IMPORTANCE Accurate measurements of choroidal thickness (CT) using enhanced-depth imaging optical coherence tomography (EDI-OCT) require a well-defined choroid-scleral junction (CSJ), which may appear in some eyes as a hyporeflective band corresponding to the suprachoroidal layer (SCL).

OBJECTIVE To identify factors associated with the presence and thickness of the SCL in healthy participants and determine how different CSJ boundary definitions impact CT measurements.

DESIGN, SETTING, AND PARTICIPANTS Secondary analysis of EDI-OCT images obtained prospectively from 74 eyes of 74 controls (mean age, 68.6 years) from the Age-Related Eye Disease Study 2 Ancillary SD-OCT Study.

MAIN OUTCOMES AND MEASURES The CSJ appearances were categorized as either having no visible SCL or a hyporeflective band corresponding to the SCL. Ocular parameters associated with the presence and thickness of the SCL were identified. Subfoveal CT was measured using 3 different posterior boundaries: (1) the posterior vessel border (vascular CT [VCT]), (2) inner border of the SCL (stromal CT [StCT]), and (3) inner border of the sclera (total CT [TCT]). Manual segmentation using custom software was used to compare VCT, StCT, and TCT across the macula.

RESULTS The SCL was visible in 33 eyes (44.6%). Factors associated with SCL presence and thickness included hyperopic refractive error ($R^2 = 0.123; P = .045$) and increased TCT ($R^2 = 0.215; P = .004$), but not age, visual acuity, intraocular pressure, retinal foveal thickness, VCT, or StCT. In eyes where the SCL was not visible, mean [SD] subfoveal VCT was 222.3 [101.5] μm, and StCT and TCT were 240.0 [99.0] μm, with a difference of 17.7 [16.0] μm ($P < .001$). In eyes where the SCL was visible, mean [SD] subfoveal VCT, StCT, and TCT were 221.9 [83.1] μm, 257.7 [97.3] μm, and 294.1 [104.8] μm, respectively, with the greatest difference of 72.2 [30.4] μm between VCT and TCT ($P < .001$). All 3 CT measurements were significantly different along all points up to 3.0 mm nasal and temporal to the fovea.

CONCLUSIONS AND RELEVANCE A hyporeflective SCL is visible at the CSJ on EDI-OCT in nearly half of healthy individuals, and its presence correlates with hyperopia. Different posterior boundary definitions may result in significant differences in CT measurements and should be explicitly identified in future choroidal studies and segmentation algorithms.

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The choroid is a complex vascular structure that supplies the outer retina. However, choroidal anatomy is poorly visualized on conventional spectral-domain optical coherence tomography (SDOCT) because of poor signal penetration through the retinal pigment epithelium and light beam defocus at the level of the choroid relative to the retina. Enhanced-depth imaging optical coherence tomography (EDI-OCT) is an imaging technique that allows conventional SDOCT devices to visualize choroidal details by taking advantage of the increased depth of field from the inverted image obtained by placing the OCT device closer to the eye. Recent studies measuring choroidal thickness (CT) using EDI-OCT have implicated a role of the choroid in the pathogenesis of various ocular diseases including central serous choriorretinopathy, polypoidal choroidal vasculopathy, Vogt-Koyanagi-Harada syndrome, and possibly age-related macular degeneration.

Although EDI-OCT has prompted a renewed interest in understanding choroidal anatomy in disease pathophysiology, CT measurements have varied considerably between different studies. Unlike retinal thickness, CT varies with age, axial length, and time of day. Moreover, differences in image acquisition techniques and CT measurement methods contribute to these discrepancies. An important factor in standardizing CT measurements is the definition of the choroidal-scleral junction (CSJ). Most EDI-OCT studies, including the original Spaide et al report of the technique, define the CSJ as the inner border of the sclera. However, some groups have also noted in some individuals a hyporeflective band, likely corresponding to the suprachoroidal layer (SCL), which may result in variability in CT measurements.

