Focal Loss Analysis of Nerve Fiber Layer Reflectance for Glaucoma Diagnosis

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Purpose: To evaluate nerve fiber layer (NFL) reflectance for glaucoma diagnosis.

Methods: Participants were imaged with 4.5 × 4.5 mm volumetric disc scans using spectral-domain optical coherence tomography. The normalized NFL reflectance map was processed by an azimuthal filter to reduce directional reflectance bias caused by variation of beam incidence angle. The peripapillary area of the map was divided into 160 superpixels. Average reflectance was the mean of superpixel reflectance. Low-reflectance superpixels were identified as those with NFL reflectance below the fifth percentile normative cutoff. Focal reflectance loss was measured by summing loss in low-reflectance superpixels.

Results: Thirty-five normal, 30 preperimetric, and 35 perimetric glaucoma participants were enrolled. Azimuthal filtering improved the repeatability of the normalized NFL reflectance, as measured by the pooled superpixel standard deviation (SD), from 0.73 to 0.57 dB (P < 0.001, paired t-test) and reduced the population SD from 2.14 to 1.78 dB (P < 0.001, t-test). Most glaucomatous reflectance maps showed characteristic patterns of contiguous wedge or diffuse defects. Focal NFL reflectance loss had significantly higher diagnostic sensitivity than the best NFL thickness parameter (from map or profile): 77% versus 55% (P < 0.001) in glaucoma eyes with the specificity fixed at 99%.

Conclusions: Azimuthal filtering reduces the variability of NFL reflectance measurements. Focal NFL reflectance loss has excellent glaucoma diagnostic accuracy compared to the standard NFL thickness parameters. The reflectance map may be useful for localizing NFL defects.

Translational Relevance: The high diagnostic accuracy of NFL reflectance may make population-based screening feasible.
a diagnostic parameter, underperformed the average NFL thickness. This poor diagnostic performance could be due to several sources of bias and noise in the measurement of NFL reflectivity. One source is the attenuation of OCT signal caused by media opacity or poor focusing, and this has been dealt with in previous literature by normalizing the NFL reflectivity by one or more outer retinal layers, but the resulting average reflectivity ratio still did not outperform NFL thickness. Combining the reflectivity ratio with NFL thickness did improve diagnostic accuracy. This was the starting point of our methodology. In this article, we developed further improvements in NFL reflectance analysis on the basis of our hypotheses regarding additional sources of measurement bias and noise that could be suppressed in automated postprocessing of OCT images.

We hypothesized that an important limitation of the diagnostic reliability of NFL reflectivity was its dependence on incidence angle. The NFL reflectivity is highest when the OCT beam is perpendicular to the nerve fibers in the plane parallel to their long axis and the reflectivity decreases rapidly with increasing off-perpendicular incidence angle. In routine clinical OCT imaging, it is very difficult for the operator to obtain uniform perpendicular beam incidence that would maximize reflectivity. Generally, beam incidence angle varies within any NFL scan circle or area, leading to reflectivity variability that reduces the diagnostic accuracy. In this study, we developed a method to suppress the reflectivity variation. The method is based on the insight that the beam incidence angle variation generally has a first-degree azimuthal dependence, which is related to the pupillary position of the OCT beam and the nasal offset of the optic nerve head relative to the optical axis of the eye. Thus azimuthal spatial frequency filtering could remove NFL reflectivity variation caused by beam incidence angle variation during OCT scanning.

We also observed that measurement artifacts and glaucoma affect the NFL reflectance pattern in different ways. Beam attenuation and off-perpendicular incidence tend to affect the OCT signal globally or over large regions. On the other hand, glaucoma tends to affect the NFL focally (arcuate bundle defects), at least in the early disease stages that pose the greatest diagnostic challenge. On the basis of these observations, we hypothesized that an algorithm to quantify focal loss in NFL reflectance could improve the sensitive detection of early glaucoma while reducing the confounding effect of measurement artifacts.

Our final NFL reflectance analysis algorithm combined the approaches outlined above: (1) normalization to an outer retinal reference layer, (2) azimuthal filtering, and (3) focal loss analysis. The diagnostic performance of this algorithm was tested in a prospective observational clinical study.

**Methods**

**Participants**

This prospective observational study was performed from January 6, 2017, to May 30, 2019, at the Casey Eye Institute, Oregon Health & Science University (OHSU), Portland, OR, USA. Research protocols were approved by the Institutional Review Board at OHSU and carried out in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from each participant. The study was in accordance with the Health Insurance Portability and Accountability Act of 1996 privacy and security regulations.

