Three-Dimensional Evaluation of the Lamina Cribrosa Using Spectral-Domain Optical Coherence Tomography in Glaucoma

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PURPOSE. To introduce a novel, digital, three-dimensional (3D) reconstruction of the optic nerve head (ONH) and to use this method to evaluate the 3D configuration of the lamina cribrosa (LC) in patients with primary open-angle glaucoma.

METHODS. Optic discs of 137 eyes of 137 patients with open-angle glaucoma were scanned with enhanced depth-imaging spectral domain-optical coherence tomography (SD-OCT). 3D images of the ONH were then reconstructed from B-scan images using maximum intensity projection (MIP) and texture-based volume rendering (VRT). The performance of the threshold segmentation by MIP and VRT was assessed by comparing the distance of the anterior LC surface from the reference line set at the Bruch’s membrane opening level (LC depth) measured within both of the 3D images and the B-scan images.

RESULTS. The LC configuration could be evaluated three dimensionally in ~95% of patients scanned with enhanced depth-imaging SD-OCT. The mean LC depth was 559.50 ± 151.98, 558.97 ± 152.38, and 560.22 ± 152.26 μm in B-scan, MIP, and VRT images, respectively. There were excellent agreements between the values (intraclass correlation coefficient = 1.00 between MIP and B-scan, and 0.999 between VRT and B-scan). The configuration of the LC varied considerably among individual glaucoma patients.

CONCLUSIONS. This method provides measurable 3D images of the LC that enable comprehensive evaluation of the LC configuration. This technique should facilitate the investigation of the LC in glaucomatous eyes. (Invest Ophthalmol Vis Sci. 2012;53:198 –204) DOI:10.1167/iovs.11-7848

The lamina cribrosa (LC) is considered to be the putative site of primary axonal injury in glaucoma.1–4 The compression and deformation of the LC are thought to promote optic neuropathy by a blockade of axoplasmic flow within the optic nerve fibers.4–7 However, changes in the LC have been evaluated largely through histopathology, and such studies are prone to the effects of tissue shrinkage or swelling from the process of fixation.5–9 Moreover, the influence of intraocular pressure (IOP) on enucleated eyes is unclear.

Several recent reports have suggested that spectral-domain optical coherence tomography (SD-OCT) imaging of the human optic nerve head (ONH) can visualize the LC. Inoue et al.8 measured the LC thickness of glaucoma patients using three-dimensional (3D) images. However, 42% of their study eyes were excluded from the analysis because the LC could not be clearly identified, either because of poor image contrast or vascular shadowing that obscured the LC. In the normal monkey, Strouthidis et al.10 compared serial ONH histology with interpolated B-scans generated from a 3D SD-OCT ONH volume acquired in vivo from the same eye. Using this method, they were able to delineate the anterior laminar surface. However, the posterior surface of the LC was not detectable as the signal faded with increasing depth through the optic nerve.

Recently, enhanced depth imaging OCT, a new imaging technique, has been described by Spaide et al.11 This technique was developed to visualize the full thickness of the choroid and involves positioning the SD-OCT device close enough to the eye to obtain an inverted representation of the fundus. We previously reported that the enhanced depth-imaging technique improves the visualization of the LC by increasing the depth of signal and image contrast.12 From the B-scan images obtained using this method in glaucoma patients, we constructed the 3D image of the ONH using 3D processing software and evaluated the structural changes in situ of the LC by using a novel imaging algorithm.

The purpose of the present study was to describe a new method of in situ, 3D visualization of the LC and to use this method to evaluate the LC’s depth in patients with primary open-angle glaucoma (POAG).

METHODS

This investigation is based on the Lamina Cribrosa Exploration Study, an ongoing study of glaucoma and healthy individuals at the Glaucoma Clinic of Seoul National University Bundang Hospital. The study was approved by the Seoul National University Bundang Hospital Institutional Review Board and conformed to the Declaration of Helsinki.

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Study Subjects
Glucoma patients were enrolled from the Glaucoma Clinic of Seoul National University Bundang Hospital.

