisition.

OCTA imaging was performed using the RTVue XR Avanti spectral-domain OCT device with AngioVue software (Optovue, Fremont, CA, USA). This software (version number 2016.200.0.37) was an updated, noncommercially available version of the software used in our previous study and was very similar to the future commercial version, as it included two important advancements: the PAR algorithm and improved segmentation. The proprietary three-dimensional PAR algorithm developed by Optovue removed projection artifacts from the OCTA volume on a per voxel basis, using information from the OCT and OCTA volume to differentiate in situ OCTA signal from projection artifacts, specifically, parameters derived from the OCTA and OCT intensity profiles anterior to, at, and around the voxel of interest (Hsiao Y, et al. IOVS 2017;58:ARVO E-Abstract 5998; Wolfson Y, et al. IOVS 2017;58:ARVO E-Abstract 1669). With three-dimensional PAR-enabled software, projection artifact was removed from en face OCTA images and B-scan OCTA images (Fig. 1). The improved segmentation was afforded by additional segmentation layers to define the en face slabs for the three plexuses, including the ICP, allowing for more consistent slab definitions across subjects. The OCTA device used a light source at 840 nm, a bandwidth of 45 nm, and an A-scan rate of 70,000 scans per second. A 3 × 3-mm and a 6 × 6-mm cube scan was acquired, each composed of 304 × 304 × 3-mm cube scan was acquired, each composed of 304 × 3-mm scans. Each B-scan was repeated at each cross-section in the fast-scan axis to separate static tissue from blood flow signals. Two OCTA volume scans with orthogonal fast-scan directions (horizontal and vertical) were acquired for each eye and then merged to minimize motion artifact. The signal-to-noise ratio was improved with split-spectrum amplitude decorrelation technology.

To define the three distinct retinal capillary plexuses, the automated segmentations of the OCTA software were adjusted by the user within the software to offset the boundaries. Several different methods of segmentation based primarily on the known anatomic locations of the plexuses and published reports were applied to a subset of eyes to determine which segmentation boundaries effectively visualized the three plexuses. The different segmentation boundaries were also qualitatively evaluated by setting a thin slab at potential boundaries and assessing which methods resulted in slabs of low flow indicating the interplexus spaces. Based on these two evaluations, we qualitatively determined the three distinct plexuses were effectively visualized as follows: the SCP en face OCTA image was segmented with an inner boundary of 3 μm below the internal limiting membrane and an outer boundary set at the inner plexiform layer (IPL)-inner nuclear layer (INL) junction, thus including the nerve fiber layer (NFL), ganglion cell layer (GCL), and IPL. The ICP en face OCTA image was segmented with an inner boundary set at 20 μm below the IPL-INL junction, thus including approximately the inner half of the INL. The DCP en face OCTA image was segmented with an inner boundary set at 20 μm below the IPL-INL junction and an outer boundary set at 15 μm below the outer plexiform layer (OPL)-outer nuclear layer (ONL) junction, thus including approximately the outer half of the INL, the OPL, and a small portion of the ONL.

Vessel density analysis was performed as previously described. The en face OCTA images were exported to an external software program (Imagej, version 1.50i; http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA) and opened in image...
analysis.17 The microvascular en face scans were binarized and skeletonized and each blood vessel was illustrated as a 1-pixel-wide line. Vessel density was calculated from the skeletonized images of all scans as ([pixels of vessels] \times [scan width in mm/304]/[area in mm²]) in mm⁻².18,19 The updated AngioVue software automatically calculated the FAZ area in mm² for each eye, using a slab from the internal limiting membrane to 75 μm above the RPE. This protocol was used on the basis of recent studies validating a single merged quantitative measurement of the FAZ.11,12,20