All participants were part of the “Functional and Structural Optical Coherence Tomography for Glaucoma” study (NIH R01 EY023285). The inclusion criteria for the PG group were (1) an optic disc rim defect (thinning or notching) or retinal NFL defect visible on slit-lamp biomicroscopy and (2) a consistent glaucomatous pattern on both qualifying Humphrey SITA 24-2 VFs. The pattern of glaucoma defect was assessed on the VF total deviation map by a glaucoma specialist. Glaucomatous VF must further meet abnormality criteria defined as either pattern standard deviation (PSD) outside normal limits ($P < 0.05$) or glaucoma hemifield test outside normal limits. Eyes in the PPG group only met the biomicroscopic criteria (1), but not the VF criteria (2).

For the normal group, the inclusion criteria were as follows: (1) No evidence of retinal pathology or glaucoma, (2) a normal Humphrey 24-2 VF, (3) intraocular pressure $< 21$ mm Hg, (4) central corneal pachymetry $> 500 \mu m$, (5) no chronic ocular or systemic corticosteroid use, (6) an open angle on gonioscopy, (7) a normal-appearing optic nerve head (ONH) and NFL, and (8) a symmetric ONH between left and right eyes.

Participants were excluded from this study if any of the following situations were observed: (1) best-corrected visual acuity less than 20/40, (2) age $<40$ or $>80$ years, (3) spherical equivalent refractive error of $>+3.00$ or $<-7.00$ diopters, (4) previous intraocular surgery except for an uncomplicated cataract extraction with posterior chamber intraocular lens implantation, (5) any other diseases that might cause VF loss or optic disc abnormalities, or (6) inability to perform reliably on automated VF testing.
One eye from each participant was scanned and analyzed. For the normal group, the eye was randomly selected. For the PPG and PG group, the eye with the worse VF mean deviation (MD) was selected.

**Data Acquisition**

Participants were scanned with a 70 kHz, 840 nm wavelength spectral-domain OCT system (Avanti; Optovue, Inc., Fremont, CA, USA). Two scan patterns, the optic disc volumetric high-definition OCT angiography (HD OCTA) scan and the structural OCT ONH scan, were used.

The optic disc volumetric HD OCTA scan covered 4.5 × 4.5 mm area centered on the disc. The cross-sectional B-frames, comprised of 400 A-lines, were repeated twice at each location to allow the computation of the angiographic flow signal. Each volume was comprised of 400 B-frame locations. Two consecutive volumetric scans, that is, a vertical-priority raster and a horizontal-priority raster, were merged using an orthogonal registration algorithm. This reduced motion artifacts and improved image quality. The merged volume provided both angiographic (flow signal) and structural (reflectance signal) images. Volumetric structural OCT images were analyzed by our novel reflectance algorithm described below.

The ONH scan was a 4.9 mm composite scan, centered on the disc. Using the Avanti software, the ONH scan provided the traditional NFL thickness profile and measurements on the circle with a diameter of 3.4 mm. Although we could obtain a similar thickness profile from the volumetric scan, we chose to use the traditional ONH scan because the diagnostic performance and quality control has been well characterized in the literature.

The VF was assessed by standard automated perimetry on the Humphrey Field Analyzer (HFA II; Carl Zeiss Meditec, Inc., Dublin, CA, USA), using the Swedish Interactive Thresholding Algorithm 24-2.

**NFL Reflectance Analysis**

**Image Segmentation**

The OCT signal of the merged volumetric HD OCTA scan was exported from the Avanti and processed by the custom software Center for Ophthalmic Optics & Lasers-Angiography Reading Toolkit (COOL-ART) that was developed in our laboratory in the MATLAB programming environment by coauthors Y.J., J.W., and others. COOL-ART automatically segmented the disc boundary and retinal layers and allowed manual correction by human graders. Grading was conducted by co-authors L.L. and Q.Y.

**Normalized NFL Reflectance Map**

The NFL reflectance (Fig. 1) was analyzed using custom software developed by the first author (O.T.). The OCT reflectance data were transformed to a linear intensity scale. The NFL band and the photoreceptor and pigment epithelium complex (PPEC) band were extracted from the OCT image. The PPEC band included the region from the anterior boundary of ellipsoid zone (EZ) to the Bruch’s membrane. The OCT intensity was axially averaged in the PPEC band to provide a reference map. The NFL reflectance was axially summed to provide the NFL reflectance map (Figs. 1A–C). Based on the data from normal subjects, the NFL/PPEC reflectance ratio map was normalized by the population average of map averages in the 1.1–2.0 mm radius analytic zone, followed by transformation to a logarithmic dB scale. For the sake of brevity, we refer to this output as the NFL reflectance map. Because large vessels displace nerve fibers and interfere with NFL reflectance analysis, the reflectance values in vessel areas were replaced with values from neighboring pixels to preserve continuity (Figs. 1D–F).

