

Variability of Foveal Avascular Zone Metrics Derived From Optical Coherence Tomography Angiography Images

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Purpose: To characterize sources of inter- and intrasubject variability in quantitative foveal avascular zone (FAZ) metrics.

Methods: Two 3×3-mm optical coherence tomography angiography scans (centered on the fovea) were acquired in both eyes of 175 subjects. An image of the superficial plexus was extracted from each scan and segmented twice by a single observer. Four quantitative FAZ morphology metrics (area, axis ratio, acircularity, major horizontal axis angle) were calculated, and a variance components analysis was performed.

Results: Mean (\pm SD) age was 27.9 ± 11.9 years, and 55% were female. Area had the largest amount of variance resulting from intersubject differences (93.1%). In contrast, there was large interocular variance for axis ratio, acircularity, and major horizontal axis angle (55.0%, 53.7%, 70.7%, respectively), though only axis ratio showed significant asymmetry between fellow eyes ($P < 0.05$). Neither repeated images from the same eye nor repeated segmentation on the same image were significant sources of variance.

Conclusions: Metrics of FAZ morphology show excellent repeatability and reliability. Excluding FAZ area, there was a high amount of variance attributed to interocular differences for the other FAZ metrics; therefore, the fellow eye should not be considered a control for FAZ studies when using these metrics.

Translational Relevance: Vision scientists must be prudent when choosing FAZ metrics, as they display varying degrees of within-subject differences relative to between-subject differences. It seems likely that different metrics will be best suited for different tasks, such as monitoring small changes over time within a single subject or assessing whether a given FAZ is abnormal.

The area devoid of retinal capillaries at the central macula defines the foveal avascular zone (FAZ). There is a variation in FAZ size across normal individuals,^{1–3} but it may be affected in a variety of diseases including diabetic retinopathy,⁴ sickle cell retinopathy,⁵ albinism,⁶ and foveal hypoplasia.^{7,8} Studies have reported a correlation between visual acuity and the enlargement of the FAZ in diabetic

retinopathy and that vascular changes occur early in the disease course.⁹ This information suggests that FAZ metrics may serve as useful biomarkers in studying disease onset and progression not only in the eye but also in systemic diseases.¹⁰

Despite the ability to measure the FAZ with a plethora of imaging modalities for the last three decades,^{11–17} it is only recently, with the advent of optical coherence tomography angiography (OCTA), that imaging the FAZ has become highly

practical in the clinic. Owing to its conceptual simplicity and the ease with which it can be measured, area has been the most studied metric for characterizing the FAZ.^{18–20} However, as with all laterally based measurements in retinal images, individual differences in ocular magnification and axial length need to be corrected in order to accurately compare area measurements between subjects or across studies.^{1,21} The need to measure axial length, as well as the large variation of area seen in normal individuals,^{1–3,19,22–25} makes it difficult to use area as a measure to compare small changes associated with disease progression between subjects or for identifying an FAZ as “abnormal.” While these limitations are less important when assessing repeatability of OCTA during a single visit,²⁶ they represent important barriers to comparing data across different studies and populations.

Due to these limitations, other metrics have been introduced to assess the relative health of the FAZ. Acircularity index (acircularity), introduced in 2011,²⁷ is defined as the perimeter shape of a given FAZ relative to a perfect circle with the same area of that FAZ. Axis ratio, introduced in 2017, is the ratio between the major and minor axis of an ellipse having the same normalized second central moments as the FAZ itself.²⁸ Prior to the axis ratio, in 2016 the major horizontal axis angle (MHAA, defined as the difference between a horizontal line and the major axis of the aforementioned ellipse) was used in a diabetes study to differentiate between normal and diseased eyes.²⁹ The major practical advantage of these proposed metrics is that axial length measurement and correction for ocular magnification is not required, making them easier to use in a clinical setting. However, the application of any metric to compare eyes in normal and diseased states requires a comprehensive understanding of the sources of variability underlying a given metric; this may help inform which metric may be more clinically useful. Sources of variability in a metric may include but are not limited to differences between subjects, interocular differences, segmentation differences, as well as differences between images of the same eye. However, there has been minimal direct comparison of different FAZ metrics regarding the factors that contribute to their respective variances. Therefore, this study was designed to assess the variance and interocular symmetry for four FAZ metrics (area, axis ratio, acircularity, and MHAA) in normal subjects.