All subjects received complete ophthalmic examinations that included visual acuity measurement, Goldmann applanation tonometry, refraction tests, slit lamp biomicroscopy, gonioscopy, dilated stereoscopic examination of the optic disc; SD-OCT, central corneal thickness, axial length, and standard automated perimetry (Humphrey Field Analyzer 750; 24-2 Swedish interactive threshold algorithm; Carl Zeiss Meditec, Dublin, CA). Perimetry and OCT examinations were conducted within a 3-month period.

To be included, eyes were required to have diagnosed POAG and to have best corrected visual acuity ≥20/40, spherical refraction within ±6.0 D, and cylinder correction within 3.0 D. Those with a history of ocular surgery other than cataract extraction and glaucoma surgery and intraocular disease (e.g., diabetic retinopathy or retinal vein occlusion) or neurologic disease (e.g., pituitary tumor) that could cause visual field loss were excluded from the study. When both eyes were eligible, the eye with the lower mean deviation was included.

POAG was defined as the presence of glaucomatous optic nerve damage (i.e., vertical cup-to-disc ratio of 0.7 or greater, or asymmetry 0.2 or more, or the presence of focal thinning, and notching) and associated visual field defect without ocular disease or conditions that may elevate the IOP. A glaucomatous visual field change was defined as (1) outside normal limits on the glaucoma hemifield test or (2) three abnormal points with P < 5% of being normal, one with P < 1% by pattern deviation; or (3) pattern SD of 5% if the visual field was otherwise normal, confirmed on two consecutive tests.

Enhanced Depth Imaging OCT of the Optic Disc
The ONH was imaged by SD-OCT (Spectralis; Heidelberg Engineering GmbH, Heidelberg, Germany) using the enhanced depth-imaging technique. The detail and advantages of this technology to evaluate the LC have been described previously. In brief, the device was positioned close enough to the eye to create an inverted image near the top of the display. Enough separation from the top of the display was used to avoid image ambiguity from image folding with respect to 0 depth. The technique provided images with a more intense signal and better image contrast in the deep ONH tissue compared to the conventional imaging techniques. Although the discernment of the full-thickness lamina was not required in this study, the images are routinely obtained using the enhanced depth-imaging technique at our institution.

The imaging was performed using a 10° × 15° rectangle covering the optic disc. This rectangle was scanned with approximately 65 sections, which were set to 30 to 34 μm apart (the slicing distance is determined automatically by the machine). Each section had 42 OCT frames averaged, which provided the best tradeoff between the image quality and patient cooperation.

In 30 subjects with stable IOP (i.e., <18 mm Hg for the last two consecutive visits), the scan was repeated on a different day within a 1-month period, to determine intersession variability.

Using the SD-OCT (Spectralis, Heidelberg Engineering), the images are obtainable only when the quality score is higher than 15. When the quality score does not reach 15, the image acquisition process automatically stops and the image of the respective section remains missing. Only acceptable scans with a good-quality image (i.e., quality score >15) obtained in more than 60 sections and allowing clear delineation of anterior and posterior borders of the LC were included.

3D Construction of the Optic Disc Image
Volumetric rendering of the 3D-OCT data set was performed with image-processing software (Amira 5.2.2; Visage Imaging, Berlin, Germany). The 3D configuration of LC was assessed by maximum intensity projection (MIP) and texture-based volume rendering (VRT). The MIP rendering allows the visualization of the highest or lowest intensity in a data volume along the current line of sight. This module is often used in the field of nuclear medicine to locate the lesion of interest three dimensionally. The VRT was constructed using a color map that was set to provide a semitransparent view of the low reflective tissue such as the outer optic nerve axons. The highly reflective tissue, such as LC and the retinal pigment epithelium (RPE)-Bruch’s membrane complex, is visualized as an opaque, bright beige image. Thus, the LC is clearly visualized amid the surrounding semitransparent ONH tissues using this color map, which enables a true 3D evaluation of the LC.