**Azimuthal Filtering**

The NFL reflectance signal in an OCT image depends on not only the intrinsic reflectivity, but also extrinsic factors, such as beam incidence angle and beam coupling factors. Generally, these extrinsic factors vary with the azimuthal angle, which is the angular position of the peripapillary retina in the polar coordinates. To reduce the effects of the extrinsic factors, we performed an azimuthal spatial frequency filtering. The details of azimuthal filtering can be found in the supplementary section. In short, the polar-coordinate reflectance map was band-stop-filtered in the azimuthal dimension to remove the first-degree angular component, which is associated with the bias caused by the incident angle. The result accentuates nerve fiber bundle defects (Fig. 1G), in which the disc area was masked out because the NFL reflectance was undefined in the disc region.

**Superpixel**

The filtered NFL reflectance map was divided into superpixels (Fig. 1H). The superpixel grid in the peripapillary area was divided into 32 tracks that ran parallel to the average nerve fiber trajectory map.
determined by the nerve fiber flux analysis described in a previous publication. Nerve fiber flux represents the NFL cross-sectional area transected perpendicular to the nerve fiber trajectory. The widths of the tracks were adjusted so that each contained the same nerve fiber flux. Thus each track contained approximately an equal number of nerve fibers. Because the NFL is thicker at the superior and inferior arcuate bundle regions, the tracks there were narrower. Thus the arcuate regions were weighed more by denser superpixels, which is appropriate as these regions are more likely to be affected by glaucoma. Each track was evenly divided into five segments in the annulus between 1.1 and 2.0 mm from the center of the disc. The region outside of the 2.0-mm radius was excluded to avoid cropping artifacts from possible scan decentration. Thirty-two tracks in five segments resulted in 160 superpixels. The NFL reflectance in each superpixel was averaged. Experimentation with different sizes of superpixels resulted in little variation in diagnostic performance. The diagnostic performance would be slightly worse if the superpixel size was much larger or smaller.

Age, Gender and Axial Length Adjustment Using Linear Mixed Effects Model

Multiple linear regression based on the linear mixed effects model was used to test the correlation between age, gender and axial length and the normalized NFL reflectance in the normal group. The superpixel location was modeled as a random effect, whereas age, axial length and gender were used as fixed effects. Age, axial length, and the interaction between them, were significant factors. Therefore the NFL reflectance of superpixels was adjusted for age and axial length using the regression model obtained from normal eyes.

Low-Reflectance Superpixel

We assumed that the normalized NFL reflectance followed a normal distribution in the normal group. This was confirmed by the Shapiro-Wilk test ($P = 0.42$). The population average and standard deviation of the adjusted NFL reflectance for each superpixel were calculated. Based on the normal distribution assumption, the 5% and 1% cutoff of reflectance values were estimated for each superpixel. Superpixels with adjusted reflectance below the 5% cutoff were considered “low-reflectance.” The number of low-reflectance superpixels was counted for each eye.

Diagnostic Parameters

Besides the low-reflectance superpixel count, two additional diagnostic parameters were calculated:
overall average reflectance and focal reflectance loss. The overall average reflectance was the average of reflectance values in all superpixels. Focal reflectance loss was the summation of reflectance deviation (difference between the tested superpixel and the normal reference, adjusted for age and axial length) over the low-reflectance superpixels. Focal reflectance loss was then normalized by the total number of superpixels ($n = 160$). Glaucoma damage manifests as more low-reflectance pixels (positive integer count), lower overall average reflectance (dB), and more negative focal reflectance loss (dB).

The above NFL reflectance parameters were compared with the two standard glaucoma diagnostic parameters already in clinical use: NFL thickness and visual field mean deviation (VF MD). The overall circular NFL thickness and quadrant NFL thickness at the 3.4-mm diameter circumpapillary circle were obtained from the ONH scan using the REVue software (version 2018.0.0.18, provided by the manufacturer). The focal loss volume of NFL thickness was calculated based on the NFL thickness profile. Superpixel average NFL thickness was also averaged from the same area of the reflectance ($D = 1.1 \sim 2\text{mm}$) and following the same superpixel dividing scheme.

### Statistical Analysis

We tested whether most NFL reflectance loss patterns were consistent with nerve fiber wedge defects characteristic of glaucoma. To perform this analysis, we categorized the loss pattern into diffuse, wedge, other grouping, isolated, or none (Fig. 2). Diffuse loss (full width defect spanning more than a quadrant of the annular analytic area) would be consistent with severe glaucoma, while wedge pattern (contiguous superpixels connecting the inner and outer edges of the annular analytic zone) would be consistent with mild or moderate glaucoma when damage was local. Reflectance loss in isolated superpixels or other grouping (3 or more contiguous superpixels in a non-wedge configuration) could indicate measurement noise or mild disease of indeterminate type. If two or more patterns were observed in same eye, the one corresponding to a more severe glaucoma category was applied.