Methods

Subjects

This study was approved by the Institutional Review Board of the Medical College of Wisconsin (PRO 23999 and PRO 17439) and was conducted in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained for all subjects once the nature and risks of the study were explained. An ocular health questionnaire (OHQ) was used to ascertain that subjects had no previous retinal disease. Inclusion criteria included 5 years of age or older, no major eye movement artifacts near the fovea, and subjectively good signal to noise ratio and overall image sharpness. Exclusion criteria included any previous history of ocular or systemic vascular disease, such as hypertension and diabetes.

Imaging

Axial length measurements were acquired using a partial-coherence interferometer (IOL Master; Carl Zeiss Meditec, Dublin, CA). Spectral domain optical coherence tomography (OCT) images were acquired from each subject (Cirrus HD-OCT; Carl Zeiss Meditec) to assess for any posterior segment anomaly and pathology by a single observer (MNM). If the images were not available, the structural OCT from the AngioVue OCTA system (Optovue, Inc., Fremont, CA) was examined instead ($n = 9$ subjects).

Subjects underwent OCTA imaging with the AngioVue OCTA system (Optovue Inc.). Two scans (one horizontal and one vertical), each consisting of 304 B-scans at 304 A-scans per B-scan, were acquired at the fovea of both eyes with a nominal scan size of 3×3 mm. The horizontal and vertical scans were then coregistered by the device (software version 2016.2.0.16) to minimize motion artifacts and create a single volume from which an image of the superficial plexus was extracted.^{30,31} The superficial plexus image was produced by integrating motion contrast data between $3 \mu\text{m}$ below the internal limiting membrane to $16 \mu\text{m}$ above the inner plexiform layer. Two such volumes for each eye of each subject were obtained for a total of four images per subject.

Quantitative FAZ Analysis

The FAZ in each superficial plexus image was manually segmented by a single observer (REL) using ImageJ software’s multipoint tool.³² Each image was

then segmented a second time, with the observer masked to their initial segmentation. The coordinates from each segmentation were entered into a custom MATLAB script similar to one previously described.^{1,12} In brief, the script used the function *poly2mask* to produce a mask defining the area and the perimeter of the FAZ. To accommodate the analysis required for this study, changes made to the script included calling for the major axis, minor axis, and MHAA (orientation) using the MATLAB function *regionprops*.¹²

The size of the FAZ is equal to the number of pixels included in the FAZ mask. The nominal image scale is taken as the nominal scan width (3000 μm) divided by the width of the image in pixels (304). The actual image scale was determined as previously described¹ by multiplying the nominal image scale by the ratio of the subject's measured axial length to that assumed by the system (23.95 mm). This image scale was used to convert FAZ mask area from pixels to absolute retinal area in square millimeters. The perimeter was scaled in a similar manner and was used to derive the acircularity of the FAZ defined as ratio of the perimeter of a given FAZ to that of a perfect circle with the same area of the FAZ (will always be ≥ 1). The major axis, minor axis, and MHAA of the ellipse with the same normalized second central moments as the FAZ masked region were output by *regionprops* (Fig. 1). Axis ratio is simply the ratio of the major axis length to minor axis length (will always be ≥ 1). The MHAA is defined as the orientation of the major axis to a horizontal line through the origin of the ellipse (ranges between -90° and 90°).

Statistical Analysis

For each metric, a paired *t*-test or Wilcoxon matched-pairs signed rank test was used to evaluate interocular symmetry (Prism; GraphPad Software, La Jolla, CA). The choice of test was based on an analysis of normality (using $P < 0.05$ as the criterion) of the differences between eyes using the D'Agostino-Pearson normality test (Prism; GraphPad Software). In addition, a variance component model was used to estimate the subject effect (differences between subjects) and the effects of an eye within a subject (intraocular differences), image within a subject (differences between repeated images of the same eye), and trial within a subject (differences between repeated segmentations by the same observer) to the overall variance of each metric (SAS v9.4; SAS Institute, Cary, NC). As above, the data were