To assess the performance of segmentation using MIP and VRT, the distance of the anterior LC surface from the level of BM0 (LC depth) was measured in the MIP and VRT images, as well as in the B-scan images, and compared. The comparison was made at three to five locations in each eye (Fig. 2; Supplementary Videos S1, S2, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7848/-/DCSupplemental). To do this, three to five B-scans around the central ONH were selected equidistantly (one from every seven scans) from the 3D image data set (Fig. 2A, top). Then, the thickness of the section of two-scan-line width (one scan width to each direction from the selected B-scan) was generated (Fig. 2A, bottom), creating three to five thin-section 3D images. The volume images can be seen only with the volume section and the minimal producible section width using the image-processing software (Amira; Visage Imaging) is 1 scan line width (approximately 30 μm). We used a two-scan-line width section because the LC was more clearly visible with this thickness because of the semitransparent nature of our 3D volume images. The LC depth was measured in each selected B-scan (Fig. 2B) and the volume sections of MIP (Fig. 2C) and VRT images (Fig. 2D). The depth was measured at three points for each area; the maximally depressed point and an additional two points (100 and 200 μm apart from the maximally depressed point in temporal direction). Only temporal adjacent points were selected, because the maximally depressed point was mostly located at the nasal side, which was often close to the central vessel trunk. In such
cases, the LC at the nasal adjacent area was obscured by the shadow of the blood vessels. The distance was measured on the line perpendicular to the reference line using a manual caliper tool of the software. The measurements obtained at the three points were averaged. The extent of visualization of the anterior LC border was compared between the B-scan images (B) and the threshold 3D images (C, D). *Orange dots*: the anterior border of the LC; *yellow squares*: the two end points of the anterior LC border visualized in each selected image. The extent of visualization of the anterior LC border was measured as the lineal distance between the two *yellow squares*, using a manual caliper tool of the software.

**Extent of Visibility of the Lamina Cribrosa as Observed in the Threshold 3D Images**

The extent of visibility of the LC observed in our 3D data sets was evaluated in two different ways in randomly selected 50 eyes. First, we assessed the ratio of the extent of visualization of the LC surface (using our threshold algorithms) to the size of Bruch’s membrane opening (BMO), by calculating the area ratio and the horizontal and vertical diameter ratio. The area parameters and the diameter parameters were measured on the en face images using Image J software (ver. 1.43u, developed by Wayne Rasband, National Institutes of Health, Bethesda, MD, http://rsb.info.nih.gov/ij) and the manual caliper tool of the image-processing software (Amira 5.2.2; Visage Imaging), respectively. The diameters were measured on the plane where the LC is maximally visualized.

Second, the extent of anterior LC border visualized in our threshold 3D data sets was compared to that visualized in individual B-scans. To do this, the distance between the nasal and temporal end point of the visualized anterior LC borders was compared between the 3D section images and the B-scan images (Figs. 2B–D). The comparison was made using the volume section images and B-scan images (that measured within the 3D volume data set) from every seven scans) from the 3D image data set (A, top). Then, the two-scan-width, thin volumetric sections around the B-scan was generated (A, bottom), creating five 3D sections. *Arrowheads*: the borders of one of the thin volumes. The LC depth was measured by manual delineation in each selected B-scan (B) and the thin volume sections of maximum intensity projection (C) and the VRT images (D). To do this, the distances from the reference line (straight line connecting the two termination points of Bruch’s membrane; *green squares*) to the anterior lamina surface was measured at three points: the maximally depressed point (white arrow) and two additional points 100 and 200 µm away from the maximally depressed point in the temporal direction. The distance was measured on the line perpendicular to the reference line using a manual caliper tool of the image-processing software (Visamir 5.2.2; Visage Imaging) and these three values were averaged. The mean distance measured at the maximally depressed plane was defined as the maximal LC depth (max D) and the distance measured at the minimally depressed plane was defined as the minimal LC depth (min D). The difference between the values (max-min D) was calculated to evaluate the surface variation of the LC (Fig. 3).

**Statistical Analysis**

The agreement of the mean LC depth obtained using MIP and VRT with manual delineation within acquired B-scans was assessed by calculating the intraclass correlation coefficient (ICC) and limits of agreement using Bland-Altman analysis. The ICC was defined as the ratio of the intersubject component of variance to the total variance. According to Fleiss et al.14 scores ≥0.75, between 0.40 and 0.75, and ≤0.4 are termed excellent, moderate, and poor, respectively.