The two-sided Wilcoxon rank sum test was used to compare the difference between the normal and glaucoma groups. The diagnostic accuracy was evaluated by the area under receiving characteristic operating curve (AROC) and by the sensitivity at the 99% specificity. The cutoff of specificity was estimated using kernel density estimation. To account for inter-eye correlation, the AROC was computed based on the formula of Obuchowski, which extended the nonparametric method of Camino et al. as applied to clustered data. The same method has been used in previous studies in ophthalmology to handle inter-eye correlation.

The sensitivity was compared using McNemar’s test. For all parameters, the age adjustment was applied to obtain equivalent value at a reference age of 50 years. Pearson correlation coefficients were calculated among NFL parameters and VF MDs. The coefficients were compared using the bootstrap method. All analysis were done in Matlab R2019a with statistics toolbox.

We used cross-validation to reduce bias in the diagnostic accuracy measurement. We chose the 0.632+ bootstrap with replacement for the age and axial length adjustment, and low-reflectance cutoff calculations. The parameters were averaged from multiple trials. In each trial the parameters were estimated on the basis of 63.2% of normal population and applied to other normal and glaucoma eyes.

### Results

#### Characteristics of the Study Participants

One eye each from normal ($n = 35$), PPG ($n = 30$), and PG participants ($n = 35$) were included in this study. Patients in both the PPG and PG groups were
Table 1. Characteristics of the Study Population

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>PPG</th>
<th>PG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye, no.</td>
<td>35</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>Age (y)</td>
<td>60.0 ± 10.8</td>
<td>65.1 ± 8.7*</td>
<td>66.9 ± 8.8*</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>8/27</td>
<td>12/18*</td>
<td>21/14*</td>
</tr>
<tr>
<td>Axial length (mm)</td>
<td>23.6 ± 0.9</td>
<td>24.7 ± 1.0*</td>
<td>24.6 ± 1.3*</td>
</tr>
<tr>
<td>VF MD (dB)</td>
<td>0.23 ± 1.24</td>
<td>−0.63 ± 1.89*</td>
<td>−6.06 ± 5.20*</td>
</tr>
<tr>
<td>VF PSD (dB)</td>
<td>1.46 ± 0.31</td>
<td>1.82 ± 0.63*</td>
<td>7.29 ± 4.30*</td>
</tr>
</tbody>
</table>

Values for continuous variables are means ± standard deviations.
VF PSD, visual field pattern standard deviation.
*P value < 0.05 compared to the normal group.

Figure 3. The normalized NFL reflectance maps averaged in the normal and glaucoma groups. The glaucoma group included both pre-perimetric and perimetric glaucoma cases. All eyes were transformed to a right-eye orientation for analysis. (Left) Average map of normal eyes. (Middle) The population SD in the normal group. (Right) The average map for the glaucoma groups were subtracted by the normal average to obtain the average loss pattern (glaucoma damage shows as negative values).

older, had longer axial lengths, thinner central cornea thickness (CCT), worse VF MDs, and worse PSDs than normal patients (P < 0.05, Table 1). Glaucoma eyes also have more myopia than normal, but not significantly (P > 0.21). In the PPG group, MD ranged from −7.3 to 2.0 dB, and PSD from 1.1 to 4.0 dB. In the PG group, VF MD ranged from −19.3 to 0.3 dB, and PSD from 1.4 to 14.7 dB.

Incidence Angle and Azimuthal Filtering

Using 20 normal eyes with two repeated OCT scans, we tested the effect of azimuthal filtering on the repeatability of NFL reflectance in the 160 superpixels. The repeatability was measured by the pooled standard deviation (SD). For the superpixels, the repeatability was improved from 0.73 ± 0.15 dB to 0.57 ± 0.11 dB (P < 0.001, paired t-test) using the azimuthal filter.

In the normal group with 35 eyes, we also compared the population SD for each superpixel. It was reduced from 2.14 ± 0.40 dB to 1.78 ± 0.34 dB using the azimuthal filter. The reduction was significant (P < 0.001, paired t-test).

Reflectance Patterns in Normal and Glaucoma Groups

The NFL reflectance map, averaged in the normal group (Fig. 3), had the highest reflectance in the inferotemporal (6:30 o’clock peak, using right eye convention) and superotemporal (11 o’clock peak) regions. There was also a secondary superonasal (1 o’clock) peak. The population SD map showed slightly higher variability in the inferotemporal and superonasal regions. The average SD was 1.8 dB, and the peak SD was 2.4 dB.