Statistical analysis was performed using Excel software (Microsoft, Redmond, WA, USA) for Macintosh (2011 version 14.5.2; Apple Inc., Cupertino, CA, USA) and Prism 7.0c for Mac OS X (GraphPad Software, La Jolla, CA, USA). Because vessel density may be correlated between the two eyes of a single patient, one primary eye was selected for analysis at random for each patient to ensure that each data point could be assumed to be independent from each other. Differences between males and females, right and left eyes, and predefined age groups for the primary eye were calculated using independent t-tests assuming unequal variances. The paired t-test was used to compare vessel densities and FAZ area between the primary and the fellow eye. A two-tailed statistic was used for all calculations. Missing variables were not imputed. Statistical significance was set at 0.05. The slope of the linear regression line for the vessel density and FAZ area versus age was used to calculate the respective annual change. An analysis of covariance was used to compare the slopes of the linear regression lines between sexes. Spearman correlation coefficient and linear regression models were used to test the association between age and SSI.

**RESULTS**

A total of 100 subjects (167 eyes) were imaged during the study period. Normal eyes (152) from 95 subjects were included in the study (Table 1) based on the quality of their OCTA images (i.e., 5 patients were excluded because of poor-quality OCTA images). The cohort included 39 males (62 eyes) and 56 females (90 eyes) with a mean age ± SD of 42 ± 25 years (range: 4-90 years). All 152 eyes had 3 × 3-mm OCTA images used for analysis, whereas only 128 eyes had 6 × 6-mm images included in the study due to insufficient image quality or absent scans. Three en face OCTA images were acquired from each scan size with segmentation at the SCP, ICP, and DCP levels (Fig. 2). The SCP anatomy consisted of predominantly radially oriented large vessels with interconnected ladder-like capillaries centered on the FAZ. The ICP and DCP anatomy illustrated a denser and more complex distribution of fine capillaries clustered in a spider or vortex-like configuration surrounding the fovea.

The mean vessel density (Table 2) of eyes included in the analysis was 15.48 ± 2.04 mm⁻¹ in the SCP, 15.28 ± 1.82 mm⁻¹ in the ICP, and 16.35 ± 2.32 mm⁻¹ in the DCP for 3 × 3-mm OCTA images. For the 6 × 6-mm OCTA images, the mean vessel density was 6.44 ± 1.04 mm⁻¹ in the SCP, 7.23 ± 1.37 mm⁻¹ in the ICP, and 7.90 ± 1.50 mm⁻¹ in the DCP. Analysis of the FAZ (Table 3) in 3 × 3-mm scans yielded a mean FAZ area of 0.270 ± 0.101 mm². For both the 3 × 3-mm and 6 × 6-mm scans, there was a trend toward a greater mean vessel density in the DCP compared with either the SCP or ICP, although these differences were not significant for nearly all age groups (Fig. 3). In the 3 × 3-mm scans there was a trend toward greater mean vessel density in the SCP compared with the ICP, whereas in the 6 × 6-mm scans there was a trend toward greater mean vessel density in the ICP compared with the SCP, although again these differences were not significant for nearly all age groups.

The values for vessel density and FAZ area were similar in left and right eyes and between males and females without significant differences (P > 0.05 for all comparisons). There were no significant differences in any of the analyzed variables between paired right and left eyes (57 pairs). A negative correlation was found between age and vessel density and a positive correlation between age and FAZ area (Fig. 4). The average reduction in vessel density per year of age with 3 × 3-mm OCTA scans was 0.04 mm⁻¹ (or 0.22%) in the SCP, 0.05 mm⁻¹ (or 0.27%) in the ICP, and 0.06 mm⁻¹ (or 0.30%) in the DCP, and was 0.02 mm⁻¹ (or 0.22%) in the SCP, 0.03 mm⁻¹ (or 0.37%) in the ICP, and 0.05 mm⁻¹ (or 0.33%) in the DCP with 6 × 6-mm OCTA scans. The average increase in FAZ area per year was 0.0015 mm² (0.72%). There were no significant differences in the annual change in mean vessel density of the SCP, ICP, or DCP for either scan size or in the annual change in FAZ area between males and females.

