

Focal Loss Analysis of Nerve Fiber Layer Reflectance for Glaucoma Diagnosis

Ou Tan¹, Liang Liu¹, Qisheng You¹, Jie Wang¹, Aiyin Chen¹, Eliesa Ing¹, John C. Morrison¹, Yali Jia¹, and David Huang¹

¹ Casey Eye Institute, Oregon Health & Science University, Portland, OR, USA

Correspondence: David Huang, Ophthalmology & Biomedical Engineering, Casey Eye Institute, Oregon Health & Science University, 515 SW Campus Dr., CEI 3154, Portland, OR, USA.
e-mail: huangd@ohsu.edu

Received: June 29, 2020

Accepted: March 17, 2021

Published: May 6, 2021

Keywords: glaucoma; optical coherence tomography; nerve fiber layer reflectance; focal loss analysis

Citation: Tan O, Liu L, You Q, Wang J, Chen A, Ing E, Morrison JC, Jia Y, Huang D. Focal loss analysis of nerve fiber layer reflectance for glaucoma diagnosis. *Transl Vis Sci Technol*. 2021;10(6):9.
<https://doi.org/10.1167/tvst.10.6.9>

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.

Introduction

Nerve fiber layer (NFL) thickness measurement by optical coherence tomography (OCT) has been widely used in the clinical management of glaucoma.^{1–7} According to the American Academy of Ophthalmology's IRIS Registry, OCT is now used in more glaucoma clinic visits than visual field (VF) analysis.⁸ NFL thickness is useful for confirming the diagnosis of glaucoma and monitoring the progression, at least in the early stage.^{9–14} However, its diagnostic sensitivity is not sufficient to be used alone for mass screening.^{10,15} At the 99% specificity diagnostic cutoff

needed for screening applications, the best single NFL thickness parameters have sensitivity of only 7% to 30% for preperimetric glaucoma (PPG) and 20% to 60% for perimetric glaucoma (PG).^{16–21} Combining diagnostic parameters from several anatomic regions could boost the sensitivity to 55% to 85% for perimetric glaucoma.^{16,18,22–26} Thus there is still room for improvement.

In this article, we explored methods of improving glaucoma diagnostic accuracy by analyzing the NFL reflectance. It is well established that the NFL reflectivity is reduced in glaucoma subjects,²⁷ presumably because of loss of axons and axonal microtubule content.^{28–30} However, the average NFL reflectivity, as

a diagnostic parameter, underperformed the average NFL thickness.²⁷ 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.^{31,32} Combining the reflectivity ratio with NFL thickness did improve diagnostic accuracy.^{32,33} 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.^{34,35} 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 μm , (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 $> +3.00\text{D}$ 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.³⁶ 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.^{36–38} 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. Good-quality images with a signal strength index of 50 (of 100) or more and a quality index of 5 (of 10) or more were used. Images not meeting the quality criteria were excluded from further analysis.

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.^{39,40} 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 environ-

ment by coauthors Y.J., J.W., and others.⁴¹ 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,⁴² 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