The average pattern of reflectance loss in the glaucoma groups (Fig. 3) showed that damage was commonly most severe in the inferotemporal region (7 o’clock peak), followed by shallower peaks superotemporally (11 o’clock) and superonasally (1:30 o’clock). The average loss was 2.2 dB in the PPG group and 5.6 dB in the PG group. The peak loss (inferotemporal) was 3.1 dB in the PPG group and 8.1 dB in the PG group.

Three eyes were selected from the normal, PPG, and PG groups to show the characteristic glaucomatous reflectance loss patterns (Fig. 4). Both PPG and
Figure 4. Representative NFL reflectance and VF maps from the normal and glaucoma groups. The significance map classifies superpixels into normal, borderline (first to fifth percentile of normal population), and abnormal (below first percentile of normal) categories.

Table 2. Eyes with Different Loss Pattern in Normal, PPG and PG Eyes

<table>
<thead>
<tr>
<th>Defect Pattern</th>
<th>Normal Eyes</th>
<th>PPG Eyes</th>
<th>PG Eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse</td>
<td>0</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>Wedge</td>
<td>4</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Other grouping</td>
<td>13</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Isolated</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>12</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

PG eyes had wedge-shaped loss patterns consistent with the nerve fiber wedge defect characteristic of glaucoma. The reflectance loss pattern correlated well with the locations of VF defects.

There was a positive correlation between the eyes with severe defects and glaucoma stages. Most PPG eyes (22 of 30) exhibited glaucomatous reflectance loss patterns (Table 2), and all PG eyes exhibited glaucomatous (diffuse or wedge) reflectance loss patterns. Nineteen normal eyes exhibited isolated or other-grouping patterns, showing that these loss patterns were not diagnostic of glaucoma. Only four of 35 normal eyes exhibited a wedge-shaped loss pattern, all in the temporal quadrant. This suggests that reflectance loss in the temporal quadrant may be a less reliable diagnostic observation. No normal eye exhibited a diffuse pattern. Overall, significantly ($P < 0.001$, $\chi^2$ test) higher percentage (88%) of glaucomatous eyes (PG and PPG) exhibited wedge-shaped or diffuse reflectance defect, compared to normal eyes (11%).

**Characteristic of Nerve Fiber Layer Parameters**

All NFL parameters, including the three reflectance and four thickness parameters, were significantly different between the normal and glaucoma groups (Table 3). The overall average thickness and reflectance were normally distributed for all groups (Fig. 5). The low-reflectance superpixel count and focal reflectance loss were not normally distributed. The normal group clustered around zero for both the low-reflectance superpixel count and the focal reflectance loss. The PPG group had a trimodal distribution for the low-reflectance superpixel count, and a bimodal distribution for the focal reflectance loss. The PG group had a bimodal distribution for both the low-reflectance superpixel count and the focal reflectance loss. The different distribution patterns for average and focal parameters suggests that the glaucoma groups may not be homogeneous, and thus, there may be distinct clusters of focal versus diffuse loss patterns.

Unsupervised cluster analysis based on Gaussian mixture models (Fig. 6) showed 3 loss patterns. In Cluster 1, most normal eyes (27/35) and 8 PPG eyes had no reflectance loss. In Cluster 2, eight normal eyes,
Table 3. Group Statistics for Nerve Fiber Layer Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NFL Normal</th>
<th>Glaucoma</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thickness profile</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average (μm)</td>
<td>102.1 ± 8.8</td>
<td>82.7 ± 14.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Focal loss (%)</td>
<td>−0.8 ± 2.4</td>
<td>−14.1 ± 12.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Inferior quadrant (μm)</td>
<td>127.7 ± 14.4</td>
<td>96.5 ± 23.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Thickness map</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average (μm)</td>
<td>141.6 ± 12.6</td>
<td>111.3 ± 22.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Reflectance map</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average loss (dB)</td>
<td>0.4 ± 1.1</td>
<td>−3.5 ± 2.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low-reflectance superpixel count</td>
<td>11.5 ± 16.1</td>
<td>106.3 ± 54.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Focal loss (dB)</td>
<td>−0.3 ± 0.4</td>
<td>−4.1 ± 2.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Thickness profile is over the circle with 3.4 mm diameter around the disc. Thickness map and reflectance map are over the peripapillary area with 1.1 mm~2 mm radii around the disc.

Figure 5. Distribution of nerve fiber layer parameters in three groups: normal (N), PPG, and PG.

18 PPG, and 26 PG eyes had equal diffuse and focal losses. In Cluster 3, four PPG and nine PG eyes had predominantly focal loss. Generally, Cluster 3 had a more severe average ($P = 0.044$) and focal ($P = 0.001$) reflectance loss than Cluster 2. This suggests that the predominantly focal pattern of loss may be associated with more aggressive disease courses.