Data from the 30 subjects having repeated SD-OCT datasets scanned on a different day were used for assessing the intersession reproducibility of our measuring the max D and max-min D. All data sets were evaluated by one observer (EJL). The intersession reproducibility was calculated using ICC and coefficient of variation (CV).

To evaluate the interobserver reproducibility of our measuring method, 15 randomly selected SD-OCT datasets based on a random sample generator (SPSS 17.0; SPSS Inc, Chicago, IL) were evaluated by two independent examiners (EJL, TWK) and the ICC and the CV were calculated. In the statistical analyses (SPSS 17.0 software; SPSS, Chicago, IL), \( P < 0.05 \) was considered to be significant.
RESULTS

One hundred forty-four patients were initially scanned using enhanced depth-imaging 3D-OCT. Of these, seven subjects were excluded because of poor image quality (i.e., poor image contrast or more than five missing sections) that did not allow detection of the anterior surface of the LC (n = 4) or decentralized images in which the nasal RPE-Bruch’s membrane complexes were not included in the scan, and the reference lines could not be set (n = 3). Of the remaining 137 patients, 57 were women and 80 were men. Seventy-six patients had normal-tension glaucoma (untreated IOP < 22 mm Hg) and 61 had high-tension glaucoma (untreated IOP ≥ 22 mm Hg). The mean ± SD age of the subjects was 58.3 ± 13.6 years (range, 21–86 years). The mean spherical equivalent refraction was −1.40 ± 2.51 D (range, −7.0 to 4.13 D). The mean baseline IOP (average of at least two measurements before IOP-lowering treatment) and the IOP at the time of SD-OCT was 20.7 ± 7.3 and 14.3 ± 4.9 mm Hg, respectively (Table 1). Twelve eyes had undergone glaucoma surgery, 86 were on topical ocular hypotensive medication, and the remaining 39 (28.5%) were treatment naive, newly diagnosed glaucomatous eyes at the time of optic disc scanning.

The LC depth was compared in five sections in 114 eyes, four sections in 19, and three sections in 4. Thus, 658 sections (5 × 114 + 4 × 19 + 3 × 4) were compared. The mean LC depth measured in the B-scan, MIP, and VRT images were 559.50 ± 151.98, 558.97 ± 152.39, and 560.22 ± 152.26 μm, respectively. The ICCs between the measurements were 1.000 between B-scan and MIP, and 0.999 between B-scan and VRT. The limit of agreement by Bland-Altman plot was −7.85 to 8.90 and −11.70 to 11.95 μm between B-scan and MIP and between B-scan and VRT, respectively (Fig. 4).

The ratio of the visualized proportion of laminar surface area to BMO area was 0.78 ± 0.08 for MIP and 0.37 ± 0.07 for VRT. The ratios of the vertical and horizontal lamina surface length to BMO diameter were 0.92 ± 0.07 and 0.86 ± 0.06, respectively, for MIP and 0.82 ± 0.07 and 0.52 ± 0.12, respectively, for VRT. The proportions of the extent of laminar delineation in the threshold 3D images to that in the B-scan images were 1.00 for MIP and 0.62 for VRT, respectively.

The LC was mostly U-shaped or nearly flat when each of the horizontal B-scan sections was viewed. However, when the 3D reconstructed images were viewed from the side, the shape of the lamina showed considerable variation. The variation was able to be classified into 5 categories; flat, U-shaped, slope configuration, focal concavity, and W-shaped (Fig. 5; Supplementary Videos S5–S9, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7848/-/DCSupplemental). Those types were identified in 18 (13.1%), 6 (4.4%), 16 (11.7%), 32 (23.4%), and 65 (47.4%), respectively.

The mean value of max D was 609.03 ± 149.33 μm. The mean value of max-min D was 107.46 ± 76.94 μm. There was excellent interobserver reproducibility, both in the measurements of max D and max-min D (ICC = 0.990 and 0.956, respectively). The interobserver CVs were 2.57% for max D and 15.97% for max-min D. The interobserver ICCs in measurement of max D and max-min D were 0.984 and 0.896, respectively. The interobserver CVs were 2.59% for max D and 18.62% for max-min D.