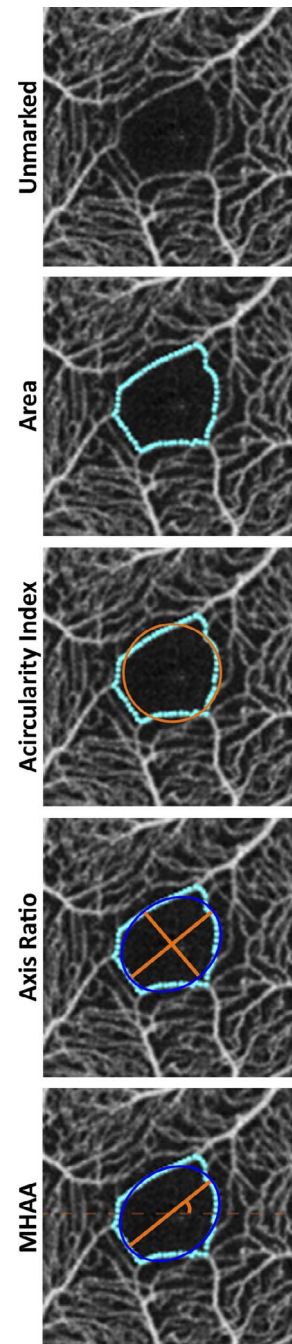


Figure 1. Finding each FAZ metric using our custom MATLAB script. All four metrics are found with a single segmentation shown in teal. Area is defined as the area within the FAZ segmentation boundary. Acircularity compares the perimeter of the FAZ segmentation to the perimeter of a perfect circle with the same area. For axis ratio and the MHAA, we use the ellipse (defined by *regionprops*) with the same normalized second central moments as the FAZ masked region. Axis ratio is ratio of the major to the minor axis of this ellipse (longest to shortest axis ratio), while the MHAA is the angle at which the major axis of the ellipse occurs relative to the horizontal axis.

Table 1. Summary of FAZ Metrics for 350 Eyes of 175 Subjects With Normal Vision

| | Area, mm ² | Axis Ratio, Log-Transformed | Acircularity | MHAA Degrees |
|---------------------|-----------------------|--------------------------------|--------------|-----------------|
| Mean, ± SD | 0.278 ± 0.101 | 1.19 ± 0.119 | 1.19 ± 0.095 | −7.00 ± 36.47 |
| Range | 0.073–0.659 | 1.02–2.16 | 1.02–1.54 | −88.11 to 85.02 |
| Median | 0.270 | 1.17 | 1.16 | −5.80 |
| Interquartile Range | 0.120 | 0.123 | 0.134 | 47.64 |

analyzed for normality, and if normality could not be confirmed for a given metric, the variance component model was run on log-transformed data.

Results

We recruited 187 “normal” subjects for the study. However, upon review of their OHQs, as well as their structural OCT results, 12 subjects were excluded due to ocular or systemic abnormalities. Four of these subjects reported a history of hypertension ($n = 2$), diabetes ($n = 1$), or histoplasmosis ($n = 1$). Others were excluded based on OCT findings as follows: optic disc abnormalities ($n = 3$), foveal hypoplasia ($n = 1$), intraretinal hyperreflective speckle lesions ($n = 1$), microhole at the fovea ($n = 1$), posterior staphyloma ($n = 1$), and vitreous debris and retinal hemorrhage ($n = 1$). Our final database consisted of two images from 350 eyes of 175 subjects (96 females) with the average age (\pm SD) of 27.9 ± 11.9 years old (range, 5–77 years old).

The population mean (\pm SD) and range for each metric is provided in Table 1. The difference between eyes for each metric was tested for normality with only the axis ratio failing. Because of this, axis ratio was log-transformed and the analysis was run on the transformed numbers. When assessing interocular symmetry of the FAZ (Fig. 2), area, acircularity, and MHAA showed no statistically significant

difference between eyes ($P = 0.11$, $P = 0.63$, $P = 0.35$, respectively) with mean differences of 0.0043 mm^2 , 0.0044 , and 3.71° , respectively. However, the log-transformed axis ratio resulted in statistically significant differences between eyes within a subject ($P = 0.033$), where the average ratio between eyes showed the right eye to be 3% larger than that of the left. However, even for the three metrics assessed as having high degree of interocular symmetry, we found that true asymmetry for each metric can exist even in healthy patients as shown in Figure 3.