In the analysis of 3 × 3-mm scans, there was a statistically significant decrease in mean vessel density between subjects 60 to 69 years of age versus those 70 to 79 years of age in the SCP (P = 0.004), ICP (P = 0.003), and DCP (P = 0.014). Additionally, a statistically significant decrease in mean vessel density was found in 6 × 6-mm scans of the SCP in a comparison of subjects 30 to 39 years of age versus 40 to 49 years of age (P = 0.035). A statistically significant increase in FAZ area was found in a comparison of subjects 30 to 39 years old versus 40 to 49 years old (P = 0.016). The remainder of the statistic comparisons between sequential decades were not significantly different. We also analyzed scan data by comparing larger near tertile age groups, that is, subjects 35 years or younger, 36 to 64 years of age, and 65 years or older. For 3 × 3-mm scans, we found a statistically significant decrease in mean vessel density between the lowest and middle tertiles in the ICP (P = 0.0001) and DCP (P = 0.001) and between the middle and highest tertiles in the SCP (P = 0.0007), ICP (P = 0.007), and DCP (P = 0.003).
and DCP (P = 0.002). For 6 × 6-mm scans, a statistically significant decrease in mean vessel density was found in a comparison of the lowest and middle tertiles in the ICP (P = 0.005) and DCP (P = 0.01). A statistically significant increase in FAZ area was found in a comparison of the lowest and middle tertiles (P = 0.04). The remainder of statistical comparisons of vessel density and FAZ area between tertile age groups were not significantly different. Analysis of the association between age and SSI determined that both scan sizes had a statistically significant Spearman correlation coefficient of −0.42, indicating that increasing age was moderately but significantly associated with decreasing SSI, although linear regression analysis indicated that both SSI (decreasing) and age (increasing) were independently associated with reductions in vessel density.

An additional repeatability analysis of a subset of 17 subjects who received follow-up scans within the study period was performed. The mean age ± SD of this group was 53 ± 20 years of age and mean ± SD follow-up time was 6.8 ± 2.0 months. Clinical examination at the follow-up visit ensured that no pathologic changes had developed in the interim. The mean vessel density was not significantly different at the level of the SCP (P = 0.359), ICP (P = 0.852), or DCP (P = 0.923) with 3 × 3-mm scans between initial and follow-up visits. The mean vessel density was statistically different at the level of the SCP (P = 0.01), ICP (P = 0.0002), and DCP (P = 0.00007) with 6 × 6-mm scans at follow-up. The FAZ area was not statistically different at the follow-up visit (P = 0.171).

### DISCUSSION

OCTA may hold great promise as an advanced imaging technology with the capability to provide detailed depth-resolved microvascular information of the retina and choroid. To effectively study pathologic eyes with this new modality, it is necessary to have normative data from healthy eyes. In this study, we reported on the retinal microvascular density and the FAZ area by decade of life in a broad healthy population. This is the first study of its size reporting on normative OCTA data from the three major retinal capillary plexuses of the macula: SCP, ICP, and DCP. This report has expanded on previous work by our group, which provided normative OCTA data from 70 patients and documented that SCP and DCP vessel density decreased with age while the FAZ area increased with age. As a result of advances in OCTA software, including a new algorithm for PAR, we have provided in this study analysis of the ICP in addition to the SCP, and a more refined analysis of the DCP due to the removal of projection artifact. For the current study, an additional 25 patients and 39 eyes were added to the original group of 70 patients and 113 eyes, for a total of 95 patients and 152 eyes studied in this report.