Histologically, the boundaries of the choroid are defined anteriorly by the lamina vitrea or Bruch's membrane and posteriorly by the lamina fusca or SCL. While Bruch's membrane is thin and compact to allow nutrient and oxygen exchange with the outer retina, the SCL is actually a zone of transition, variable in thickness, consisting of interconnected lamellar fibers extending from the choroidal stroma to the sclera. The lamellae are in close apposition to each other but may become separated by fluid or blood in pathologic states, resulting in "suprachoroidal" effusion or hemorrhage. The varied appearance of the SCL may therefore confound accurate determination of the CSJ and CT measurements on EDI-OCT.

In this study, we characterized the different appearances of the CSJ seen on EDI-OCT obtained from healthy volunteers participating as controls in the Age-Related Eye Disease Study 2 (AREDS2) Ancillary SDOCT study (A2A-SDOCT) and identified ocular parameters associated with the presence and thickness of the SCL. We measured CT using different landmarks as the posterior boundary and determined how different CSJ definitions impact CT measurements. Anatomic comparisons between the SCL seen on EDI-OCT and histological sections are also presented.

Methods

Patient Selection
All participants for this study were control participants in the prospective A2A-SDOCT study (ClinicalTrials.gov identifier NCT00734487). Participants were recruited on a voluntary basis from outside the Duke University Eye Center patient population. Written informed consent was obtained from all study participants. The study followed the tenets of the Declaration of Helsinki and was approved by the institutional review board at the Duke University Eye Center. Inclusion criteria for controls have been previously described and include healthy participants aged 50 to 85 years with normal ophthalmologic examination findings including dilated ophthalmoscopy. Exclusion criteria include known or discovered retinal or choroidal disease, glaucoma, or high myopia (>6 diopters D spherical equivalent). As part of the A2A-SDOCT study, all participants had best-corrected visual acuity measured as Early Treatment Diabetic Retinopathy Study letter score, with refraction performed and intraocular pressure (IOP) measured by a certified ophthalmic technician. Lens opacity was graded using the AREDS Clinical Lens Grading System, which measures degree of nuclear sclerosis on a scale from 0 to 3 in 0.5 step. The EDI-OCT imaging was performed on both eyes in all the participants included in this study.

Image Capture Protocol
Enhanced-depth imaging OCT and standard volumetric SDOCT were performed on each eye using the Heidelberg Spectralis SD-OCT (870-nm) device (Heidelberg Engineering). The EDI-OCT scans were captured using the Spectralis EDI mode, a preset, software-driven algorithm that places the retinal pigment epithelium near the zero-delay line while producing an upright enhanced choroidal image. In EDI-OCT mode, a single 30° horizontal line scan (approximately 8.9 mm) captures 1536 A-scans per B-scan with 40 averaged B-scans per image, using the automatic averaging and eye tracking features. For standard volumetric SDOCT of the macula, 20° horizontal line scans covered an approximate 5 × 5 mm area centered on the fovea and consisted of 97 horizontal B-scan lines with 512 A-scans per B-scan.

CT and SCL Measurements
The EDI-OCT images were viewed and measured using Heidelberg Eye Explorer software (version 1.7.0.0; Heidelberg Engineering) and manually segmented using DOCTRAP, a customized software with a graphical user interface designed using MATLAB (MathWorks). Two experienced masked observers (G.Y. and P.P.) independently categorized the CSJ as either a single distinct junction with no visible SCL (SCL absent) (Figure 1A) or a widened hyporeflective band likely corresponding to the SCL (SCL present) (Figure 1B). Images in which the observers did not agree on the presence of a SCL, or which either observer deemed to be ungradable because of poor visualization of the CSJ, were removed from analysis. Both observers also measured the subfoveal CT from the outer border of the hyperreflective line corresponding to the retinal pigment epithelium to each of 3 posterior boundaries: (1) the posterior large-vessel border (vascular CT [VCT]), (2) the inner border of the SCL (stromal CT [StCT]), or (3) the inner border of the sclera (total CT [TCT]), which includes the SCL and is distinct from the StCT only in eyes where the SCL is visible (Figure 1C).
For eyes with a visible SCL, manual segmentation was also performed using each of these 3 posterior boundaries to measure CT at the fovea and in 5-μm intervals up to 3.0 mm nasal and temporal to the fovea. The SCL thickness was calculated as the difference between TCT and StCT. Measurements taken by the 2 observers were averaged for statistical analyses. Central foveal thickness (CFT) of the retina was determined by Heidelberg Eye Explorer software. Anatomic comparisons were made using histological sections of a nonstudy adult postmortem eye after standard fixation and staining with hematoxylin-eosin (Figure 1D).

