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Separation and thickness measurements of superficial and deep slabs of the retinal nerve fiber layer in healthy and glaucomatous eyes

Luis E Vazquez, MD, PhD¹, Jean-Claude Mwanza, MD, MPH, PhD², Giacinto Triolo, MD¹, Pedro Monsalve, MD¹, William J Feuer, MS¹, Richard K Parrish II, MD¹, Douglas R Anderson, MD¹, Donald L Budenz, MD, MPH²

¹Bascom Palmer Eye Institute, Department of Ophthalmology, University of Miami Miller School of Medicine, Miami, Florida, United States

²Department of Ophthalmology, University of North Carolina School of Medicine, Chapel Hill, North Carolina, United States

Abstract

Purpose: Describe a new method to analyze retinal nerve fiber layer (RNFL) thickness maps.

Design: Cross-sectional study.

Subjects: RNFL thickness maps of healthy and glaucomatous eyes.

Methods: Optical coherence tomography (OCT) RNFL raster scans from 98 healthy and 50 glaucomatous eyes were analyzed. The RNFL thickness maps were separated into superficial (SNFL) and deep (DNFL) slabs through a partial thickness plane set at the modal thickness (mode). Association between mode and OCT signal strength (SS), age, axial length, and visual field mean deviation (VFMD) was tested (Pearson coefficient, r). Thicknesses of inferior and superior SNFL regions (i-,s-SNFL), and inferior, superior, nasal, and temporal DNFL regions (i-,s-,n-,t-DNFL) were calculated. The regions thicknesses were compared between healthy and glaucomatous eyes (t-test) and between glaucomatous eyes with early, moderate, and severe disease (ANOVA and linear regressions of thickness on VFMD). Diagnostic accuracy and correlation with VFMD of RNFL regions thicknesses were calculated as the area under the

Corresponding Author Luis E. Vazquez, MD, PhD, Address: 900 NW 17th Street, Miami, FL 33136, Phone: 305-482-4528, Fax: 305-326-6306, LVazquez@med.miami.edu.

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^cOther

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Conflict of Interest

No conflicting relationship exists for any author

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receiver operating characteristic curve (AUC) and Pearson r , respectively. $P < 0.05$ was considered significant.

Main outcome: Thickness of regions in SNFL and DNFL slabs.

Results: The mode was not associated with SS, age, axial length, or VFMD, it circumscribed the thicker RNFL around the optic disc of healthy and glaucomatous eyes, and it was used to separate the SNFL and DNFL slabs of RNFL thickness maps. The thickness of the SNFL slab was less in glaucomatous eyes than in healthy eyes ($P < 0.001$). S-SNFL and i-SNFL thicknesses (respectively) were $86.0 \pm 8.2 \mu\text{m}$ and $87.3 \pm 9.6 \mu\text{m}$ in healthy eyes vs. $66.1 \pm 9.1 \mu\text{m}$ and $63.4 \pm 8.2 \mu\text{m}$ in glaucomatous eyes ($P < 0.001$ for both). The thickness of the DNFL slab was similar between groups ($P = 0.19$). T-DNFL thickness was $37.0 \pm 5.3 \mu\text{m}$ in healthy eyes vs. $33.9 \pm 5.0 \mu\text{m}$ in glaucomatous eyes ($P < 0.001$); thicknesses of all other DNFL regions were similar. The SNFL regions only thinned with progressively worse glaucoma severity, had excellent AUCs (AUC 0.95, $P < 0.001$), and correlated strongly with VFMD ($r = 0.60$, $P < 0.001$).

Conclusions: Glaucomatous RNFL thinning is predominantly detected within a slab with thickness greater than the mode. SNFL thickness has great AUC and correlation with VFMD in glaucomatous eyes. The usefulness for diagnosis and monitoring of glaucoma needs further study.

Precis

We measured the thicknesses of superficial and deep slabs of OCT-RNFL scans. Glaucomatous loss is predominantly detected within the superficial slab. Superficial slab thickness has strong diagnostic capacity and correlation with VFMD in glaucomatous eyes.

Introduction

Optical coherence tomography (OCT) has improved our ability to detect structural changes around the optic disc in glaucoma. Circumpapillary measurement of the retinal nerve fiber layer (RNFL) thickness was developed in the 1990s and remains a widely used tool to analyze the RNFL in glaucoma.^{1,2} Circumpapillary RNFL thickness is extracted from a single circular scan with a fixed diameter ($\sim 3.5 \text{ mm}$) centered on the optic nerve head (ONH). The data are reported numerically as the average thicknesses of the entire circle, and of sections of the circle, as well as graphically as a “TSNIT” plot. Relative to normative data, thinner RNFL values - especially in the inferior and superior quadrants - are highly sensitive for the detection of glaucoma.^{3, 4}