This study demonstrated that vessel density in the SCP, ICP, and DCP decreased with increasing age, whereas FAZ area increased with age. This is consistent with our previous study that reported the same trends when the retinal capillary plexuses were divided into SCP and DCP. The current results were also quite similar to our previous study in terms of mean vessel density, vessel density change per year, FAZ area, and FAZ area change per year. The average annual reduction in vessel density with 3 × 3-mm scans in the SCP was 0.0393 mm⁻¹ (or 0.26%) in our original study and 0.04 mm⁻¹ (or 0.22%) in this present study, whereas the average annual reduction in the DCP was 0.0574 mm⁻¹ (or 0.27%) in our original study and 0.06 mm⁻¹ (or 0.30%) in this present study. The average annual increase in FAZ area in our original study was 0.0014 mm² (or 0.63%) and 0.0011 mm² (or 0.20%) at the levels of the SCP and DCP, respectively, and 0.0015 mm²

| TABLE 2. Quantitative Analysis of Vessel Density (mm⁻¹) According to Age and Sex |
|-----------------------------------|----------------|----------------|----------------|
|                                   | SCP            | ICP            | DCP            |
| Overall                           |                |                |                |
| Age, y                            |                |                |                |
| 60–69                             | 12             | 15.70 ± 0.67   | 15.87 ± 1.34   |
| 70–79                             | 9              | 13.60 ± 1.52   | 14.25 ± 1.34   |
| ≥80                               | 8              | 12.54 ± 3.09   | 12.61 ± 2.99   |
| Sex                               |                |                |                |
| Male                              | 39             | 15.40 ± 1.91   | 16.41 ± 2.27   |
| Female                            | 56             | 15.55 ± 2.15   | 16.28 ± 2.38   |
| Data are mean ± SD.               |                |                |                |

Table 3. Quantitative Analysis of FAZ Area in 3 × 3-mm OCTA Scans According to Age and Sex

<table>
<thead>
<tr>
<th>No. of Subjects</th>
<th>FAZ Area, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>0.270 ± 0.101</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
</tr>
<tr>
<td>60–69</td>
<td>0.291 ± 0.105</td>
</tr>
<tr>
<td>70–79</td>
<td>0.289 ± 0.117</td>
</tr>
<tr>
<td>≥80</td>
<td>0.361 ± 0.115</td>
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<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.273 ± 0.107</td>
</tr>
<tr>
<td>Female</td>
<td>0.265 ± 0.093</td>
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<tr>
<td>Data are mean ± SD.</td>
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</tbody>
</table>
Quantitative Analysis Using OCTA

FIGURE 3. Line graphs show the relationship between mean vessel density and age in decades with error bars set to standard error.

FIGURE 4. Scatterplots of data according to age illustrate the relationship between vessel density and FAZ area with increasing age (years). The dotted lines represent the corresponding linear regression line with respect to age.

(0.72%) in this present study, which reports a single FAZ for all retinal capillary plexuses.

Previous studies by others have demonstrated a decrease in total retinal blood flow associated with an increase in FAZ area with increasing age.\(^{21-23}\) In a study of Chinese patients with healthy eyes using OCTA, Yu et al.\(^{21}\) found decreases in the SCP parafoveal flow index and vessel area density with increasing age at a rate of 0.6% and 0.4% per year, respectively. Shahlaee et al.\(^{24}\) reported that parafoveal vascular densities in the SCP and DCP decrease with age, and in a separate report\(^{25}\) measured a mean superficial FAZ area of 0.27 mm\(^2\) and deep FAZ area of 0.34 mm\(^2\). Coscas et al.\(^{26}\) also reported on the age-related decrease in vessel density in the SCP and DCP of Caucasian eyes with OCTA.

A trend was noted in which the DCP displayed a slightly greater vessel density than the SCP or ICP with both scan sizes, although differences in the relative vessel densities among the three plexuses were not significant for nearly all comparisons. Although some reports have noted the SCP to have the greatest vessel density of the retinal plexuses,\(^{5,6,12}\) later reports by two of these groups failed to duplicate these findings.\(^{26,27}\) These varying results may reflect the differing imaging modalities and nonstandardized methodologies used to determine vessel density in each of these reports. Comparing the relative...
densities of the three plexuses may be especially challenging with OCTA, as projection artifacts have greater effects on deeper layers.