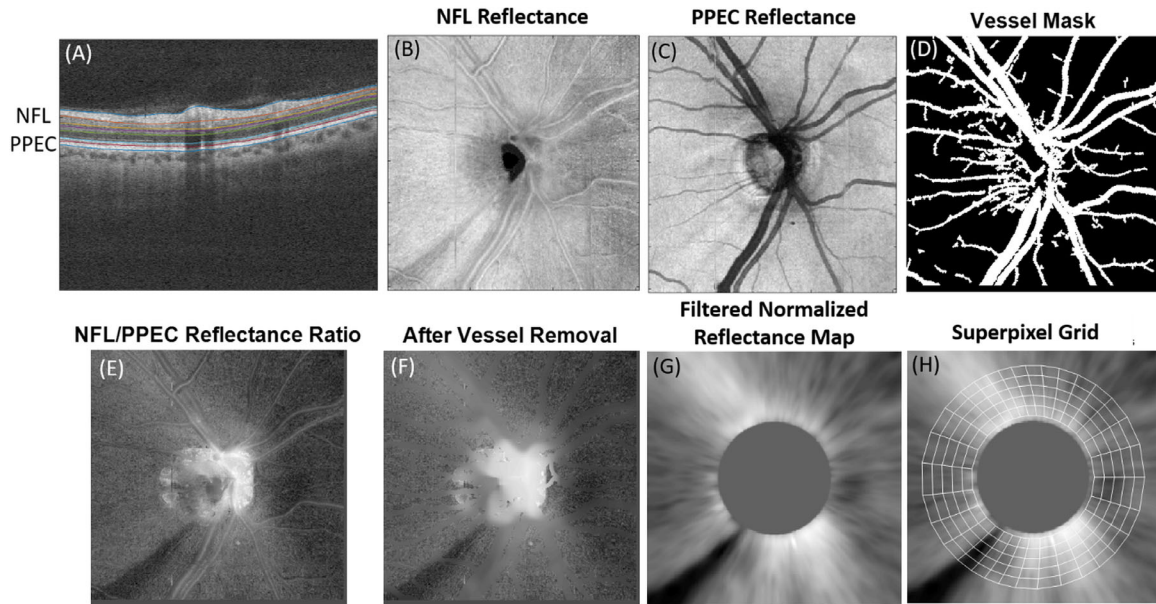


Figure 1. Calculation of the NFL reflectance map (4.5×4.5 mm) in a glaucomatous right eye with an inferotemporal nerve fiber bundle defect. (A) OCT sections were segmented to identify the topmost NFL and a reference layer called the PPEC. (B) Summed OCT signal intensity map in the NFL band. (C) Average OCT signal intensity map in the PPEC band. (D) Large vessel mask. (E) NFL/PPEC reflectance ratio map. (F) Ratio map with vessels removed. (G) Formation of the normalized NFL reflectance map by normalization of the ratio map against the average value from the normal population and then performing spatial frequency filtering in the polar coordinate. (H) Reflectance map is overlaid with a superpixel grid.

determined by the nerve fiber flux analysis described in a previous publication.⁴³ 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^{44,45} 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:

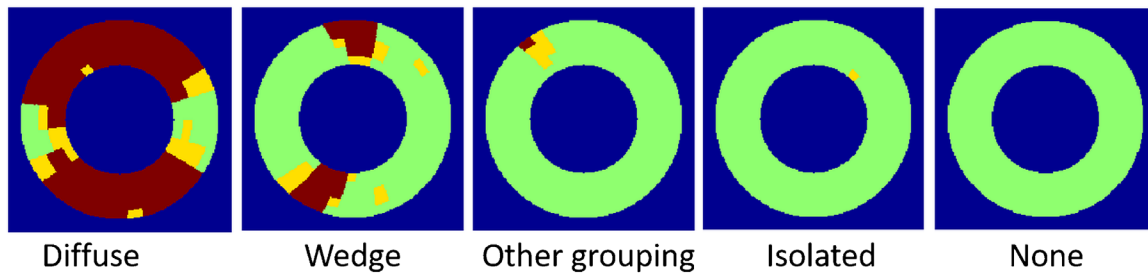


Figure 2. Five types of NFL reflectance loss patterns.

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.⁴⁶ 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)¹⁷ 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,³³ which extended the nonparametric method of Camino et al.³⁸ as applied to clustered data. The same method has been used in previous studies in ophthalmology^{16,39} 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.⁴⁷ Pearson correlation coefficients were calculated among NFL parameters and VF MDs. The coefficients were compared using the bootstrap method.⁴⁸ 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^{49–51} 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

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

Values for continuous variables are means ± standard deviations.

VF PSD, visual field pattern standard deviation.

*P value < 0.05 compared to the normal group.

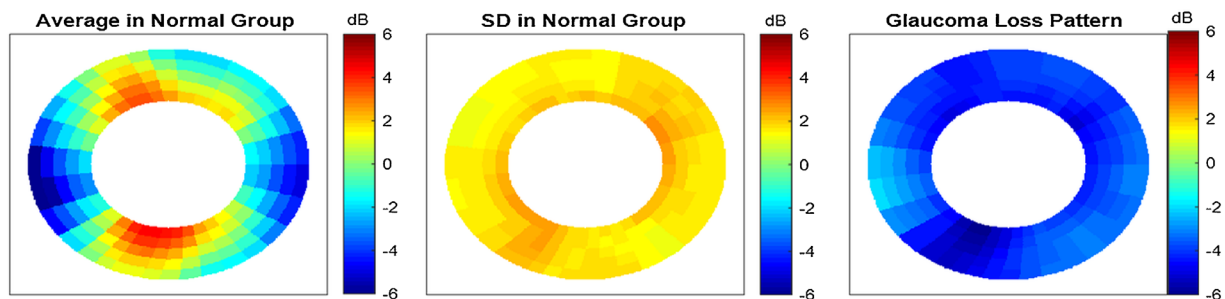


Figure 3. The normalized NFL reflectance maps averaged in the normal and glaucoma groups. The glaucoma group included both pre-perimetric and perimetric glaucoma cases. All eyes were transformed to a right-eye orientation for analysis. (Left) Average map of normal eyes. (Middle) The population SD in 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