Alternatively, a peripapillary raster scan acquired with spectral-domain OCT (SD-OCT) generates an RNFL image cube, from which a topographical RNFL thickness map is derived. About 1% of the thickness data is extracted in a circular fashion from the RNFL thickness map of some commercial instruments to mimic traditional circumpapillary RNFL analysis. Use of a greater number of RNFL thickness data points from the raster scan improves the ability of OCT to differentiate glaucoma from healthy eyes compared to the traditional circular line scan.^{5, 6}

The RNFL thickness map data can also be used to differentiate normal anatomical variants from disease in eyes with suspicion of glaucoma. Anatomical variants, such as optic disc anomalies, very low or high axial lengths, ONH tilt, and temporalization of vessels, can

displace RNFL thickness around the ONH.⁷⁻¹³ These examples account for some OCT false positive and false negative results, because the topographical distribution of RNFL thickness deviates from that found in other normal eyes.¹⁴ Inspection of the RNFL thickness map helps clinicians identify displaced (physiologic) vs. thinned (pathologic) RNFL in such cases,¹⁵ and future development of OCT analyses that accurately characterize RNFL thinning may further improve our ability to recognize early glaucomatous degeneration.¹⁶

More than half of all RGCs are located in the macula, and macular RGC arcuate projections and large blood vessels contribute to thickened superior and inferior peripapillary RNFL.¹⁷⁻¹⁹ This thickened inferior and superior RNFL and macular projections within them are frequently vulnerable to glaucomatous damage.^{20, 21} Therefore, we developed a method to isolate these naturally thickened regions by separation of the RNFL thickness map into superficial and deep slabs (SNFL and DNFL, respectively). The average thicknesses of the SNFL and DNFL were determined and compared between healthy and glaucomatous eyes. This study supports that substantial glaucomatous damage detected with OCT localizes to the thickened RNFL regions, captured within the SNFL of OCT RNFL thickness maps.

Methods

Study Cohort

This cross-sectional study was performed in compliance with the Health Insurance Portability and Accountability Act and adhered to the tenets of the Declaration of Helsinki. The University of Miami Miller School of Medicine Institutional Review Board ruled that approval was not required. We analyzed de-identified clinical data from 2 studies,^{22, 23} which included SD-OCT scans of one eye from 98 subjects with healthy eyes and 50 subjects with glaucoma of different disease subtypes (primary open angle, normal tension, pseudoexfoliation, and uveitic glaucomas) and with a spectrum of disease severity. A diagnosis of glaucoma was based on characteristic glaucomatous structural damage to the optic disc detected with dilated fundus examination and a visual field defect detected with a Humphrey Visual Field Analyzer (Carl Zeiss Meditec, Dublin, CA) using the Swedish Interactive Thresholding Algorithm standard program. Criteria for glaucomatous visual field defect were as follows: glaucoma hemifield test outside normal limits, pattern standard deviation with a P value <5%, or a cluster of 3 points in the pattern deviation plot in a single hemifield (superior or inferior) with a P<5%, one of which with a P value of <1%. Inclusion and exclusion criteria, acquisition parameters used to obtain reliable OCT scans and visual fields, and the method to determine glaucoma severity from the VFMD score were previously described.^{22, 23} The following data were retrieved: age, visual field mean deviation (VFMD) score, glaucoma severity (glaucoma eyes only) axial length (healthy eyes only), and *Cirrus* OCT ONH scan image files, as well as the signal strength (SS), ONH and RNFL data from the RNFL and ONH Analysis report (*Cirrus* SD-OCT, software version 6.0, Carl Zeiss Meditec (CZM), Dublin, California).

Image Analysis

RNFL thickness maps were generated from *Cirrus* SD-OCT ONH 200×200 pixel cube scans and with use of *Cirrus* software version 6.0 (CZM) as routinely done for clinical use. Briefly,

the automated segmentation algorithm determines the boundaries between retinal layers of 200 horizontal b-scans, and the RNFL thickness is quantified as the distance in microns between the internal limiting membrane and the interface between the nerve fiber and ganglion cell layers in each a-scan; the disc margin is determined by the ends of Bruch's membrane from the b-scans, and the optic disc region is removed. The RNFL thickness maps were exported and converted from a CZM file format to an 8-bit Tag Image File Format (TIFF) with the help of the manufacturer (CZM). These images were imported into Image J software version 1.49 (National Institutes of Health, NIH) and scaled from 200×200 pixels to 6×6 mm (*xy* dimensions). The RNFL thickness (*z* dimension) was represented by image brightness (greyscale values between 0-255 multiplied by 1.954). A jet color scale was applied to visualize and confirm the appearance of the TIFF to the RNFL thickness map as in the *Cirrus RNFL and ONH analysis* (Figure 1A, *left*).