**Diagnostic Accuracy**

Focal reflectance loss and low-reflectance pixel had significantly higher AROC (0.93 and 0.92, $P < 0.023$) than NFL thickness profile parameters (0.86), but not significantly higher than the NFL thickness map average (0.88, $P = 0.070$; Table 4). Focal reflectance loss and low-reflectance pixel count had higher AROC than the average reflectance, but the differences were not significant.

In the overall glaucoma group, all reflectance parameters ($P < 0.013$) had significantly higher glaucoma diagnostic sensitivity (0.68~0.77) than all of the thickness parameters (0.40~0.55) when the specificity was fixed at 99% (Table 5). In subgroup analysis, focal reflectance loss and low-reflectance superpixel count (0.53 and 0.50) had significantly higher ($P < 0.043$) sensitivity than all of the thickness parameters (0.10~0.23) in the PPG group. In the PG group, focal reflectance analysis had the highest sensitivity.

Using either the 5% or 1% cutoff, focal reflectance loss detected more glaucoma eyes than the NFL thickness profile average ($P \leq 0.023$) and the NFL thickness map average ($P < 0.074$). Venn diagrams (Fig. 7)
Figure 6. Unsupervised cluster analysis of focal versus overall reflectance loss revealed three clusters (C1–C3): C1, no loss (green); C2, equal diffuse and focal loss (blue), and C3, predominantly focal loss (red). These clusters were only partially correlated with the clinical diagnostic grouping: normal (circles), PPG (cross), and PG (square).

showed that nearly all eyes with abnormally thin NFL thicknesses also had abnormally large focal reflectance loss, but not vice versa. Thus NFL thickness would not be needed if focal reflectance loss was already used as the primary diagnostic parameter.

Correlation With Visual Field

All NFL parameters had moderate Pearson correlation with VF MD (Pearson \( r \) between 0.52 and 0.61, Table 6). Focal reflectance loss had the highest correlation \( (r = 0.61) \), but it was not significantly higher than the NFL thickness profile \( (r = 0.56) \) or NFL thickness map \( (r = 0.58) \). The NFL reflectance parameters were highly correlated with NFL thickness \( (r \) between 0.79 and 0.85). All of the correlations with VF MD and NFL thickness were statistically highly significant \( (P < 0.001) \).

Two-segmented piecewise linear regression showed that all NFL reflectance and thickness parameters had good correlation with VF MD for eyes with no or mild

### Table 4. Diagnostic Accuracy of Nerve Fiber Layer Parameters

<table>
<thead>
<tr>
<th>NFL</th>
<th>AROC</th>
<th>Confidence Interval (95%)</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thickness profile</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.859 ± 0.037</td>
<td>0.788–0.931</td>
<td>N/A</td>
</tr>
<tr>
<td>Focal loss</td>
<td>0.861 ± 0.032</td>
<td>0.799–0.923</td>
<td>0.958</td>
</tr>
<tr>
<td>Inferior quadrant</td>
<td>0.862 ± 0.036</td>
<td>0.792–0.931</td>
<td>0.928</td>
</tr>
<tr>
<td><strong>Thickness map</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.882 ± 0.033</td>
<td>0.818–0.947</td>
<td>0.234</td>
</tr>
<tr>
<td><strong>Reflectance map</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average loss</td>
<td>0.910 ± 0.029</td>
<td>0.853–0.967</td>
<td>0.047</td>
</tr>
<tr>
<td>Low-reflectance superpixel count</td>
<td>0.921 ± 0.026</td>
<td>0.870–0.973</td>
<td>0.023</td>
</tr>
<tr>
<td>Focal loss</td>
<td>0.925 ± 0.025</td>
<td>0.876–0.974</td>
<td>0.022</td>
</tr>
</tbody>
</table>

\( P \) values for comparison of AROC between NFL thickness profile average and other parameters.

### Table 5. Diagnostic Sensitivity of Nerve Fiber Layer Parameters at 99% Specificity

<table>
<thead>
<tr>
<th>NFL</th>
<th>PPG</th>
<th>( P ) Value*</th>
<th>PG</th>
<th>( P ) Value*</th>
<th>All Glaucoma</th>
<th>( P ) Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thickness profile</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.233</td>
<td>N/A</td>
<td>0.714</td>
<td>0.041</td>
<td>0.492</td>
<td>0.343</td>
</tr>
<tr>
<td>Focal loss</td>
<td>0.100</td>
<td>0.134</td>
<td>0.657</td>
<td>0.013</td>
<td>0.400</td>
<td>0.009</td>
</tr>
<tr>
<td>Inferior quadrant</td>
<td>0.167</td>
<td>0.167</td>
<td>0.800</td>
<td>0.371</td>
<td>0.507</td>
<td>0.450</td>
</tr>
<tr>
<td><strong>Thickness map</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.167</td>
<td>0.167</td>
<td>0.886</td>
<td>N/A</td>
<td>0.554</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Reflectance map</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average loss</td>
<td>0.367</td>
<td>0.289</td>
<td>0.943</td>
<td>0.480</td>
<td>0.677</td>
<td>0.013</td>
</tr>
<tr>
<td>Low reflectance superpixel count</td>
<td>0.500</td>
<td>0.043</td>
<td>0.971</td>
<td>0.248</td>
<td>0.754</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Focal loss</td>
<td>0.533</td>
<td>0.027</td>
<td>1.000</td>
<td>0.137</td>
<td>0.769</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Differences between NFL reflectance parameters and best single NFL thickness parameter.
Figure 7. Venn diagrams of glaucoma detection with NFL parameters with either 5% or 1% specificity cutoff. Numbers in the circle are the eyes detected by either NFL parameter or both, whereas the number out of box is the eyes missed by both parameters. The PPG and PG groups were combined for this analysis.