The results of the variance component analysis assessing the contributions to the variance for each of the four metrics are shown in Table 2. The axis ratio failed the normality test, thus the analysis was run on the log-transformed numbers. When comparing between the metrics, area had the largest contribution of variance due to subjects, whereas there was less contribution from subjects for the other three metrics. In addition, the area had significantly less variance caused by both interocular variability within subjects (between the two eyes of a subject) and residual component (variance not attributed to the other variables). In contrast, axis ratio, acircularity, and MHAA had greater than 50% of its variance between the fellow eyes. Also, the measurement error for axis ratio, acircularity, and MHAA varied between 20% and 30%. Variation arising from image segmentation and trial were negligible for all metrics. Thus, there

Table 2. Component Variance Analysis for Each FAZ Metric With Amount of Variance Attributed to Each Possible Variable for Both Exact and Percentage

| Component Variance Analysis | Area, mm ² | Axis Ratio, Log-Transformed | Acircularity | MHAA Degrees |
|-----------------------------|-----------------------|--------------------------------|------------------|-----------------|
| Subject (%) | 0.009614 (93.11) | 0.001538 (13.22) | 0.002063 (18.17) | 0.00 (0.00) |
| Image (%) | 0.00 (0.00) | 0.000010 (0.08) | 0.00 (0.00) | 3.2206 (0.19) |
| Trial (%) | 0.000007 (0.07) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| Eye (%) | 0.00060 (5.76) | 0.006399 (55.00) | 0.006093 (53.67) | 1202.85 (70.72) |
| Residual (%) | 0.000109 (1.06) | 0.003687 (31.69) | 0.003196 (28.15) | 494.89 (29.09) |
| Total (%) | 0.01033 (100) | 0.011634 (100) | 0.011352 (100) | 1690.9606 (100) |