**Eye Selection and Statistical Analysis**

Only 1 eye from each participant was designated as the study eye for analysis. The study eye was chosen if only 1 eye qualified based on inclusion/exclusion criteria. If both eyes qualified, the right eye was designated the study eye for participants with an even-number birth month, and the left eye was selected for those with an odd-number birth month. In participants where both eyes qualified, the fellow eye was used for comparisons between right and left eyes. The significance of the difference between participants with or without a visible SCL was assessed using t tests for scale variables (age, refractive error, best-corrected visual acuity, IOP, CFT, VCT, StCT, and TCT) and Mann-Whitney U test for the ordinal variable (lens opacity). The difference in time of day when the images were obtained was evaluated by Wilcoxon rank sum analysis. The relationship of SCL thickness with each ocular parameter was assessed by univariate linear regression analysis. The VCT, StCT, and TCT were compared using paired-samples t tests. In participants where both eyes qualified for analysis, comparisons between study and fellow eyes were measured using the κ statistic for binomial factors (presence or absence of SCL) and intraclass correlations for scale factors (VCT, StCT, TCT, and SCL thickness). Interobserver correlations between the 2 observers were also determined using intraclass correlations according to Bland and Altman. All statistical analyses were performed using SPSS software (IBM Corp).
Results

Demographics

Seventy-nine healthy controls underwent EDI-OCT imaging of both eyes as part of the A2A-SDOCT study. Seventeen eyes were excluded: 3 because of high myopia (>6 D spherical equivalent), 1 because of a new diagnosis of myopic foveoschisis, and 13 because of poor visualization of the CSJ using criteria described in the Methods section. Of the 141 remaining eyes eligible for analysis, 74 eyes from 74 participants were designated as the study eye. Among qualifying participants, the mean age was 68.6 years (range, 55 to 85 years), 30 participants (40.5%) were male, and 72 (97.3%) were white, while the remaining 2 participants were Asian. Of the study eyes, 34 were right eyes (45.9%), 72 were phakic (97.3%), and 2 were pseudophakic.

Factors Associated With Visible SCL

The SCL was visible at the CSJ in 33 study eyes (44.6%). Among the ocular parameters evaluated, eyes with a visible SCL were associated with more hyperopic refractive error (Table 2). The mean (SD) refractive error of eyes with a visible SCL was +0.70 (2.20) D, compared with −0.85 (2.08) D in those without (P = .003). There was no significant difference in age (mean [SD], 68.9 [8.0] years vs 68.4 [6.7] years; P = .78), IOP (mean [SD], 15.9 [3.2] mm Hg vs 15.6 [2.6] mm Hg; P = .60), best-corrected visual acuity (mean [SD] Early Treatment Diabetic Retinopathy Study letter score, 82.6 [4.9] [Snellen equivalent 20/22] vs 84.6 [4.0] [Snellen equivalent 20/20]; P = .06), or retinal CFT (mean [SD], 284.8 [21.5] μm vs 281.2 [25.2] μm; P = .51) between the 2 groups. The VCT (mean [SD], 221.9 [83.1] μm vs 222.3 [101.5] μm; P = .99) and StCT (mean [SD], 257.7 [97.3] μm vs 240.0 [99.0] μm; P = .44) were also similar, while the TCT was significantly thicker in eyes with a visible SCL compared with those without (mean [SD], 294.1 [104.8] vs 240.0 [99.0]; P = .03). This difference was expected, because the TCT measurement includes the SCL, while VCT and StCT do not. These results suggest that the SCL is more likely to be visible in more hyperopic eyes and eyes with a higher TCT. In phakic eyes (n = 72), SCL visibility was not affected by the severity of cataracts, because grade of lens opacity was similar between the 2 groups (mean [SD], 1.54 [0.79] vs 1.69 [0.55]; P = .50). The median time at which the EDI-OCT scan was obtained also did not differ significantly between the 2 groups (11:44 AM vs 11:18 AM; P = .51).