The RNFL thickness maps were horizontally sliced through a partial RNFL thickness plane with the “thresholding tool” of Image J (Figure 1A, *right*). The regions superficial to this plane formed a slab of thick RNFL (SNFL) that corresponds to the yellow and red regions of the RNFL thickness map (Figure 1B). The SNFL slab was divided into hemi-fields through the center of the disc, and the SNFL islands superiorly and inferiorly were defined as the s- and i-SNFL regions, respectively. The regions deeper to the horizontal plane in all 200 line-scans combined formed a slab of thin RNFL (DNFL), which surrounds the SNFL and corresponds to the blue regions of the RNFL thickness map (Figure 1C). The DNFL slab was divided obliquely into quadrants through the center of the disc, and the DNFL in the temporal, superior, nasal, and inferior quadrants were defined as the t-, s-, n-, and i-DNFL regions, respectively. The width (*xy* dimension) and thickness (*z* dimension) of each SNFL and DNFL region from all the study eyes were calculated with the “measure” tool in Image J, and the values were exported to Excel.

We explored several potential depths (*z* axis) to use as the partial-thickness plane to separate the SNFL from the DNFL slab of each eye with the understanding that eye-specific and acquisition-specific factors influence the overall RNFL thickness of individual eyes (such as OCT SS, age, and glaucoma severity).²⁴⁻²⁸ We studied the distribution of thicknesses from all pixels within each image file as histograms, and the mean, median, mode, minimum, and maximum thickness were retrieved from each histogram with Image J. We confirmed that the minimum and maximum thickness values corresponded to the thinnest and thickest pixels of each image file, respectively. We then traced and inspected the pixels with either modal, median, and mean RNFL thicknesses to determine which established a robust useful plane that separates the SNFL from the DNFL slab of that eye.

Among the modal, mean, and median RNFL thicknesses, the modal RNFL thickness (peak of the histogram curve of RNFL thickness against number of pixels) of each eye accurately and reproducibly circumscribed the thicker RNFL superiorly and inferiorly (see results section). This value (obtained individually for each eye) was used to slice RNFL thickness maps into SNFL and DNFL slabs as described above. In addition, we sliced the RNFL thickness maps of all eyes with a single partial-thickness cut-off. We tested several arbitrary partial-thickness cut-offs, including one that represented the mean of the modal thicknesses

of the cohort studied. These measurements were used to study glaucoma diagnostic accuracy of the SNFL slab thickness and its correlation to VFMD.

Statistical Analysis

Age, VFMD, and ONH/RNFL parameters from the *Cirrus* report were compared between healthy and glaucoma eyes with Student's t-test. Age, VFMD, and ONH/RNFL parameters were compared between early, moderate, and severe glaucoma eyes with a one-way ANOVA.

The histogram skewness and kurtosis, and minimum, modal, median, mean, and maximum RNFL thicknesses were compared between healthy and glaucoma eyes with Student's t-test. Pearson correlation (r) coefficients between modal RNFL thickness and OCT SS, VFMD, age, and axial length of each eye were determined to study the association of the slice plane with factors that influence RNFL thickness.

The thicknesses of each SNFL and DNFL region were compared between healthy and glaucoma eyes with Student's t-test. The deviation from normal thickness of each region was calculated with the following formula (expressed as percentage loss from normal RNFL thickness): $100 - 100 \times (\text{region thickness} / \text{mean region's RNFL thickness in healthy eyes})$. This deviation from normal RNFL thickness was compared between regions with a paired t-test. The mean thickness of each RNFL region among glaucoma eyes were determined for each stage of disease severity (early, moderate, and severe) and compared with a one-way ANOVA. Additionally, the slope of each RNFL region thickness as a function of VFMD was calculated with linear regression analysis in glaucoma eyes.

The diagnostic capacity of the SNFL slab was further investigated with use of a standard partial thickness plane (fixed cut-off) applied across all eyes. The SNFL thickness values (acquired with an individualized cut-off) were compared to standardized SNFL thickness values (acquired with a fixed cut-off) with a paired t-test. The deviation from normal thickness measured with different cut-offs of each SNFL region were also compared with paired t-tests. Standardized s- and i-SNFL thickness values were used to test diagnostic accuracy with area under the ROC curve (AUC) and Pearson correlation with VFMD analyses; r and AUC strengths were determined.²⁹

All statistical analyses were performed with *Prism* software (GraphPad); the statistical test performed was specified, except for instances where Student's t-test was used. Mean $\pm$ SD values for the healthy and glaucoma groups are reported; $P < 0.05$ was considered statistically significant.