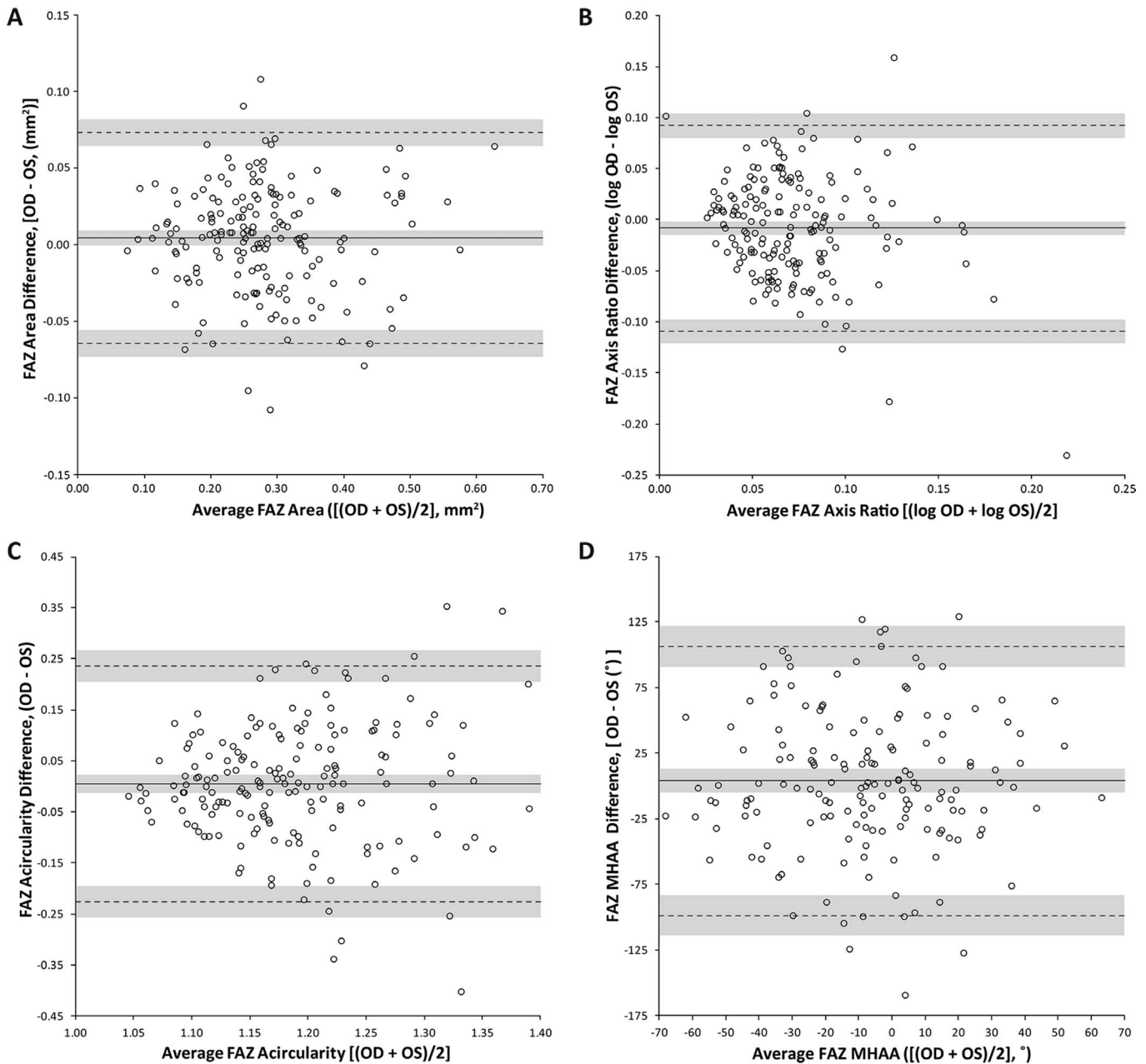


Figure 2. Interocular symmetry of FAZ metrics. Data are expressed in Bland-Altman plots to show interocular differences for (A) area, (B) axis ratio, (C) acircularity, and (D) MHAA. *Solid lines* represent average difference (bias) between the eyes, while *dotted lines* represent limits of agreement (LOA). The *gray shading* represents the confidence intervals for the bias and LOAs for each metric.

was good repeatability between two images taken for each eye and reliability in segmentation of images. However, the repeatability and reliability for the MHAA can be decreased by small changes in head or eye positioning or rotation (Fig. 4), while the wide range of normal angles makes classifying angles as pathologically difficult (Fig. 5).

Discussion

Our large normative database allowed us to analyze four FAZ metrics (area, axis ratio, acircularity, and MHAA) that have been used in various OCTA studies.^{7,9,27–29,33–46} We evaluated whether there was