Table 1. Study Demographics

| Age, y (range) | 68.6 (55-85) |
| Sex | M 30 | F 44 |
| No. of Participants (N = 74) | 34 | 40 |
| Phakic/pseudophakic | 72/2 |
| Refractive error, D, mean (SD) | −0.15 (2.26) |
| BCVA (ETDRS letter score), mean (SD) | 83.7 (4.5) |
| IOP, mm Hg, mean (SD) | 15.7 (2.9) |
| Abbreviations: BCVA, best-corrected visual acuity; D, diopter; ETDRS, Early Treatment Diabetic Retinopathy Study; IOP, intraocular pressure. |

Factors Correlated With SCL Thickness

The mean (SD) subfoveal SCL thickness was 36.4 (17.8) μm in eyes where the SCL was visible (Table 2). The SCL thickness was similar when measured in 5-μm intervals up to 3.0 mm nasal and temporal to the fovea, except at the far nasal area near the optic nerve where the thickness is reduced (Figure 2A). Subfoveal SCL thickness was variable among individuals but showed a slight association with more hyperopic refractive error, with borderline significance (R² = 0.123; P = .045) (Figure 2B), but not with age

Table 2. Factors Associated With Visible SCL in Aged Eyes

<table>
<thead>
<tr>
<th>Mean (SD)</th>
<th>SCL Present (n = 33)</th>
<th>SCL Absent (n = 41)</th>
<th>P Value</th>
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<tr>
<td>Age, y</td>
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<td>68.4 (6.7)</td>
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<td>Refractive error, D</td>
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<td>BCVA (ETDRS letter score)</td>
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<td>84.6 (4.0)</td>
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<td>IOP, mm Hg</td>
<td>15.9 (3.2)</td>
<td>15.6 (2.6)</td>
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<td>CFT, μm</td>
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<td>281.2 (25.2)</td>
<td>.51</td>
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<td>VCT, μm</td>
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<td>StCT, μm</td>
<td>257.7 (97.3)</td>
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<td>.44</td>
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<tr>
<td>TCT, μm</td>
<td>294.1 (104.8)</td>
<td>240.0 (99.0)</td>
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</tr>
<tr>
<td>SCL thickness, μm</td>
<td>36.4 (17.8)</td>
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</table>

Abbreviations: BCVA, best-corrected visual acuity; CFT, central foveal thickness; D, diopter; ETDRS, Early Treatment Diabetic Retinopathy Study; NA, not applicable; IOP, intraocular pressure; SCL, suprachoroidal layer; StCT, stromal choroidal thickness; TCT, total choroidal thickness; VCT, vascular choroidal thickness. a Significant.
Figure 2. Factors Associated With Suprachoroidal Layer (SCL) Thickness in Healthy Participants

(A) The SCL thickness across the macula in participants where the SCL is visible. B and C, Subfoveal SCL thickness vs refractive error (B) and total choroidal thickness (TCT) (C). The curved lines are the 95% confidence intervals for the trend line (straight). D indicates diopter.

(R\(^2\) = 0.007; \(P = .65\)), visual acuity (R\(^2\) = 0.003; \(P = .78\)), IOP (R\(^2\) = 0.027; \(P = .36\)), or CFT (R\(^2\) = 0.005; \(P = .69\)). Subfoveal SCL thickness also correlated with increased CT but was only statistically significant when measured to the sclera as the TCT (R\(^2\) = 0.240; \(P = .004\)) (Figure 2C). Both VCT (R\(^2\) = 0.110; \(P = .06\)) and StCT (R\(^2\) = 0.119; \(P = .05\)) showed only a weak correlation with borderline significance. These results suggest that SCL thickness contributes to CT measurements only when it is included in the measurement (TCT) but may not independently correlate with the underlying thickness of the vascular or stromal choroid (VCT or StCT).