As the ability to visualize the ICP with OCTA is a recent advancement, there are relatively few studies describing the ICP using this nascent imaging modality.\textsuperscript{10,12} The appearance of the ICP in our study as a complex distribution of capillaries in a spider or vortex-like pattern was consistent with images of the ICP in other studies.\textsuperscript{10,12} To our knowledge, there are no other studies of this size quantifying vessel density of the ICP with OCTA. The ICP is a distinct physiological, metabolic, and genetic vascular network whose formation has been shown to depend more on hypoxia-induced factor than the other capillary systems.\textsuperscript{28} The ability of OCTA to visualize the ICP may have clinical implications for ischemic conditions of the middle retina, such as AMN and PAMM, which may be due to capillary ischemia at the ICP and/or DCP levels, as well as diabetic retinopathy and macular telangiectasia type 2, which may preferentially affect the ICP.\textsuperscript{1,7–10}

This study reported on a single FAZ for each eye. Prior OCTA studies have often distinguished separate FAZs for each of the capillary plexuses. Histologic analysis of the three vascular plexuses has indicated that they merge at the edge of the FAZ.\textsuperscript{3,29} A finding also noted with careful observation of OCTA images. Therefore, separating the plexuses into different segments at the FAZ margin may lead to increased variability of measurements, a concern noted by others.\textsuperscript{11,12,20} Furthermore, the FAZ was originally defined in terms of dye-based fluorescein angiography,\textsuperscript{23,30} which does not provide separation of individual capillary plexuses. We therefore believe that for purposes of OCTA studies, a single FAZ for all the retinal vascular plexuses may provide more accurate and reproducible measurements, a conclusion also noted by Campbell et al.\textsuperscript{12}

Our measured mean FAZ area of 0.270 mm\textsuperscript{2} was quite consistent with previous studies of FAZ area using a variety of imaging techniques, including OCTA.\textsuperscript{18,25,30–32} As a new technology, OCTA studies have been limited by a variety of factors, including various imaging artifacts. Projection artifacts\textsuperscript{1,5} in particular pose a significant challenge, causing superficial vessels to be incorrectly detected as deeper vessels. Several approaches have been developed to reduce projection artifact, such as slab subtraction, but they have drawbacks including removal of the true DCP. The current study used AngioAnalytics research software (Optovue), which was very similar to the future commercial version that will include the same PAR algorithm and improved segmentation used in the current study. This PAR algorithm has been shown to effectively reduce projection artifact with both en face and cross-sectional angiograms (Fig. 1), allowing for improved visualization of the three distinct capillary plexuses. Studies have demonstrated that with PAR-enabled software there is a decreased two-dimensional correlation coefficient between the plexuses (indicating the plexuses are more distinct) and suppression of projection artifacts with reduced vessel density measurements in the deeper layers; that is, the layers most affected by projection artifact (Garrity ST, et al., unpublished data, 2017) (Hsiao Y, et al. IOVS 2017;58:ARVO E-Abstract 5998; Wolfson Y, et al. IOVS 2017;58:ARVO E-Abstract 1669). Notably, this PAR algorithm is distinct from the projection-resolved (PR) algorithm used in other similar studies.\textsuperscript{11,12}