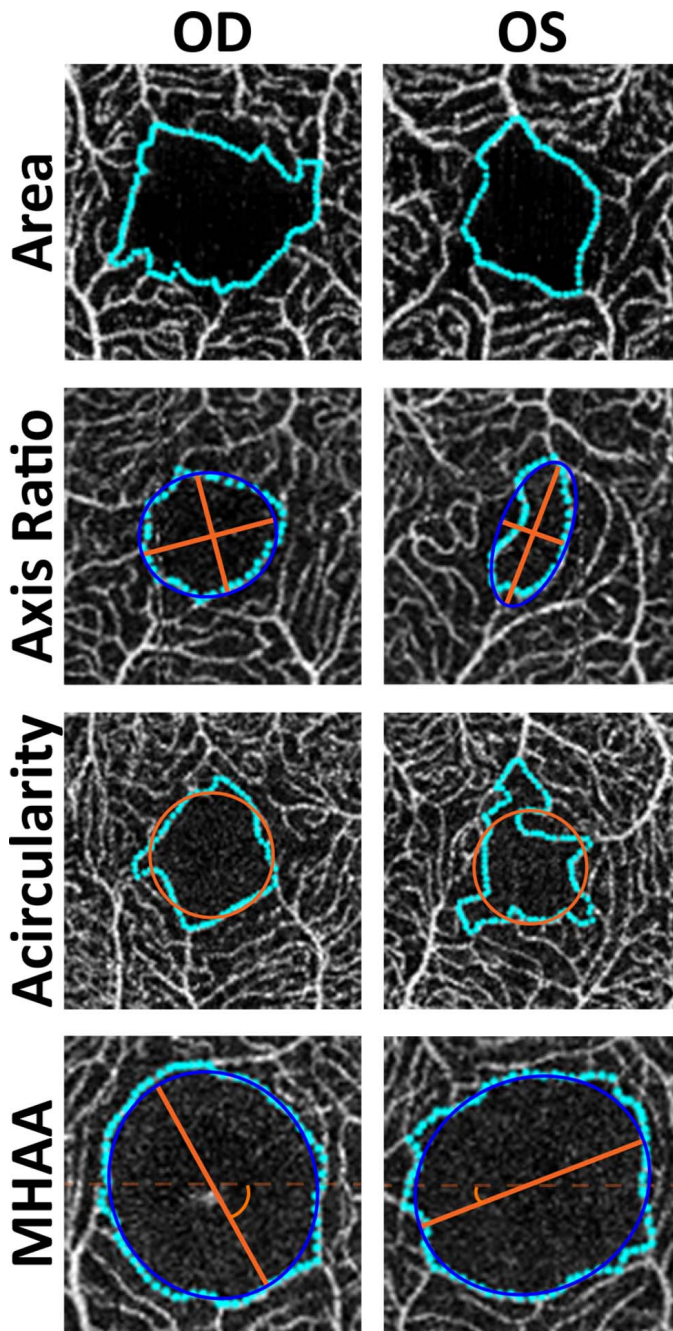


Figure 3. Examples of interocular asymmetry in FAZ morphology. From *top to bottom*, subject JC_11327 had an interocular difference of 0.11 mm² for area, subject JC_10672 had an interocular difference of 0.59 for the log of the axis ratio (~80%), subject JC_1246 had an interocular difference of 0.402 for acircularity, and subject JC_10579 had an interocular difference of 88.75° for the MHAA. Such large differences were not common but can occur, even in patients with no ocular pathology.

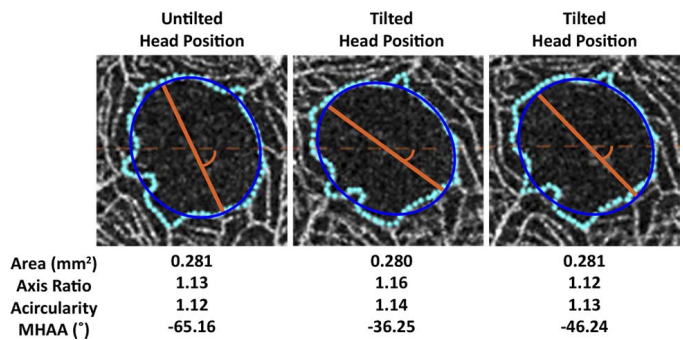


Figure 4. Illustrating the effect of head tilt on FAZ metrics. Shown on the *left* is an image from the right eye of a subject (JC_10567) acquired using the patient's normal head posture. The patient was then instructed to tilt the head, after which two additional images were acquired (*middle* and *right panels*). The area, axis ratio, and acircularity did not change substantially (less than 3%), whereas the MHAA changed by 44% (*middle panel*) and 29% (*right panel*).

interocular symmetry for each of the four metrics and assessed how different sources of variation contribute to the overall variance of each metric. These analyses are important for clinicians and imaging researchers to consider when deciding on the appropriate metric to be used as an imaging biomarker in a clinical setting or in research trials utilizing OCTA. It is important to note that our results are limited to the device used here (Optovue AngioVue). Other OCTA devices may yield different results, especially as the within-image distortions can be substantial in devices that scan the retina in only a single direction.^{47–50} Such distortions would be unique between repeated images of the same subject and thus would affect the absolute accuracy of the FAZ metric(s) being examined. This would also increase the contribution of the image to the overall variance of a given metric, thereby reducing the ability to detect real differences between subjects, between eyes, or between images of the same eye taken over time.

Area remains the most widely used and easily understood FAZ metric of the four studied here. The mean (\pm SD) area in our study of 0.278 ± 0.101 mm² is very similar to that reported in other recent studies of 0.266 to 0.284 (± 0.09 – 0.11) mm².^{19,22–25,51} However, accuracy of the reported area is dependent on multiple factors, including the method used to segment the FAZ^{1,24,49,52} and whether or not the image scale was corrected for ocular magnification due to individual differences in axial length.^{1,21} Not correcting for axial length can result in errors in the estimated FAZ area of up to 51%.^{1,21} A limitation of our study is that the majority of subjects were Caucasian (78.9%). Area is known to be larger in