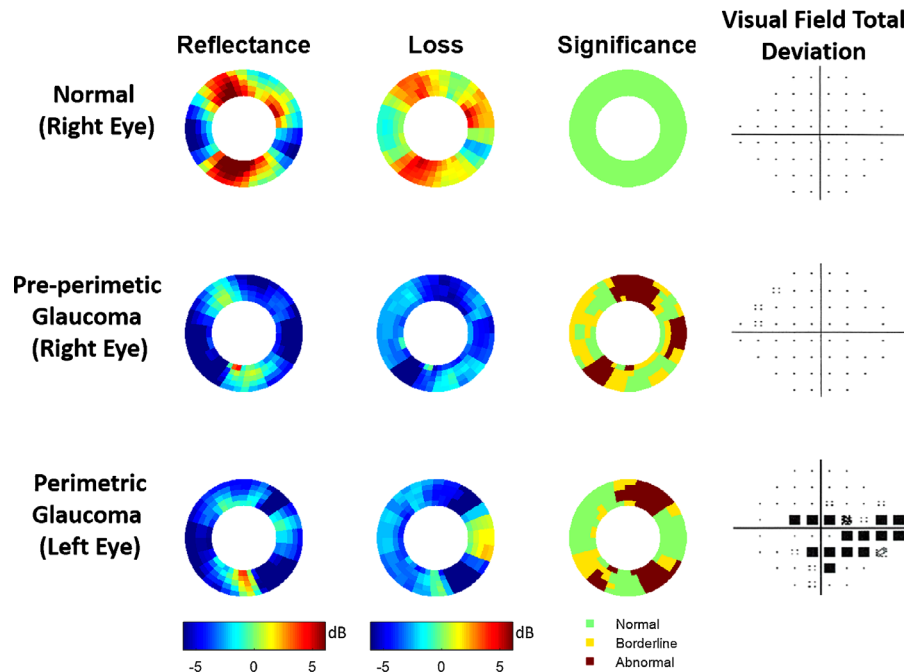


Figure 4. Representative NFL reflectance and VF maps from the normal and glaucoma groups. The significance map classifies superpixels into normal, borderline (first to fifth percentile of normal population), and abnormal (below first percentile of normal) categories.

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

Defect Pattern	Normal Eyes	PPG Eyes	PG Eyes
Diffuse	0	10	24
Wedge	4	12	11
Other grouping	13	4	0
Isolated	6	3	0
None	12	1	0

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 ($P < 0.001$, χ^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

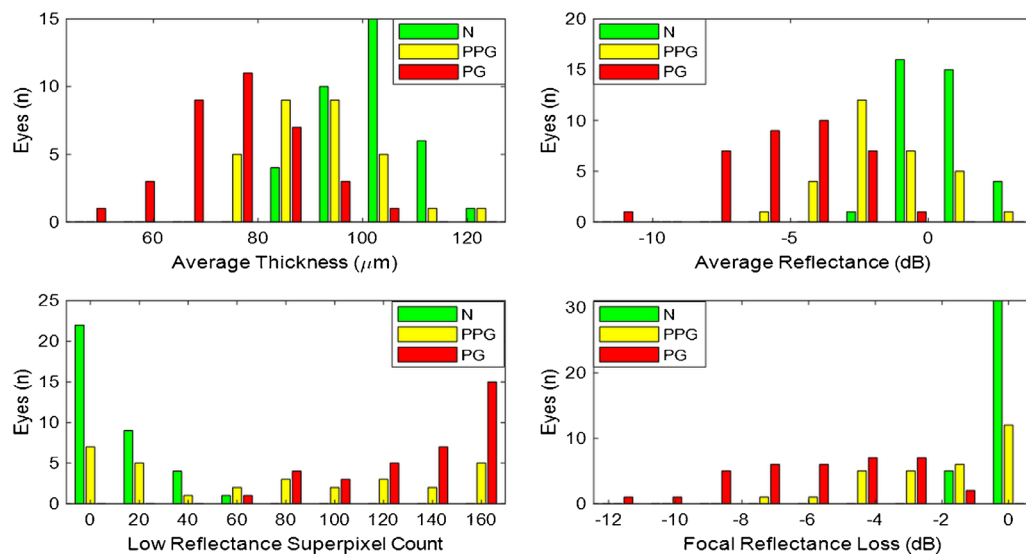
All NFL parameters, including the three reflectance and four thickness parameters, were significantly different between the normal and glaucoma groups (Table 3). The overall average thickness and reflectance were normally distributed for all groups (Fig. 5). The low-reflectance superpixel count and focal 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 models⁵² (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

NFL	Normal	Glaucoma	P Value
Thickness profile			
Average (μm)	102.1 ± 8.8	82.7 ± 14.7	<0.001
Focal loss (%)	-0.8 ± 2.4	-14.1 ± 12.8	<0.001
Inferior quadrant(μm)	127.7 ± 14.4	96.5 ± 23.2	<0.001
Thickness map			
Average (μm)	141.6 ± 12.6	111.3 ± 22.4	<0.001
Reflectance map			
Average loss (dB)	0.4 ± 1.1	-3.5 ± 2.7	<0.001
Low-reflectance superpixel count	11.5 ± 16.1	106.3 ± 54.7	<0.001
Focal loss (dB)	-0.3 ± 0.4	-4.1 ± 2.9	<0.001

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)