Table 6. Pearson Correlation Matrix of OCT and Visual Field Diagnostic Parameters

<table>
<thead>
<tr>
<th>Pearson r</th>
<th>NFL Reflectance</th>
<th>Low-Reflectance Superpixel Count</th>
<th>Focal Reflectance Loss</th>
<th>NFL Thickness Profile Average</th>
<th>NFL Thickness Map Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>VF MD</td>
<td>0.593</td>
<td>-0.519</td>
<td>0.612</td>
<td>0.560</td>
<td>0.584</td>
</tr>
<tr>
<td>NFL thickness profile average</td>
<td>0.854</td>
<td>-0.815</td>
<td>0.790</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>NFL thickness map average</td>
<td>0.913</td>
<td>-0.849</td>
<td>0.846</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

VF loss ($r$ between 0.48 and 0.60, Fig. 8). However, they were poorly correlated for eyes with moderate to severe loss ($r$ between 0.03 and 0.16), in which the NFL parameters reach floor levels and no longer change with disease severity. This floor effect suggests that all NFL parameters may be suitable for glaucoma monitoring in only the early stages.

Discussion

NFL reflectivity loss probably precedes thinning because the decrease of axonal microtubes occurs before loss of axons and NFL thinning. Microtubule content can also be measured by birefringence measured by polarimetry or polarization-sensitive OCT. Indeed, loss of NFL birefringence precedes thinning by three months in monkeys and by one week in rats. So theoretically these approaches could improve the early detection of glaucoma. However, clinical measurements of both NFL birefringence and reflectivity are very challenging because of many extrinsic factors that introduce noise and bias. For reflectivity measurements based on OCT, important extrinsic factors include beam coupling and incidence angle. The goal of our investigation and algorithm development effort was to reduce the effects of these extrinsic noises and more cleanly recover the diagnostic information in OCT scans of the peripapillary NFL. Beam coupling refers to the efficiency with which the tissue reflection is coupled back to the OCT detection system. Coupling is reduced by defocus, astigmatism, higher-order aberrations, iris vignetting, media opacity (cataract, vitreous floaters), and polarization mismatch (corneal birefringence and other factors). Generally, variation in beam coupling is best compensated by the normalization of NFL reflectance against a reference layer that would be equally affected. We previously described normalization of NFL reflectivity by that of the retinal pigment epithelium (RPE), and found it improved glaucoma diagnostic accuracy. Gardiner reported that normalization improved the repeatability of reflectivity measurements. Liu et al. combined normalized NFL reflectivity with thickness to generate a reflectance index, and found it further improved diagnostic sensitivity in glaucoma suspects. Our approach here was similar to that of Liu et al. because we integrated reflectivity over the NFL to produce a normalized reflectance. We made a slight change in that we expanded the reference layer to include the ellipsoid band as well as the RPE to improve robustness. A drawback to this approach is that peripapillary atrophy of the outer retinal layers...
could artifactually increase the normalized reflectance and interfere with the detection of NFL loss in these areas. However, previous studies and this study showed that overall this approach increased glaucoma diagnostic accuracy.

Incidence angle variation is a more subtle issue. Knighton et al.\(^{35}\) showed that reflectivity of nerve fibers was negatively related to the incident angle (with the angle defined as zero at perpendicular incidence), and the relationship was shaped like a Gaussian curve. In OCT scanning, the incidence angle depends on the beam location in the pupil, the axial length, and the curvature of the retina. The OCT operator could adjust the positioning of the machine until the retinal cross section appears as flat as possible, thus reducing the variation of the incidence angle. However, this is difficult to achieve while avoiding iris vignetting and while keeping the retina within the image frame. The effect of incidence angle variation on NFL reflectance cannot be reduced by using the RPE as a reference layer because RPE reflectivity is not similarly affected by incidence angle.\(^{39, 60}\)

As far as we know, our method of azimuthal filtering is the first attempt to reduce the effect of incidence angle variation on NFL reflectance measurement. Our results showed that azimuthal filtering improved the repeatability of NFL reflectance measurement, reduced inter-individual variation among normal subjects, and improved glaucoma diagnostic accuracy. The main disadvantage of azimuthal filtering is the reduction of diagnostic information associated with asymmetric NFL loss in glaucoma. However, our results showed that overall the approach improved repeatability, reduced population variation and increased diagnostic accuracy. A better solution would be to maintain perpendicular incidence while scanning the NFL, but none of the commercial OCT systems on the market has this functionality.