Results

The cohort age (mean $\pm$ SD) was 62.3 ± 9.6 (range, 44–81) years for healthy subjects and 70.7 ± 11.1 (range, 46–87) years for glaucoma subjects ($P < 0.001$; Table 1). The VFMD was 0.09 ± 1.10 dB for healthy eyes and -8.44 ± 6.98 dB for glaucomatous eyes ($P < 0.001$; Table 1). The *Cirrus* ONH cup:disc ratio was greater and ONH rim area and RNFL thickness were

lesser in glaucomatous eyes ($P<0.001$ for all; Table 1). OCT SS was 9.08 ± 0.70 for healthy eyes and 7.08 ± 0.72 for glaucomatous eyes ($P<0.001$).

Determination of a partial-thickness plane to separate the SNFL and DNFL slabs from RNFL thickness maps

Histogram curves of RNFL thickness had unimodal thickness distribution with positive skewness and kurtosis in both healthy and glaucoma eyes (Figure 2). However, the frequency distribution of RNFL thickness in glaucomatous eyes shifted from the histogram's tail toward the mode, which resulted in a curve with significantly greater skewness and kurtosis ($P<0.001$ for both; Table 2). The minimum, median, mean, and maximum RNFL thicknesses were also significantly lower in glaucomatous eyes ($P<0.001$ for all; Table 2). In contrast, the modal RNFL thickness was similar between healthy and glaucomatous eyes ($44.0 \pm 6.9 \mu\text{m}$ and $43.0 \pm 7.0 \mu\text{m}$, respectively; $P=0.40$), and there was no lateral shift in the peak of histogram curve of glaucomatous eyes. Thus, modal thickness lacked bias as a plane that separates thick from thin values of an RNFL thickness map, regardless of whether the eye is healthy or glaucomatous. Moreover, modal RNFL thickness was not significantly correlated with OCT SS ($r=-0.01$, $P=0.90$), age ($r=0.12$, $P=0.14$), axial length ($r=-0.07$, $P=0.47$), or VFMD ($r=-0.07$, $P=0.38$) (Supplementary Figure S1). This suggests that modal RNFL thickness is robust as a standard reference depth plane.

The distribution of RNFL thickness was also observed in individual RNFL thickness maps from healthy and glaucomatous eyes. Compared to healthy eyes, there were fewer yellow-to-red colored pixels and more blue colored pixels in RNFL thickness maps of glaucomatous eyes, consistent with a shorter histogram tail and a taller histogram belly (Figure 3A). In addition, the more prevalent blue colored pixels in glaucomatous RNFL thickness maps had a similar hue (i.e., similar thickness values) to those in healthy thickness maps, consistent with a lack in lateral shift of the glaucoma histogram curve. Pixels with the modal thickness value naturally circumscribed the thicker yellow-to-red regions of the RNFL thickness map of each eye, regardless of group or glaucoma severity (Figure 3B). We therefore used the modal thickness value to slice objectively and reproducibly the thicker SNFL from the thinner DNFL slab of each RNFL thickness map.

Regional RNFL loss in glaucomatous eyes

The mean thickness of the SNFL slab was $86.6 \pm 8.3 \mu\text{m}$ in healthy eyes and $64.8 \pm 8.2 \mu\text{m}$ in glaucomatous eyes ($P<0.001$), and the thickness of the DNFL slab was $39.2 \pm 5.5 \mu\text{m}$ in healthy eyes and $37.9 \pm 5.2 \mu\text{m}$ in glaucomatous eyes ($P=0.19$). The thicknesses of each SNFL and DNFL region in healthy and glaucomatous eyes are presented in Table 3. The s- and i-SNFL region thicknesses were significantly less in glaucomatous eyes ($P<0.001$). The deviation from normal of the i-SNFL region was greater than that of the s-SNFL region in glaucomatous eyes ($27.4 \pm 9.5\%$ vs. $23.1 \pm 10.7\%$, respectively; $P<0.001$, paired T-test). Both s- and i-SNFL regions incrementally thinned with glaucoma disease severity (Table 3; $P<0.05$ for both, one-way ANOVA). The slope of RNFL loss in glaucomatous eyes was $0.67 \pm 0.16 \mu\text{m}/\text{dB}$ for the s-SNFL region and 0.50 ± 0.16 for the i-SNFL region ($P<0.005$ for both, linear regression analysis; Supplementary Figure S2).

In the glaucomatous eyes, the SNFL slab was not only thinner, but also smaller in width (Figure 4). The average area of the SNFL slab was $13.57 \pm 1.2 \text{ mm}^2$ in healthy eyes and $11.14 \pm 1.9 \text{ mm}^2$ in glaucomatous eyes ($P < 0.001$); both the s- and i-SNFL regions were significantly smaller in glaucomatous eyes. It was also noted that the normal i-SNFL region was significantly smaller than the s-SNFL region within healthy eyes ($P < 0.001$, paired T-test).