DISCUSSION

We evaluated in situ the 3D configuration of the LC, both qualitatively and quantitatively. To our knowledge, this is the first report of an evaluation of the 3D configuration of the LC in glaucoma patients.

A distinctive feature of glaucoma is the cupping of the ONH, which results from the posterior displacement and com-
pression of the LC and its disinsertion from the scleral rim. Recent studies using animal glaucoma models demonstrated that the deep ONH structure including the LC is altered significantly from the very early stage of disease. The structural change in the LC may cause the laminar pores to deform. This process may impose extension, compressive, and shearing stress on the axons passing through the pores, resulting in the loss of neuronal function of the axons. In addition, the LC provides vascular supply to the ONH. Thus, deformation and compression of the lamina may alter blood supply and consequence nutrient delivery to the axons, which also contributes to axonal injury. As such, a large body of research, aimed at understanding the pathophysiology of glaucomatous optic neuropathy, has been centered on the evaluation of the LC deformation.

Until now, evaluation of the LC deformation has been mainly performed using histologic methods or finite element modeling which was based on the findings obtained from histologic specimens. However, histologic methods are prone to effects of tissue swelling or tissue shrinkage in the process of fixation. In addition, the LC provides vascular supply to the ONH. Thus, deformation and compression of the lamina may alter blood supply and consequence nutrient delivery to the axons, which also contributes to axonal injury. As such, a large body of research, aimed at understanding the pathophysiology of glaucomatous optic neuropathy, has been centered on the evaluation of the LC deformation.

In the present study, we described a new method of visualizing the LC within the volume set of the ONH image obtained using SD-OCT. This technology is robust in that it enables the clinical observation of the LC in patients. Furthermore, this technique allows observation of the regional deformation of the LC in a true 3D manner and rapid detection of the most and least displaced point. This characteristic not only provided instantaneous, comprehensive understanding of the whole configuration of LC in individual eyes, but also expedited the quantitative measurement of the LC configuration.

The LC depth measured using our threshold data set showed almost perfect agreement with that measured by manual delineation on B-scan images (ICCs = 0.999–1.000). Because the histologic comparison was not possible in living patients, we used the manual delineation of the laminar border within B-scan images as a reference standard in evaluating the LC depth. It has been shown that the anterior LC surface delineated by manual delineation on SD-OCT B-scan images corresponds well with that identified in histologic sections. Recently, Agoumi et al. successfully demonstrated the change of LC and prelaminar tissue after IOP elevation, using manual delineation for evaluating the laminar and prelaminar surface. The excellent agreement between the measurement in the 3D images and the manual delineation on B-scan images suggests that our threshold algorithm has a good performance in defining the anterior laminar surface.

LC depth was measured using the BMO plane as a reference plane. The plane may be different from that of the optic disc border in eyes with parapapillary atrophy (PPA), as the PPA plane. The plane may be different from that of the optic disc in defining the anterior laminar surface. The excellent agreement between the measurement in the 3D images and the manual delineation on B-scan images suggests that our threshold algorithm has a good performance in defining the anterior laminar surface.

### Figure 4

Bland-Altman plots of the difference against the mean magnitude of the LC depth, as measured by manual delineation within B-scans versus MIP (A) and VRT (B). The plane line represents the mean difference (A. $0.52 \pm 4.27 \mu m$; B. $0.13 \pm 6.03 \mu m$) and the two dotted lines represent the 95% limits of agreement (A. $-7.85$ to $8.90 \mu m$; B. $-11.70$ to $11.95 \mu m$).