The azimuthal filtering is robust to axial length and CCT variation. Longer axial length causes the OCT to scan area at larger radii, and likely causes thinner NFL and lower NFL reflectance. This problem was addressed in our scheme. First, our filter only removed the first frequency component in azimuthal direction,
thus its performance did not change along the radial direction. Secondly, the thinning caused by axial length was compensated by the linear mixed model in our method. On the other hand, thinner CCT may be associated with thinner NFL. The thinning is likely to be evenly applied to whole profile. Thus it would not be affected by the azimuthal filter. As CCT is also a risk factor of glaucoma, no compensation should be applied as it may reduce the diagnostic accuracy.

Another strategy that we successfully employed was the algorithm to measured focal NFL reflectance loss. Focal loss is measured in areas that have sufficiently severe loss that measurement noise is insignificant by comparison. Our results showed that this strategy further improved diagnostic accuracy. With the focal reflectance loss parameter, we were able to detect a majority of PPG eyes and almost all PG eyes at a specificity level of 99%. This is a major improvement over the NFL thickness parameter and may be sufficiently high to be useful in the population-based screening of at-risk patients. However, we cannot be sure that the excellent results we obtained here would fully generalize to populations with different characteristics. Even though we had used a cross-validation technique to reduce bias in our diagnostic accuracy assessment, our study population is different from the general population in that it had been selected to reduce confounding factors. In the general population, common pathologies such as epiretinal membrane, high refractive error, retinal edema, and retinal hemorrhage might interfere with reflectance analysis. Patients with other types of glaucoma may have different patterns of reflectance loss. Thus independent population-based studies would be needed to validate our findings.

An added bonus in our focal loss analysis is the emergence of a class of glaucoma patients in which focal loss predominates over diffuse loss. This cluster had significantly more severe disease in our study population, suggesting that disease progression in these patients may be more rapid. Thus focal NFL reflectance loss may be a valuable prognostic biomarker for the speed of glaucoma progression. This agrees with our previous results in the Advanced Imaging for Glaucoma study, in which we found that focal loss in macular GCC and peripapillary NFL thickness were the best predictors of future VF progression. We hypothesize that predominantly focal NFL reflectance loss may be an indication of a local defect in the structure or perfusion of the optic nerve head, similar to those found in eyes with disc hemorrhage, laminar defect, or peripapillary choroidal defect. A longitudinal study is needed to assess this prognostic potential.

Beyond focal loss analysis, other patterns in the normalized NFL reflectance map may offer additional diagnostic information. We found that diffuse and wedge-shaped reflectance defects were characteristic of glaucoma. Our superpixel grid, which followed the trajectory of nerve fibers, facilitated the detection of the wedge patterns. These patterns could be automatically analyzed with machine learning methods, including deep learning. Indeed, other investigators have found deep learning to be useful in analyzing OCT images to detect glaucoma. The sample size of this study is too small to train a deep learning neural network, but the potential exists to apply this methodology to the analysis of normalized reflectance maps when a larger sample of clinical data becomes available.

A major limitation of NFL reflectance parameters is the presence of a floor effect. This limitation is well known for NFL thickness parameters. Both reflectance and thickness decrease with disease severity as measured by VF MD, but only in mild glaucoma. In moderate to severe glaucoma stages, both NFL reflectance and thickness reach a floor value that do not reflect further gradations. This means that NFL reflectance may be less useful in the staging and monitoring of glaucoma beyond the early onset of the disease. Fortunately, other objective measures of glaucoma, such as macular ganglion cell complex thickness and OCT angiography perfusion measurements, may be better for this purpose.

### Conclusions

We have shown that azimuthal filtering and focal loss analysis improves the glaucoma diagnostic value of NFL reflectance measurements to a level that is significantly higher than the widely used NFL thickness parameter. Subjects with predominantly focal rather than diffuse reflectance loss tend to have more severe glaucoma. Focal NFL reflectance loss is a promising OCT-derived diagnostic biomarker for the early detection of glaucoma and a prognostic biomarker to predict the rate of disease progression. However, because of the floor effect, NFL reflectance loss is only suitable for monitoring disease progression in the early stages.

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