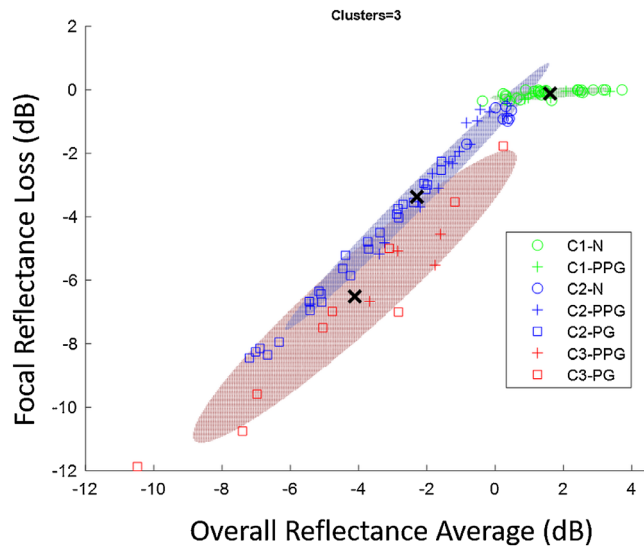


Figure 6. Unsupervised cluster analysis of focal versus overall reflectance loss revealed three clusters (C1–C3): C1, no loss (green); C2, equal diffuse and focal loss (blue), and C3, predominantly focal loss (red). These clusters were only partially correlated with the clinical diagnostic grouping: 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

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

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

NFL	PPG	P Value*	PG	P Value*	All Glaucoma	P Value*
Thickness profile						
Average	0.233	N/A	0.714	0.041	0.492	0.343
Focal loss	0.100	0.134	0.657	0.013	0.400	0.009
Inferior quadrant	0.167	0.167	0.800	0.371	0.507	0.450
Thickness map						
Average	0.167	0.167	0.886	N/A	0.554	N/A
Reflectance map						
Average loss	0.367	0.289	0.943	0.480	0.677	0.013
Low reflectance superpixel count	0.500	0.043	0.971	0.248	0.754	<0.001
Focal loss	0.533	0.027	1	0.137	0.769	<0.001

* Differences between NFL reflectance parameters and best single NFL thickness parameter.

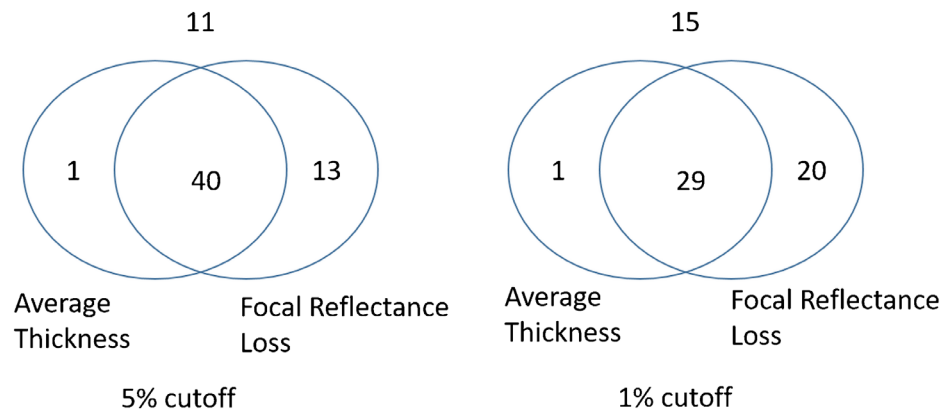


Figure 7. Venn diagrams of glaucoma detection with NFL parameters with either 5% or 1% specificity cutoff. Numbers in the circle are the eyes detected by either NFL parameter or both, whereas the number out of box is the eyes missed by both parameters. The PPG and PG groups were combined for this analysis.

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

Pearson r	NFL Reflectance	Low-Reflectance Superpixel Count	Focal Reflectance Loss	NFL Thickness Profile Average	NFL Thickness Map Average
VF MD	0.593	−0.519	0.612	0.560	0.584
NFL thickness profile average	0.854	−0.815	0.790	N/A	N/A
NFL thickness map average	0.913	−0.849	0.846	N/A	N/A

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 microtubules occurs before loss of axons and NFL thinning.^{28–30} Microtubule content can also be measured by birefringence measured by polarimetry or polarization-sensitive OCT.^{53–56} Indeed, loss of NFL birefringence precedes thinning by three months in monkeys⁵⁷ and by one week in rats.⁵⁸ 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.³¹ Gardiner reported that normalization improved the repeatability of reflectivity measurements.³² Liu et al.³³ 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.³³ 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