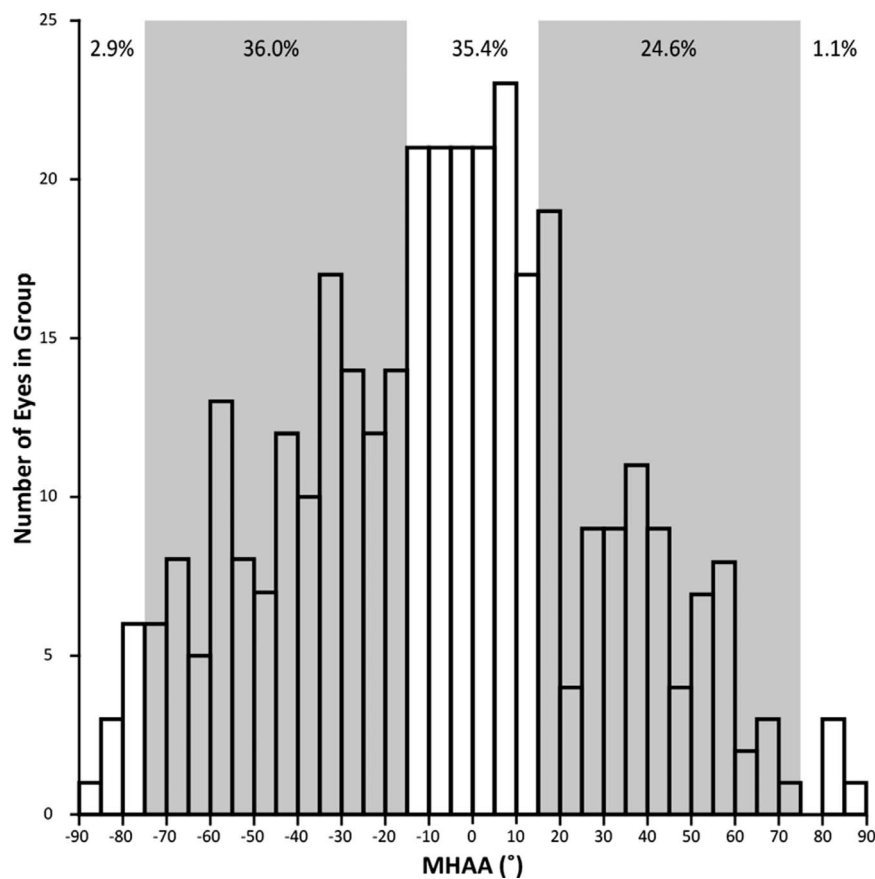


Figure 5. Distribution of MHAAs observed in 350 eyes with no known pathology. Freiberg et al.²⁹ suggested that a normal MHAAs for the FAZ should fall within 15° of either the horizontal (0°) or vertical (90°) axis. However, in our data, 60.6% of the eyes had angles that fell out of this proposed normal range (shown by the shaded gray regions in the histogram).

African,⁵³ Japanese,⁵⁴ and Chinese^{51,55,56} subjects compared to Caucasian subjects. It seems likely that race/ethnicity-specific normative databases will be required to fully leverage area (and possibly other FAZ metrics) as biomarkers for detecting and monitoring retinal and systemic vascular diseases. Finally, while our study has a large age range (5–77 years), the majority of our subjects were 20 to 50 years old (87.4%). There is still debate on whether area changes naturally with aging, with some reports showing no change^{1,19,20,25} and others showing enlargement.^{3,56,57} Regardless, more work should be done to increase the number of younger and older subjects to better understand how these metrics may change among age groups, as it may also be necessary to utilize age-specific normative databases.

Acircularity and other shaped-based metrics have been proposed as potential biomarkers for diseases impacting the FAZ, as small capillary dropout at the FAZ can lead to large changes in the perimeter but not to the area.^{27,28,39} Researchers have shown that it is a

more sensitive metric than area in detecting microvascular dropout.^{28,39} Conversely, this sensitivity itself may represent a potential liability as small errors in FAZ segmentation would have a more significant impact on the acircularity than on the area as well as cause a decrease in repeatability.^{1,51} Similar issues relating to metric sensitivity are seen with adaptive optics retinal images of the cone mosaic, where true cone degeneration is confounded by errors in cone identification. To fully characterize cone mosaic metrics, Cooper et al.⁵⁸ simulated variable loss of cones from real images of the cone mosaic to monitor how different metrics respond to that loss, enabling a head-to-head comparison of the relative sensitivity of each metric. Similar simulation studies will be required to fully assess the relative sensitivity of each FAZ metric to capillary dropout and segmentation errors, which will help inform which metric may be most appropriate for a given study. It seems likely that a combination of metrics may, in fact, be the best approach.