The t-DNFL was also significantly thinner in glaucomatous eyes (Table 3; $P < 0.001$). However, t-DNFL deviation from normal was relatively small, and significantly less compared to the deviation of SNFL regions ($8.4 \pm 13.5\%$, $P < 0.001$ compared to s- and i-SNFL deviations from normal). There was no significant difference in the thicknesses of neither the s-, i-, nor n-DNFL regions between healthy and glaucomatous eyes (Table 3). In contrast to SNFL, none of the DNFL regions incrementally thinned with glaucoma disease severity (Table 3; $P > 0.05$ for all, one-way ANOVA). Moreover, the thickness of none of the DNFL regions decayed as a function of VFMD unlike the SNFL regions (Supplementary Figure S2). The measurements of regional SNFL and DNFL thickness, as well as regional SNFL loss in glaucomatous eyes agreed with the pattern of glaucomatous damage observed in individual RNFL thickness maps (examples shown in figure 3).

Thickness of the SNFL slab had a great ability to differentiate healthy from glaucomatous eyes (AUC=0.963, $P < 0.001$) in contrast to thickness of the DNFL slab (AUC=0.559, $P = 0.24$) (Figure 5A). The s-SNFL (AUC=0.949, $P < 0.001$) and i-SNFL (AUC=0.964, $P < 0.001$) regions each had excellent diagnostic ability, unlike any of the DNFL regions. The t-DNFL (AUC=0.667, $P = 0.001$) and s-DNFL (AUC=0.600, $P = 0.05$) had poor diagnostic ability, while the n-DNFL (AUC=0.571, $P = 0.12$) and i-DNFL (AUC=0.576, $P = 0.12$) regions failed to differentiate healthy from glaucomatous eyes (Figure 5B).

Diagnostic capacity of SNFL thickness over a fixed plane to detect glaucoma

The SNFL regions thicknesses over a fixed cut-off of healthy and glaucomatous eyes are presented in Table 4. With a $44\mu\text{m}$ cut-off (average modal thickness of the entire cohort), the s-SNFL region thickness was $86.75 \pm 6.59 \mu\text{m}$ in healthy eyes and $67.59 \pm 8.22 \mu\text{m}$ in glaucomatous eyes, while the i-SNFL region thickness was $88.86 \pm 7.46 \mu\text{m}$ in healthy eyes and $65.27 \pm 7.77 \mu\text{m}$ in glaucomatous eyes ($P < 0.001$ for both). Regional SNFL thicknesses over $44\mu\text{m}$ were similar to those shown in the section above, consistent with the unbiased nature of an individualized modal thickness cut-off ($P = 0.72, 0.06, 0.37$, and 0.18 for healthy s- and i-SNFL, and glaucoma s- and i-SNFL thicknesses, respectively). Regional SNFL thicknesses over fixed cut-offs of $51\mu\text{m}$ (mean modal thickness + 1 standard deviation) and $58\mu\text{m}$ (mean modal thickness + 2 standard deviations) proportionately increased in both healthy and glaucomatous eyes as a function of greater thickness cut-offs. However, a significantly greater deviation from normal s- and i-SNFL thickness was observed in glaucomatous eyes with use of the $44\mu\text{m}$ cut-off (Table 4, $P < 0.001$ repeated measures ANOVA).

The AUCs for SNFL region thickness over a fixed cut-off are presented in Table 4. AUCs were similarly excellent for all SNFL region thicknesses regardless of cut-off (0.968, $P < 0.001$ for all). Additional cut-offs to those shown in Table 4 were evaluated (data not

shown), but a thickness cut-off of 44 μ m cut-off produced the highest AUCs for the entire SNFL slab (AUC=0.989, $P<0.001$), the s-SNFL region (0.974, $P<0.001$), and the i-SNFL region (0.981, $P<0.001$). The AUC for average thickness of the SNFL slab (0.989) was as high as the AUC for the entire RNFL thickness map (0.986, $P<0.001$) and slightly higher than the AUC for *Cirrus* circumpapillary average RNFL thickness (AUC=0.978, $P<0.001$) of this cohort. The AUC for average thickness of the s-SNFL (0.974) and i-SNFL (0.981) regions were slightly higher than the AUCs for *Cirrus* superior quadrant (AUC=0.959, $P<0.001$), and inferior quadrant (AUC=0.967, $P<0.001$) circumpapillary RNFL thickness of this cohort, respectively.

Standardized SNFL thickness also correlated strongly with VFMD in glaucomatous eyes regardless of cut-off ($r=0.60$, $P<0.001$ for all). Both the s-SNFL region thickness ($r=0.674$, $r=0.682$, and $r=0.646$) and the i-SNFL region thickness ($r=0.595$, $r=0.604$, and $r=0.593$) had moderate-to-high correlation with VFMD over all cut-offs (44, 51, and 58 microns, respectively). Thickness over the 51 μ m cut-off produced the strongest Pearson correlation coefficient for the entire SNFL ($r=0.704$), s-SNFL region (0.682), and i-SNFL region (0.604) against VFMD. All the Pearson coefficients were somewhat higher than those for the *Cirrus* circumpapillary average ($r=0.570$), superior quadrant ($r=0.574$), and inferior quadrant ($r=0.486$) RNFL thickness against VFMD of this glaucoma cohort.