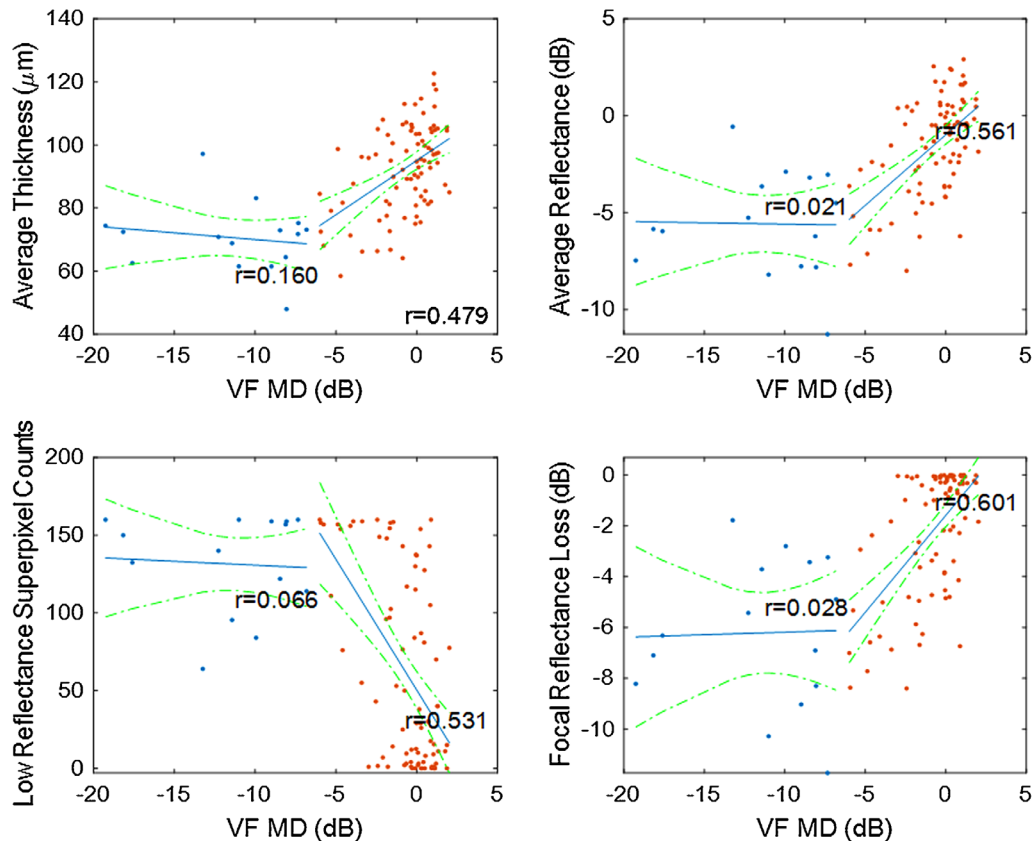


Figure 8. Two-segment piecewise linear regression of NFL parameters against VF MD. The plots combine normal, PPG, and PG groups. The segments for all NFL parameters in normal and early glaucoma eyes (VF MD > -6 dB), marked by red dots, were significantly correlated with VF MD ($P < 0.001$). The segments for moderate and severe glaucoma (VF MD < -6 dB), marked by blue dots, were not correlated with VF MD ($P > 0.05$).

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.^{59,60}

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.⁶¹ 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,⁶² in which we found that focal loss in macular GCC and peripapillary NFL thickness were the best predictors of future VF progression.^{14,46,63} 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.^{64–66} 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.^{22,67} 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.^{9,68–70} 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 thickness¹³ and OCT angiography perfusion measurements,^{42,71} 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.

Acknowledgments

Disclosure: **O. Tan**, Optovue, Inc. (P); **L. Liu**, None; **Q. You**, None; **J. Wang**, None; **A. Chen**, None; **E. Ing**,

None; **J.C. Morrison**, None; **Y. Jia**, Optovue, Inc. (F, P); **D. Huang**, Optovue, Inc. (F, I, C, P)