Impact on CT Measurements
Subfoveal CT varied significantly depending on the posterior boundary used. In participants where the SCL was not visible, mean (SD) VCT was 222.3 (101.5) \(\mu\)m while mean (SD) StCT and TCT were 240.0 (99.0) \(\mu\)m (Figure 3A), with a mean (SD) difference of 17.7 (16.0) \(\mu\)m (\(P < .001\)). In eyes where the SCL was visible, mean (SD) VCT was 221.9 (83.1) \(\mu\)m, mean (SD) StCT was 257.7 (97.3) \(\mu\)m, and mean (SD) TCT was 294.1 (104.8) \(\mu\)m (Figure 3A), resulting in mean (SD) differences of 35.8 (19.9) \(\mu\)m between VCT and StCT (P < .001), 36.3 (17.8) \(\mu\)m between StCT and TCT (P < .001), and 72.2 (30.4) \(\mu\)m between VCT and TCT (P < .001). These results suggest that CT measurements using different posterior boundaries differ most in eyes with...
a visible SCL. All 3 CT measurements measured in 5-μm intervals up to 3.0 mm nasal and temporal to the fovea were also significantly different (P < .001 at all points) in eyes with a visible SCL (Figure 3B).

### Fellow Eye and Interobserver Agreements

For participants in whom both eyes qualified for analysis (n = 67), study eyes were compared with the fellow eyes (eTable in the Supplement). The SCL was visible in 29 study eyes (43.3%), 36 fellow eyes (53.7%), and both eyes in 28 participants (41.8%). There was high agreement in SCL visibility between study and fellow eyes (κ coefficient, 0.734; P < .001). Study and fellow eyes also had high agreement in both retinal and CT measurements by intraclass correlations, with correlation coefficients of 0.880 for CFT (P < .001), 0.856 for VCT (P < .001), 0.870 for StCT (P < .001), and 0.872 for TCT (P < .001). Of pairs in which the SCL was visible in both eyes (n = 28), there was no correlation between SCL thickness in the study and fellow eyes, with a correlation coefficient of 0.169 (P = .32). Interestingly, while SCL presence among the 67 fellow eyes was also associated with a more hyperopic refractive error (mean [SD], +0.47 [1.95] D vs −0.62 [1.99] D; P = .03), the relation of SCL thickness to refractive error in fellow eyes with a visible SCL (36 eyes) did not reach statistical significance (R² = 0.084; P = .09). Interobserver reliability for all measurements by intraclass correlations was 0.996 (P < .001) for VCT, 0.996 (P < .001) for StCT, 0.997 (P < .001) for TCT, and 0.953 (P < .001) for SCL thickness measurements.

### Discussion

In this study, we characterized the CSJ seen on EDI-OCT across healthy participants and identified a visible SCL in 45% of eyes in our study population. The presence and thickness of the SCL were associated with hyperopic refractive error and increased CT only if the SCL was included in the measurement. The SCL did not appear to be affected by age, time of day of measurement, IOP, visual acuity, retinal thickness, or thickness of the vascular or stromal choroid in our cohort, possibly because of the narrow range of these parameters among healthy individuals. Using different landmarks as the posterior boundary, we found the greatest differences in CT measurements in participants with a visible SCL. These data suggest that CT measurements can be substantially affected by how the CSJ is defined.

Recent advances in OCT technology and emergence of the “enhanced-depth” imaging technique have spurred a renewed interest in understanding the choroid’s role in ocular disease pathophysiology. Many recently published reports evaluate CT as a surrogate marker for choroidal perfusion, but accurate measurements require precise anterior and posterior boundaries. Unlike Bruch’s membrane, which in non-pathological states is compact and well defined on OCT, the CSJ may appear in some healthy individuals as a broad hyporeflective band. This band is unlikely to be an imaging artifact, such as that resulting from averaging multiple scans, since it is reproducible from multiple repeated scans from the same eye (data not shown), and its presence was significantly correlated between right and left eyes in each participant from our study.