Discussion

We describe a new method to measure the thickness of specific regions within superficial and deep slabs of the RNFL thickness map. With this method, glaucomatous defects observed in OCT RNFL are predominantly detected within a superficial slab with thickness greater than the modal RNFL thickness. SNFL thickness decays with disease severity and is worse for the i-SNFL than for the s-SNFL, which is consistent with the well-described pattern of glaucomatous structural damage. The DNFL regions, on the other hand, did not follow this pattern. The t-DNFL region only was slightly thinner in glaucomatous eyes, and there was a trend for all DNFL regions to thicken with glaucoma disease severity.

Out of various options to split the SNFL and DNFL, we found that none was more robust than using a 44 micron cut-off for thickness, which represents the average modal RNFL thickness in both the glaucomatous and healthy cohorts. Modal RNFL thickness was found to be independent of OCT SS, age, axial length, or glaucoma severity (factors that can affect RNFL thickness measurements). Modal RNFL thickness values are not found in the SNFL or DNFL slabs, but rather forms a boundary between the slabs. We propose that modal RNFL thickness serves as an objective and robust reference plane that naturally separates the thickened peripapillary RNFL regions of the eye. This reference may provide an alternative approach to estimate the volume (thickness x width) of the various anatomical RNFL axonal bundles of the eye measured with OCT; the SNFL contains axonal bundles associated with the main vascular branches that emanate from the disc, which includes the arcuate bundles. The maculopapillary bundle is not represented in the SNFL, but in the t-DNFL, which is interestingly thinner in glaucoma (Table 3).

This new method produced very high AUCs that were slightly higher but not statistically different than the AUC of circumferential RNFL thickness. However, we did not design this study to evaluate superiority between RNFL analyses, and statistical power was low, because thickness measurements between methods of analysis are strongly correlated, and because AUC values are very close to 1 with all methods, which results from the inclusion of eyes with moderate and severe glaucoma.²⁰ Further refinements to this new method and a proper comparison study are needed with adequate power and cohort demographics.

Several features of the new method could contribute to better diagnostic ability. First, the RNFL thickness is measured over a broad area, and the average of the larger number of measurements should improve precision. Second, the full breadth and extent of the arcuate bundles (which are vulnerable to glaucoma²¹) is captured within the SNFL. Third, disc size, location of the arcuate bundles, and the angle at which they approach the edge of the optic disc, do not affect measurement. In addition, the strong structure-function correlation between SNFL thickness and VFMD supports a potential use in staging and monitoring for progression, since many glaucomatous visual field defects are associated with OCT defects within the extent of the arcuate regions.³⁰

We believe that our findings may help improve the interpretation of RNFL thickness maps in glaucomatous eyes. For example, a decrease in the number of yellow-to-red pixels in the arcuate regions of the *Cirrus* RNFL thickness map represents SNFL loss and glaucomatous damage, and the extent of SNFL loss reflects disease severity. Moreover, the pattern of SNFL loss can give insight into location and breadth of visual field damage of that eye (Supplementary Figure S3).

Study limitations include that a diagnostic improvement was not demonstrated by the AUCs with the number of subjects studied, the test-retest reproducibility of our measurements was not tested, nor did we test this method in other OCT instruments. We have also not considered how to adjust the average thickness to the width of the bundle, as the average thickness would be less in bundles that are spread out, as might occur myopia, nor yet determined how diseases other than glaucoma would be represented in this type of image analysis. We believe, however, that the study of RNFL loss in specific RGC axon bundle regions is feasible with this method, and the study limitations are fertile ground for further refinements and studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

(OCT)	Optical coherence tomography
(SD-OCT)	Spectral-domain OCT
(ONH)	Optic nerve head
(RGC)	Retinal ganglion cell
(RNFL)	Retinal nerve fiber layer
(SNFL)	Superficial slab of the RNFL
(DNFL)	Deep slab of the RNFL
(s-SNFL)	Superior region of the SNFL
(i-SNFL)	Inferior region of the SNFL
(s-DNFL)	Superior region of the DNFL
(i-DNFL)	Inferior region of the DNFL
(n-DNFL)	Nasal region of the DNFL
(t-DNFL)	Temporal region of the DNFL
(GCIPL)	Macular ganglion cell and inner plexiform layers
(VFMD)	Visual field mean deviation
(AUC)	Area under the (receiver operating characteristic) curve
(<i>r</i>)	Pearson correlation coefficient
(SS)	Signal strength

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Figure 1.