References

- Schuman JS, Hee MR, Puliafito CA, et al. Quantification of nerve fiber layer thickness in normal and glaucomatous eyes using optical coherence tomography. *Arch Ophthalmol*. 1995;113:586–596.
- Weinreb RN, Shakiba S, Sample PA, et al. Association between quantitative nerve fiber layer measurement and visual field loss in glaucoma. *Am J Ophthalmol*. 1995;120:732–738.
- Leung CK, Chiu V, Weinreb RN, et al. Evaluation of retinal nerve fiber layer progression in glaucoma: a comparison between spectral-domain and time-domain optical coherence tomography. *Ophthalmology*. 2011;118:1558–1562.
- Ye C, To E, Weinreb RN, et al. Comparison of retinal nerve fiber layer imaging by spectral domain optical coherence tomography and scanning laser ophthalmoscopy. *Ophthalmology*. 2011;118:2196–2202.
- Buchser NM, Wollstein G, Ishikawa H, et al. Comparison of retinal nerve fiber layer thickness measurement bias and imprecision across three spectral-domain optical coherence tomography devices. *Invest Ophthalmol Vis Sci*. 2012;53:3742–3747.
- Kotowski J, Wollstein G, Ishikawa H, Schuman JS. Imaging of the optic nerve and retinal nerve fiber layer: an essential part of glaucoma diagnosis and monitoring. *Surv Ophthalmol*. 2014;59:458–467.
- Stein JD, Talwar N, Laverne AM, Nan B, Lichter PR. Trends in use of ancillary glaucoma tests for patients with open-angle glaucoma from 2001 to 2009. *Ophthalmology*. 2012;119:748–758.
- American Academy of Ophthalmology. 2017 AAO IRIS Registry database results in the David E. I. Pyott Glaucoma Education Center on AAO website. Available at: <https://app.powerbi.com/view?r=eyJrIjoiNmYwYjQ3MTgtY2YxNy00ZDNmLTg3MGMtZmU1ZTUxYzhiOTU1IiwidCI6IjkwMjJmZGQzLTc2MmMtNDViZS1hY2Q1LTkzNGY3MTc1YWQ1OSIsImMiOiJZ9> : David E.I. Pyott Glaucoma Education Center. Accessed May 29, 2019.
- Bowd C, Zangwill LM, Weinreb RN, Medeiros FA, Belghith A. Estimating optical coherence tomography structural measurement floors to improve detection of progression in advanced glaucoma. *Am J Ophthalmol*. 2017;175:37–44.
- Bussell II, Wollstein G, Schuman JS. OCT for glaucoma diagnosis, screening and detection of glaucoma progression. *Br J Ophthalmol*. 2014;98(Suppl. 2):ii15–ii19.
- Kotowski J, Wollstein G, Folio LS, Ishikawa H, Schuman JS. Clinical use of OCT in assessing glaucoma progression. *Ophthalmic Surg Lasers Imaging*. 2011;42(Suppl.):S6–S14.
- Lavinsky F, Wollstein G, Tauber J, Schuman JS. The future of imaging in detecting glaucoma progression. *Ophthalmology*. 2017;124:S76–S82.
- Zhang X, Dastiridou A, Francis BA, et al. Comparison of glaucoma progression detection by optical coherence tomography and visual field. *Am J Ophthalmol*. 2017;184:63–74.
- Zhang X, Parrish RK, 2nd, Greenfield DS, et al. Predictive factors for the rate of visual field progression in the advanced imaging for glaucoma study. *Am J Ophthalmol*. 2019;202:62–71.
- Nakano T, Hayashi T, Nakagawa T, et al. Applicability of automatic spectral domain optical coherence tomography for glaucoma mass screening. *Clin Ophthalmol*. 2017;11:97–103.
- Loewen NA, Zhang X, Tan O, et al. Combining measurements from three anatomical areas for glaucoma diagnosis using Fourier-domain optical coherence tomography. *Br J Ophthalmol*. 2015;99:1224–1229.
- Tan O, Chopra V, Lu AT, et al. Detection of macular ganglion cell loss in glaucoma by Fourier-domain optical coherence tomography. *Ophthalmology*. 2009;116:2305–2314.e2301–2302.
- Chang RT, Knight OJ, Feuer WJ, Budenz DL. Sensitivity and specificity of time-domain versus spectral-domain optical coherence tomography in diagnosing early to moderate glaucoma. *Ophthalmology*. 2009;116:2294–2299.
- Oddone F, Lucenteforte E, Michelessi M, et al. Macular versus retinal nerve fiber layer parameters for diagnosing manifest glaucoma: a systematic review of diagnostic accuracy studies. *Ophthalmology*. 2016;123:939–949.
- Kuryshva NI, Parshunina OA, Shatalova EO, Kiseleva TN, Lagutin MB, Fomin AV. Value of structural and hemodynamic parameters for the early detection of primary open-angle glaucoma. *Curr Eye Res*. 2017;42:411–417.
- Bhagat PR, Deshpande KV, Natu B. Utility of ganglion cell complex analysis in early diagnosis and monitoring of glaucoma using a different spectral domain optical coherence tomography. *J Curr Glaucoma Pract*. 2014;8:101–106.