Comparisons with histological sections suggest that this hyporeflective band likely corresponds to the SCL, also known anatomically as the lamina fusca. However, the SCL is not often well defined on histological section. Particularly in the central macula, the SCL appears as a transition zone consisting of lamellae of loose collagen fibers extending from the choroidal stroma to the sclera, where the collagen fibers become more compact. In this way, we would expect the SCL to exhibit similar reflectivity on EDI-OCT as the extravascular stromal tissue of the choroid. The fact that the band appears so distinctly hyporeflective in some eyes suggests that there may be fluid filling the lamellae of the SCL, even in the absence of ocular disease. This mild form of suprachoroidal effusion likely allows the SCL in some eyes to become visible on imaging.

Interestingly, we found in this study that the presence and thickness of the SCL correlated with hyperopia. One possible explanation for this phenomenon may relate to the osmotic and hydrostatic forces in uveal effusion syndrome, which occurs more commonly in highly hyperopic or nanophthalmic eyes. Gass hypothesized that the normal egress of proteins leaked from choroidal vessels across the sclera is impaired in uveal effusion syndrome, resulting in osmotic fluid retention in the SCL. An alternate theory suggests that compression of vortex veins by abnormally thickened sclera increases hydrostatic pressure and leads to transudation into the SCL. The fact that highly hyperopic or nanophthalmic eyes are predisposed to uveal effusion syndrome supports our hypothesis that similar forces may occur in some healthy eyes to a lesser degree, resulting in a subclinical suprachoroidal effusion. Additional studies to evaluate the presence or thickness of the SCL in pathological states, particularly in choroidal diseases, may help elucidate these hypotheses. The possible association of hyperopia with age-related macular degeneration also warrants further investigation into the relevance of the SCL in age-related macular degeneration pathogenesis.

One limitation of this study is its restricted generalizability because of the older age of the study participants. Studies have shown that CT decreases by approximately 15.6 μm per decade in human adults. The thinner choroid in this aged population likely allows better visualization of the CSJ, since it is closer to the zero-delay line. Moreover, the absence of overlying retinal pathology in this healthy cohort avoids artifacts or shadowing that may impair CSJ visibility. These factors together may explain the high frequency of identifying the SCL in this study. In fact, we also performed a secondary analysis of 21 healthy young participants (mean age, 29.3 years) who underwent EDI-OCT as part of a comparative study of choroid imaging techniques across different OCT platforms and found no participants in whom the SCL could be visualized (data not shown). This preliminary investigation suggests that the SCL may become more visible on EDI-OCT with advanced age, possibly because of mild suprachoroidal effusion resulting from gradual loss of vessel wall integrity with age. Other factors to consider include IOP, which although not correlated with SCL presence or thickness in the individuals with...
normal blood pressure in our study, may potentially be affected in conditions such as glaucoma or hyptony.

An important question stemming from our analysis is how the CSJ should be defined in future studies involving CT measurements. From a histological standpoint, the lamina fusca contains elements of both choroid and sclera, including collagen fibers, fibroblasts, and melanocytes.27 While the predomiance of melanocytes in this layer accounts for the origin of its Latin name (from fuscus, meaning “dark”), its function is largely unknown, leaving it unclear whether it can be functionally considered a part of the choroid. From a practicality standpoint, it may be reasonable to only measure the vascular perfusion, as the choroid and sclera border also makes sense from a functional standpoint, since CT is usually measured as a marker for choroidal perfusion.

The relationship between CT and perfusion remains unclear. Some studies suggest an association with ocular blood flow,28-29 In fact, highly myopic individuals often have intact visual function despite significant choroidal thinning.14,15 It is possible that choroidal thickness may better predict outer retinal perfusion than CT measurements that include the medium and large vessels.30 Advances in choroidal imaging and segmentation algorithms will help address these issues in the future. Until then, the choice of how the CSJ should be defined or whether the SCL should be included in CT measurements will depend on the disease process being studied and whether the SCL is hypothesized to be involved.

Conclusions

Studies involving choroidal imaging and segmentation algorithms should address how the CSJ is defined. Further understanding of the SCL not only helps refine the boundaries used for measuring CT, but may also improve our understanding of how this potential space contributes to the pathophysiology of retinal and choroidal diseases.

REFERENCES