SNFL and DNFL regions of the RNFL thickness map. A. *Left*, example *Cirrus* RNFL thickness map of a healthy eye; thicker RNFL regions are shown in red and thinner RNFL regions are shown in blue (micron thickness indicated by the jet map color scale). The optic disc region is excluded from the image and analysis, shown in black. *Right*, two linear scans, superiorly and inferiorly, are shown, their locations are indicated with horizontal grey arrows over the RNFL thickness map. A partial-thickness slice (thickness plot's horizontal line at 44 microns) through each line scan of the RNFL thickness map separates the thicker SNFL slab from the thinner DNFL slab. B. The SNFL slab is horizontally divided into hemi-fields through the ONH center (dashed line) to separate the s- and i-SNFL regions. C. The DNFL is obliquely divided into quadrants through the ONH center (dashed line) to separate the t-, s-, n-, and i-DNFL regions. White regions in B and C do not contain RNFL thickness information.

Figure 2.

Thickness distribution within RNFL thickness maps of healthy and glaucomatous eyes. The frequency distributions of RNFL thickness of both healthy and glaucomatous eyes have a unimodal histogram curve with positive skewness and kurtosis. Compared to healthy eyes, there is an accumulation of pixel thicknesses from the tail toward the mode without a lateral shift in the peak of the histogram curve of glaucomatous eyes. Values obtained from the histogram analysis (skewness, kurtosis, minimum, modal, median, mean, and maximum RNFL thicknesses) are shown in Table 1.

Figure 3.

Distribution of thickness values in RNFL thickness maps of individual eyes. A. Example thickness maps from healthy and glaucomatous eyes with a wide range of disease severity. Relative to the RNFL thickness maps from healthy eyes (*top*), glaucomatous eyes (*bottom*) have fewer yellow-to-red colored pixels and a large number of blue colored pixels; the differences match those noted between histogram curves of healthy and glaucomatous eyes. B. Examples of an RNFL thickness map from a healthy eye (*left*), and eyes with early glaucoma (*middle left*), moderate glaucoma (*middle right*), and severe glaucoma (*right*). Pixels that have the modal RNFL thickness value are shown in black and circumscribe the thicker SNFL slab in individual eyes irrespective of health status. Modal thickness (top right) and VFMD (bottom right) values for each example are shown.

Figure 4.

Width of the SNFL regions of healthy (white bars) and glaucomatous eyes (black bars). In contrast to SNFL thickness (z dimension), the width (xy dimension) of the i-SNFL is smaller than that of the s-SNFL in healthy eyes (comparison between white bars); ** paired t-test, $P < 0.001$. The width of both s- and i-SNFL regions is smaller in glaucomatous eyes; * t-test, $P < 0.001$.

Figure 5.

SNFL and DNFL thickness area under the ROC curve (AUC). A. The AUC for the SNFL slab (0.963, $P < 0.001$) is significantly greater than the AUC for the DNFL slab (AUC=0.559, $P=0.243$). B. The AUCs for both the s-SNFL (0.949) and the i-SNFL (0.964) regions were similarly excellent, in contrast to all DNFL regions. The t-DNFL (0.667) and the s-DNFL (0.600) regions had poor diagnostic value, and the n-DNFL (0.571) and i-DNFL (0.576) regions failed to differentiate healthy and glaucomatous eyes.

Table 1.

Baseline characteristics of healthy and glaucoma eyes subdivided by disease severity

	Healthy	Glaucoma	<i>P</i> ^a	Early Glaucoma	Moderate Glaucoma	Severe Glaucoma	<i>P</i> ^b
N (eyes)	98	50		25	11	14	
Age	62.3 ± 9.60	70.7 ± 11.10	<0.0001	70.20 ± 10.90	70.45 ± 15.59	71.93 ± 7.35	0.16
VFMD	0.09 ± 1.10	-8.44 ± 6.98	<0.0001	-2.90 ± 1.49	-8.84 ± 1.22	-18.01 ± 4.52	<0.001
ONH							
Rim Area	1.32 ± 0.25	0.70 ± 0.24	<0.0001	0.74 ± 0.25	0.71 ± 0.22	0.61 ± 0.24	0.28
CDR	0.50 ± 0.17	0.77 ± 0.09	<0.0001	0.75 ± 0.11	0.77 ± 0.08	0.80 ± 0.08	0.43
VCDR	0.47 ± 0.16	0.78 ± 0.09	<0.0001	0.75 ± 0.10	0.79 ± 0.06	0.83 ± 0.05	0.03
RNFL							
I quadrant	117.25 ± 15.09	69.38 ± 17.02	<0.0001	76.49 ± 18.18	66.65 ± 16.31	58.83 ± 8.69	0.005
S quadrant	115.10 ± 13.35	75.43 ± 16.89	<0.0001	82.63 ± 15.97	76.80 ± 17.04	61.51 ± 9.67	<0.001
N quadrant	67.68 ± 10.51	58.46 ± 7.81	<0.0001	61.33 ± 8.39	53.90 ± 5.86	56.92 ± 6.49	0.02
T quadrant	64.26 ± 10.55	46.57 ± 10.69	<0.0001	46.99 ± 11.20	49.22 ± 12.69	43.74 ± 8.32	0.44

^a Comparison between healthy and glaucoma eyes, Student's T-test^b Comparison among glaucoma eyes subdivided by disease severity, one-way ANOVA

Table 2.