22. Muhammad H, Fuchs TJ, De Cuir N, et al. Hybrid deep learning on single wide-field optical coherence tomography scans accurately classifies glaucoma suspects. *J Glaucoma*. 2017;26:1086–1094.
23. Larrosa JM, Moreno-Montanes J, Martinez-de-la-Casa JM, et al. A diagnostic calculator for detecting glaucoma on the basis of retinal nerve fiber layer, optic disc, and retinal ganglion cell analysis by optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2015;56:6788–6795.
24. Mwanza JC, Warren JL, Budenz DL. Ganglion Cell Analysis Study G. Combining spectral domain optical coherence tomography structural parameters for the diagnosis of glaucoma with early visual field loss. *Invest Ophthalmol Vis Sci*. 2013;54:8393–8400.
25. Baskaran M, Ong E-L, Li J-L, et al. Classification algorithms enhance the discrimination of glaucoma from normal eyes using high-definition optical coherence tomography. *Investigative Ophthalmology & Visual Science*. 2012;53:2314–2320.
26. Huang JY, Pekmezci M, Mesiwala N, Kao A, Lin S. Diagnostic power of optic disc morphology, peripapillary retinal nerve fiber layer thickness, and macular inner retinal layer thickness in glaucoma diagnosis with Fourier-domain optical coherence tomography. *J Glaucoma*. 2011;20:87–94.
27. Pons ME, Ishikawa H, Gurses-Ozden R, Liebmann JM, Dou HL, Ritch R. Assessment of retinal nerve fiber layer internal reflectivity in eyes with and without glaucoma using optical coherence tomography. *Arch Ophthalmol*. 2000;118:1044–1047.
28. Huang XR, Knighton RW, Cavuoto LN. Microtubule contribution to the reflectance of the retinal nerve fiber layer. *Invest Ophthalmol Vis Sci*. 2006;47:5363–5367.
29. Huang XR, Knighton RW, Zhou Y, Zhao XP. Reflectance speckle of retinal nerve fiber layer reveals axonal activity. *Invest Ophthalmol Vis Sci*. 2013;54:2616–2623.
30. Knighton RW, Huang X, Zhou Q. Microtubule contribution to the reflectance of the retinal nerve fiber layer. *Invest Ophthalmol Vis Sci*. 1998;39:189–193.
31. Jia Y, Morrison JC, Tokayer J, et al. Quantitative OCT angiography of optic nerve head blood flow. *Biomed Opt Express*. 2012;3:3127–3137.
32. Gardiner SK, Demirel S, Reynaud J, Fortune B. Changes in retinal nerve fiber layer reflectance intensity as a predictor of functional progression in glaucoma. *Invest Ophthalmol Vis Sci*. 2016;57:1221–1227.
33. Liu S, Wang B, Yin B, et al. Retinal nerve fiber layer reflectance for early glaucoma diagnosis. *J Glaucoma*. 2014;23:e45–52.
34. Tan O, Liu L, Zhang X, Morrison JC, Huang D. Glaucoma increases retinal surface contour variability as measured by optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2016;57:OCT438–443.
35. Knighton RW, Huang XR. Directional and spectral reflectance of the rat retinal nerve fiber layer. *Invest Ophthalmol Vis Sci*. 1999;40:639–647.
36. Jia Y, Wei E, Wang X, et al. Optical coherence tomography angiography of optic disc perfusion in glaucoma. *Ophthalmology*. 2014;121:1322–1332.
37. Kraus MF, Liu JJ, Schottenhamml J, et al. Quantitative 3D-OCT motion correction with tilt and illumination correction, robust similarity measure and regularization. *Biomed Opt Express*. 2014;5:2591–2613.
38. Camino A, Zhang M, Gao SS, et al. Evaluation of artifact reduction in optical coherence tomography angiography with real-time tracking and motion correction technology. *Biomed Opt Express*. 2016;7:3905–3915.
39. Zhang X, Iverson SM, Tan O, Huang D. Effect of signal intensity on measurement of ganglion cell complex and retinal nerve fiber layer scans in Fourier-domain optical coherence tomography. *Transl Vis Sci Technol*. 2015;4:7.
40. Zhang X, Francis BA, Dastiridou A, et al. Longitudinal and cross-sectional analyses of age effects on retinal nerve fiber layer and ganglion cell complex thickness by Fourier-Domain OCT. *Transl Vis Sci Technol*. 2016;5:1.
41. Zhang M, Wang J, Pechauer AD, et al. Advanced image processing for optical coherence tomographic angiography of macular diseases. *Biomed Opt Express*. 2015;6:4661–4675.
42. Liu L, Edmunds B, Takusagawa HL, et al. Projection-resolved optical coherence tomography angiography of the peripapillary retina in glaucoma. *Am J Ophthalmol*. 2019;207:99–109.
43. Tan O, Liu L, Liu L, Huang D. Nerve fiber flux analysis using wide-field swept-source optical coherence tomography. *Transl Vis Sci Technol*. 2018;7:16.
44. Huang J, Huang J, Chen Y, Ying GS. Evaluation of approaches to analyzing continuous correlated eye data when sample size is small. *Ophthalmic Epidemiol*. 2018;25:45–54.
45. Ying G-s, Maguire MG, Glynn R, Rosner B. Tutorial on biostatistics: linear regression analysis of