### Figure 5

The volume rendered 3D optic disc images viewed from the side. The shape of the lamina was classified into five categories: (A) flat, max D = 663.90 μm, max-min D = 35.95 μm; (B) U-shaped, max D = 679.05 μm, max-min D = 247.54 μm; (C) slope configuration, max D = 926.80 μm, max-min D = 182.59 μm; (D) focal concavity, max D = 526.98 μm, max-min D = 192.77 μm; and (E) W-shaped, max D = 771.44 μm, max-min D = 341.78 μm.
scanning electron microscopy, other investigators17,18 examined the change in the LC after exposing the enucleated eyes to elevated IOP. It is likely that the response of enucleated eyes differs from eyes studied in situ due to the absence of retro-laminar pressure which counteracts the IOP.36–38 Moreover, physical properties of the lamina and the sclera should be different in enucleated eyes from those in situ. In the present study, which examined in vivo images, the change of the lamina was highly variable among glaucoma patients, and the W shape was the most prevalent. Such variation suggests that the material properties of the LC have a large degree of regional variability among individual eyes. Further study is needed to correlate the lamina configuration with clinical characteristics and disease course.

It has been demonstrated that clinically detectable deformation of the ONH structure may precede early surface structural damage,16,22 and early retinal nerve fiber layer loss,39 in experimental glaucoma. The data suggest that investigation of the 3D architecture of the deep ONH will provide an additional index to diagnose early glaucoma, and be useful to predict patients who are at greater risk of developing glaucoma among glaucoma suspects. Moreover, it is considered that eyes with severely damaged LC are more susceptible to further glaucomatous damage.29,40 In this regard, it is possible that the magnitude of the LC deformation is associated with the disease prognosis in patients with established glaucoma as well. A longitudinal study is needed to investigate the influence of the LC deformation on glaucoma progression.

It is acknowledged that the LC is compressed in glaucoma as the disease progresses.4,5,18,29,34,40,41 The resulting LC thinning may also affect the disease prognosis, because the thinner LC may lead to a steepening of the translaminar pressure gradient. Studies have shown that LC thickness may be successfully measured using SD-OCT images.4,5,9,12 Together with thickness measurement, evaluation of the LC deformation using SD-OCT would provide comprehensive information on the biomechanical condition of the ONH.

There are a few limitations in the present study. First, the VRT image using the color map did not allow the visualization of the whole lamina, because the LC has variable reflectivity depending on the thickness of the overlying neuroretinal rim. Thus, some parts of the lamina may have reflectivity lower than the threshold set to identify the lamina. Yet, the image provides almost the complete range of laminar cribrosa vertically (vertical laminar surface to BMO ratio, 0.82), and classifying the type of damage was feasible in almost all eyes. Moreover, in eyes in which such classification was difficult, MIP image complemented the evaluation. Although the MIP was not as straightforward as the VRT image, because of the overlapping nature of the high reflective points, it was useful to evaluate the part of the lamina where the lamina was not definitely identified in the VRT image. Using the MIP section image, the whole range of the anterior border of LC delineable in B-scan images was visualized. Second, one quantitative measurement was performed by measuring max D and max-min D. This method does not take full advantage of the 3D imaging algorithm. However, it is difficult to develop a standardized method that can be applied in each individual, since the visible portion of the lamina is variable in each person. We believe that our summary parameters (i.e., max D and max-min D) are still useful, given the limitation of the current technology, because the lamina is displaced in a regional fashion. Thus, a large max D suggests that the lamina is largely depressed in some region (not in one point). Similarly, a large max-min D suggests that there is a large regional variation on the lamina surface. VRT facilitated the measurement of the summary parameters as it allowed the maximally and minimally depressed plane to be readily determined. Otherwise, one should measure the distance from the reference plane to the anterior surface of the lamina in all B-scans. Further study is needed to correlate these parameters and the clinical characteristics. Last, factors influencing the max D and max-min D were not analyzed in the present study. This was because most the patients had undergone surgical treatment and/or on medical IOP lowering treatment (including medical or surgical treatment) at the time of LC imaging. It is possible that some of these patients had restored the laminar deformation as the IOP lowered with the treatment, resulting in underestimation of the effect of IOP-related mechanical stress on LC changes.12–46 A study to investigate influencing factors on LC change is currently under way enrolling treatment-naive patients.

In conclusion, we introduce a new imaging algorithm that allows the 3D in situ clinical evaluation of LC configuration. This method may facilitate research to elucidate the mechanism of glaucomatous optic nerve damage, as well as our understanding of the role of the LC in the onset and progression of the glaucomatous optic neuropathy.

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