OCTA analyses require segmentation of the layers within the OCT volumes to identify the three distinct capillary plexuses. Various segmentation techniques have been proposed to limit projection artifact and correctly visualize the desired plexuses, such as segmenting external to known anatomical boundaries or segmenting based on known anatomic locations.\textsuperscript{10,11,34} In a recent study of nine normal eyes, Campbell et al.\textsuperscript{12} used a PR OCTA algorithm to effectively demonstrate three distinct retinal capillary plexuses in the macula, using a segmentation scheme slightly different from ours. In the current study, we qualitatively evaluated several segmentation methods to determine which method effectively visualized the three plexuses. Our method was based primarily on thresholding the segmentation of the three capillary plexuses.\textsuperscript{6} In our study, the SCP was captured using a slab that included the NFL, GCL, and the IPL. We did not include any portion of the IPL in our ICP segment as Campbell et al.\textsuperscript{12} did. Although setting the SCP-ICP boundary at this lower level (as we did) may have reduced inclusion of image artifacts in our ICP analysis, it may have resulted in including part of the ICP in our SCP analysis, although our qualitative analysis of different segmentation methods did not seem to suggest this. Our DCP segmentation included the outer half of the INL as well as the OPL again to reduce any contribution of vessels from the ICP. Analysis of vessel density using the segmentation boundaries proposed by Campbell et al.\textsuperscript{12} performed in a subset of eyes (10 eyes) from our study demonstrated less than 13% change, with most eyes changing by 5% to 9%, in the measured vessel density for each SCP and ICP segmentation and no change in the DCP measurements, differences that we believe would not change the main findings of this study. Although further work and consensus is needed to identify the most appropriate segmentation boundaries across different OCTA systems, which may slightly change the quantitative analysis of vessel density, our results clearly demonstrate that vessel density decreases with increasing age in all three plexuses.

Reproducibility and repeatability of any nascent imaging technology must be verified before it can be applied in large-scale research trials and clinical practice. A repeatability analysis was performed in a subset of 17 eyes (18%) to confirm the reliability of vessel density and FAZ area measurements with OCTA analysis. The results from this analysis validated the intervisits repeatability of capillary density and FAZ area measurements with the 3 × 3-mm OCTA scans (i.e., there was agreement of measurements from different sessions of scans of the same eye), but this was not demonstrated with the 6 × 6-mm scans, which displayed a significant change in measurements at the follow-up sessions; these same trends were seen in our prior report analyzing the SCP and DCP in normal eyes.\textsuperscript{13} These findings may be attributed to insufficient pixel number across a larger sample area with the 6 × 6-mm scans, which could either result in smaller capillaries being excluded from analysis or result in inflated vascular density measurements, depending on several factors, including the exact vascular density parameter being measured. Thus, 3 × 3-mm scans may distinguish or resolve the retinal vasculature more accurately but at the expense of a smaller study area, illustrating the need for high-definition imaging with larger scan sizes. Overall, our results suggest that the 3 × 3-mm scans are more repeatable and likely more accurate than the 6 × 6-mm scans.

Our study has several advantages. Our normal cohort included a relatively large group of healthy study subjects from various ethnic backgrounds and from nearly all decades of life. In addition, our study was one of the first to include normative data across nine decades of life for all three major retinal capillary plexuses using novel PAR technology with excellent repeatability for the 3 × 3-mm scans. Nevertheless, there are several limitations to our study. First, present OCTA technology is inherently susceptible to motion artifact from movement of the patient’s head or eyes during image acquisition, complications that were noted more often in the extreme age groups. Second, although the new software included PAR, this feature is unlikely to completely eliminate projection artifact. Third, despite the repeatability of our results and concordance with other studies, we did not validate
our quantification data with another method of vascular density assessment. Fourth, a reduction in SSI was found to be correlated with age, although linear regression analysis indicated that both SSI (decreasing) and age (increasing) were independently associated with reductions in vessel density. Fifth, this report used a slightly different segmentation method and vessel density analysis method than was used in other OCTA studies that evaluated the three retinal capillary plexuses. Sixth, although we did not include patients with significant refractive error, we did not account for axial length in our analysis. Additionally, this study effectively segmented the capillary plexuses in healthy eyes, which may be challenging in pathologic eyes in which the retinal architecture may be disrupted.

In conclusion, this study presented, for the first time, quantitative OCTA analysis of vessel density and FAZ area of the three major retinal capillary plexuses in the macula (parafovea) of healthy eyes across nearly all decades of life, and demonstrated that vessel density decreased with age while FAZ area increased with age. This analysis has provided important standardized values for the three major retinal capillary plexuses, including the ICP, and has demonstrated the need for standardization and consensus in OCTA acquisition procedures to obtain reliable and accurate results.

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