For acircularity, we found no statistically significant

difference between the eyes; yet when looking at our variance component model, over 53% of the variance for acircularity was attributed to differences between eyes. This apparent discrepancy is most likely due to the minimal amount of variance found in the metric itself across all subjects. This result is similar to that of Fang et al.⁵¹ as they found no statistically significant difference in FAZ circularity between eyes. However, they reported no significant correlation between eyes (Pearson $r = 0.148$, $P = 0.411$). While not our primary analysis, we also examined the interocular correlation and found a weak but statistically significant correlation (Spearman $r = 0.274$, 95% confidence interval = [0.1269–0.4098], $P = 0.0002$). The strength of correlation is comparable between our study and that of Fang et al.⁵³; however, we had over five times as many subjects. When comparing FAZ area, both our study and that of Fang et al. found no significant difference between eyes; however, Fang et al. reported a larger mean FAZ area in their subjects. This is most likely due to difference in racial makeup of our populations, furthering the point that race and ethnicity should be considered when comparing FAZ metrics.

We identified an important confound in assessing MHAA, which has important implications for clinical use of this metric. Owing to the lack of an external anchor on which to base the measurement, small changes in head or eye positioning and rotation will manifest as real changes in the MHAA (Fig. 4). This means that FAZ images acquired over time should be registered to a baseline image to minimize this effect, though changes in the retinal vasculature and the FAZ itself may complicate this process. For comparing different subjects, however, it would be impossible to know if any difference in MHAA was due to differences in FAZ morphology or differences in relative head positioning of the two subjects during image acquisition. It is important to note that this does not impact the other three FAZ metrics.

Asymmetry of image-based measures of retinal and ocular anatomy has been shown to be a useful biomarker for retinal nerve fiber layer thickness,⁵⁹ disc cupping,⁶⁰ or axial length,⁶¹ as it can be an early indicator of pathology. While we found no significant asymmetry in area, acircularity, or MHAA, we did observe significant interocular asymmetry for axis ratio. This asymmetry may be caused by an oversimplification of the FAZ shape, since it requires an ellipse that represents the FAZ rather than using the true segmentation of the FAZ. Alternatively, it could be a more sensitive metric that shows true differences between eyes that the other metrics were not able to

detect. Regardless, axis ratio has been suggested as a possible biomarker for diabetic retinopathy due to its ability to capture the enlargement of the FAZ occurring in retinopathies and does not require axial length.²⁸ These factors make the axis ratio ideal for clinical practice, yet the significant asymmetry between fellow eyes in our normal population brings its clinical utility into question.

It is important to note that we used a single observer to manually segment the FAZs. We elected to use manual segmentation as prior studies in our lab demonstrated that manual segmentation had lower measurement error and better repeatability compared to two versions of automated segmentation provided by the device manufacturer.¹ This is also reflected in the present study where the contribution of trial to the overall variance component model was near zero for all metrics (Table 2), indicating excellent repeatability of this observer. Our study was designed to examine interocular symmetry and relative between-subject variation in the four FAZ metrics used; having such highly repeatable segmentation allowed us to isolate these biological variables. Future studies seeking to characterize the effect of different algorithms or different manual graders will be needed to make generalized conclusions about a given method's ability to detect interocular or between-subject differences.

There is no ideal metric for assessing the FAZ, as all the metrics studied here have important limitations. While the variation in area was largely driven by real differences between subjects, it could be that the differences between eyes or images were too subtle to be picked up by such a high-level descriptor of the FAZ. Moreover, area must be scaled to axial length, a manual process that limits clinical applicability. Acircularity is a more sensitive measure when evaluating for minor changes in FAZ morphology. Yet, small differences in segmentation can produce unfounded, significant changes. In summary, each metric has beneficial and limiting characteristics. Therefore, clinicians and vision science researchers must use care when deciding which metric to use for a particular clinical application. A combination of metrics may be required to capture local and global aspects of the FAZ.

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