- continuous correlated eye data. *Ophthalmic Epidemiol.* 2017;24:130–140.
46. Zhang X, Dastiridou A, Francis BA, et al. Baseline Fourier-domain optical coherence tomography structural risk factors for visual field progression in the advanced imaging for glaucoma study. *Am J Ophthalmol.* 2016;172:94–103.
 47. Bengtsson B, Olsson J, Heijl A, Rootzen H. A new generation of algorithms for computerized threshold perimetry, SITA. *Acta Ophthalmol Scand.* 1997;75:368–375.
 48. Wilcox RR. Comparing dependent robust correlations. *Br J Math Stat Psychol.* 2016;69:215–224.
 49. Efron B. Estimating the error rate of a prediction rule: improvement on cross-validation. *J Am Statistical Assoc.* 1983;78:316–331.
 50. Efron B, Tibshirani R. Improvements on cross-validation: the 632+ bootstrap method. *J Am Statistical Assoc.* 1997;92:548–560.
 51. Medeiros FA, Zangwill LM, Bowd C, Bernd AS, Weinreb RN. Fourier analysis of scanning laser polarimetry measurements with variable corneal compensation in glaucoma. *Invest Ophthalmol Vis Sci.* 2003;44:2606–2612.
 52. McLachlan GJ, Peel D. *Finite mixture models.* Hoboken, NJ: John Wiley & Sons; 2000.
 53. Zaveri MS, Conger A, Salter A, et al. Retinal imaging by laser polarimetry and optical coherence tomography evidence of axonal degeneration in multiple sclerosis. *Arch Neurol.* 2008;65:924–928.
 54. Pocock GM, Aranibar RG, Kemp NJ, Specht CS, Markey MK, Rylander HG, 3rd. The relationship between retinal ganglion cell axon constituents and retinal nerve fiber layer birefringence in the primate. *Invest Ophthalmol Vis Sci.* 2009;50:5238–5246.
 55. Jiang H, Chen W, Delgado S, Liu Y, Lin Y, Wang J. Altered birefringence of peripapillary retinal nerve fiber layer in multiple sclerosis measured by polarization sensitive optical coherence tomography. *Eye Vis (Lond).* 2018;5:14.
 56. Fortune B, Wang L, Cull G, Cioffi GA. Intravitreal colchicine causes decreased RNFL birefringence without altering RNFL thickness. *Invest Ophthalmol Vis Sci.* 2008;49:255–261.
 57. Fortune B, Burgoyne CF, Cull G, Reynaud J, Wang L. Onset and progression of peripapillary retinal nerve fiber layer (RNFL) retardance changes occur earlier than RNFL thickness changes in experimental glaucoma. *Invest Ophthalmol Vis Sci.* 2013;54:5653–5661.
 58. Huang XR, Kong W, Qiao J. Response of the retinal nerve fiber layer reflectance and thickness to optic nerve crush. *Invest Ophthalmol Vis Sci.* 2018;59:2094–2103.
 59. Meleppat RK, Zhang P, Ju MJ, et al. Directional optical coherence tomography reveals melanin concentration-dependent scattering properties of retinal pigment epithelium. *J Biomed Opt.* 2019;24:1–10.
 60. Gao W, Cense B, Zhang Y, Jonnal RS, Miller DT. Measuring retinal contributions to the optical Stiles-Crawford effect with optical coherence tomography. *Opt Express.* 2008;16:6486–6501.
 61. Arranz-Marquez E, Lauzirika G, Teus MA, Katsanos A. Thinner retinal nerve fibre layer in healthy myopic eyes with thinner central corneal thickness. *Graefes Arch Clin Exp Ophthalmol.* 2020;258:2477–2481.
 62. Le PV, Zhang X, Francis BA, et al. Advanced imaging for glaucoma study: design, baseline characteristics, and inter-site comparison. *Am J Ophthalmol.* 2015;159:393–403.e392.
 63. Zhang X, Loewen N, Tan O, et al. Predicting development of glaucomatous visual field conversion using baseline Fourier-domain optical coherence tomography. *Am J Ophthalmol.* 2016;163:29–37.
 64. Akagi T, Saunders LJ, Shoji T, et al. Association between rates of retinal nerve fiber layer thinning and previous disc hemorrhage in glaucoma. *Ophthalmol Glaucoma.* 2018;1:23–31.
 65. Lee EJ, Kim T-W, Kim H, Lee SH, Girard MJA, Mari JM. Comparison between lamina cribrosa depth and curvature as a predictor of progressive retinal nerve fiber layer thinning in primary open-angle glaucoma. *Ophthalmol Glaucoma.* 2018;1:44–51.
 66. Lee EJ, Kim J-A, Kim T-W. Influence of choroidal microvasculature dropout on the rate of glaucomatous progression: a prospective study. *Ophthalmol Glaucoma.* 2020;3:25–31.
 67. Hood DC, Fortune B, Mavrommatis MA, et al. Details of glaucomatous damage are better seen on OCT en face images than on OCT retinal nerve fiber layer thickness maps. *Invest Ophthalmol Vis Sci.* 2015;56:6208–6216.
 68. Mwanza JC, Kim HY, Budenz DL, et al. Residual and dynamic range of retinal nerve fiber layer thickness in glaucoma: comparison of three OCT platforms. *Invest Ophthalmol Vis Sci.* 2015;56:6344–6351.
 69. Tan O, Greenfield DS, Francis BA, Varma R, Schuman JS, Huang D. Estimating visual field mean deviation using optical coherence

- tomographic nerve fiber layer measurements in glaucoma patients. *Sci Rep*. 2019;9:18528.
70. Wollstein G, Kagemann L, Bilonick RA, et al. Retinal nerve fibre layer and visual function loss in glaucoma: the tipping point. *Br J Ophthalmol*. 2012;96:47–52.
71. Yarmohammadi A, Zangwill LM, Diniz-Filho A, et al. Relationship between optical coherence tomography angiography vessel density and severity of visual field loss in glaucoma. *Ophthalmology*. 2016;123:2498–2508.