Thickness distribution within RNFL thickness maps of healthy and glaucoma eyes

	Healthy (N=98)	Glaucoma (N=50)	<i>P</i> ^a
Skewness	2.21 ± 0.45	2.93 ± 0.56	<0.001
Kurtosis	4.69 ± 2.51	8.51 ± 3.90	<0.001
Min	24.41 ± 3.91	14.69 ± 6.26	<0.001
Mode	44.02 ± 6.94	42.99 ± 7.12	0.40
Median	64.26 ± 5.53	49.59 ± 7.12	<0.001
Mean	77.47 ± 6.53	55.36 ± 6.75	<0.001
Max	252.1 ± 51.5	181.4 ± 47.7	<0.001

^aComparison between healthy and glaucoma eyes, Student's T-test

Table 3.

Thickness of each RNFL thickness map region of healthy and glaucoma eyes

	Healthy (N=98)	Glaucoma (N=50)	<i>P</i> ^a	Early Glaucoma (N=25)	Moderate Glaucoma (N=11)	Severe Glaucoma (N=14)	<i>P</i> ^b
s-SNFL	85.97 ± 8.20	66.10 ± 9.09	<0.001	70.04 ± 8.79	64.32 ± 8.25	60.46 ± 7.47	0.004
i-SNFL	87.31 ± 9.60	63.41 ± 8.35	<0.001	66.51 ± 8.18	61.35 ± 10.14	59.48 ± 4.49	0.02
t-DNFL	36.97 ± 5.30	33.87 ± 4.95	<0.001	32.80 ± 4.18	32.40 ± 4.08	36.92 ± 5.95	0.09
s-DNFL	40.93 ± 5.98	39.01 ± 5.52	0.06	38.58 ± 5.00	36.76 ± 4.55	41.55 ± 6.61	0.07
n-DNFL	39.06 ± 5.72	40.40 ± 5.98	0.19	40.62 ± 5.85	37.08 ± 4.95	42.63 ± 6.38	0.08
i-DNFL	40.30 ± 5.93	38.66 ± 5.75	0.11	38.21 ± 5.18	36.27 ± 5.70	41.34 ± 6.29	0.02

^aComparison between healthy and glaucoma eyes, Student's T-test; P<0.025 and P<0.0125 (Bonferroni corrections for SNFL and DNFL, respectively) are considered significant for the s-SNFL, i-SNFL, and t-DNFL regions.

^bComparison among glaucoma eyes subdivided by disease severity, one-way ANOVA; P<0.025 and P<0.0125 (Bonferroni corrections for SNFL and DNFL, respectively) are considered significant for the s-SNFL and i-SNFL regions.

Table 4.

SNFL thickness over fixed cut-offs of healthy and glaucoma eyes

Parameter ^a	Healthy (N=98)	Glaucoma (N=50)	P ^b	Deviation (%) ^c	AUC	P ^d
s-SNFL (44µm)	86.75 ± 6.59	67.59 ± 8.22	<0.001	22.1 ± 9.5	0.974 ± 0.010	<0.001
s-SNFL (51µm)	91.85 ± 6.69	72.78 ± 8.27	<0.001	20.8 ± 9.0	0.968 ± 0.012	<0.001
s-SNFL (58µm)	99.50 ± 6.84	80.06 ± 8.19	<0.001	19.5 ± 8.2	0.968 ± 0.013	<0.001
i-SNFL (44µm)	88.86 ± 7.46	65.27 ± 7.77	<0.001	26.5 ± 8.8	0.981 ± 0.010	<0.001
i-SNFL (51µm)	95.30 ± 7.83	70.93 ± 8.42	<0.001	25.6 ± 8.8	0.978 ± 0.012	<0.001
i-SNFL (58µm)	103.80 ± 8.28	78.88 ± 8.70	<0.001	24.0 ± 8.4	0.974 ± 0.013	<0.001

^aMeasurements of SNFL thickness over fixed cut-offs of 44, 51, and 58 microns are shown.

^bComparison between healthy and glaucoma eyes, Student's T-test

^cDeviation from normal SNFL thickness of glaucomatous eyes (in percent); $P < 0.001$ for both s- and i-SNFL regions, repeated measures ANOVA (comparison of deviations measured with different cut-offs).

^dAUC P value