Does Optic Nerve Head Size Variation Affect Circumpapillary Retinal Nerve Fiber Layer Thickness Measurement by Optical Coherence Tomography?

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PURPOSE. To determine the relationship between retinal nerve fiber layer (RNFL) thickness, optic disc size, and image magnification.

METHODS. The cohort consisted of 196 normal eyes of 101 participants in the Advanced Imaging for Glaucoma Study (AIGS), a multicenter, prospective, longitudinal study to develop advanced imaging technologies for glaucoma diagnosis. Scanning laser tomography was used to measure disc size. Optical coherence tomography (OCT) was used to perform circumpapillary RNFL thickness measurements using the standard fixed 3.46-mm nominal scan diameter. A theoretical model of magnification effects was developed to relate RNFL thickness (overall average) with axial length and magnification.

RESULTS. Multivariate regression showed no significant correlation between RNFL thickness and optic disc area (95% confidence interval [CI] = -0.9 to $4.1 \mu\text{m}/\text{mm}^2$, $P = 0.21$). Linear regression showed that RNFL thickness depended significantly on axial length (slope = $-3.1 \mu\text{m}/\text{mm}$, 95% CI = -4.9 to -1.3 , $P = 0.001$) and age (slope = $-0.3 \mu\text{m}/\text{y}$, 95% CI = -0.5 to -0.2 , $P = 0.0002$). The slope values agreed closely with the values predicted by the magnification model.

CONCLUSIONS. There is no significant association between RNFL thickness and optic disc area. Previous publications that showed such an association may have been biased by the effect of axial length on fundus image magnification and, therefore, both measured RNFL thickness and apparent disc area. The true diameter of the circumpapillary OCT scan is larger for a longer eye (more myopic eye), leading to a thinner RNFL measurement. Adjustment of measured RNFL thickness by axial length, in addition to age, may lead to a tighter

normative range and improve the detection of RNFL thinning due to glaucoma. (*Invest Ophthalmol Vis Sci.* 2012;53:4990-4997) DOI:10.1167/iovs.11-8214

Optical coherence tomography (OCT)¹ is widely used in the evaluation and management in retinal and optic nerve disorders. It uses low-coherence interferometry to measure time-of-flight delay of backscattering light and determines the depth of reflections from retinal layers. This principle produces cross-sectional images with higher resolution than is possible with other noninvasive imaging modalities.

Glaucomatous optic neuropathy is characterized by a progressive loss of retinal ganglion cells and their respective axons, which comprise the retinal nerve fiber layer (RNFL).² For glaucoma evaluation, OCT provides reproducible quantitative measurements of RNFL around the optic nerve head (ONH)³ and in the macular region.⁴⁻⁸ Retinal nerve fiber layer assessment is important because structural damage to the ONH and RNFL often precede functional changes detected by perimetry.⁹⁻¹⁸

OCT systems are widely used for RNFL imaging. Commercial OCT systems generally provide an age-stratified normative database of circumpapillary RNFL thickness. Circular RNFL scans around the ONH are commonly used. Previously, Schuman et al.⁵ demonstrated that the 3.4-mm fixed-diameter scan was more accurate and reproducible when compared with smaller or larger diameter scans. Thus, the 3.4-mm scan has been adopted as the standard circular scan in clinical practice and research studies.

The strategy of using a fixed-scan diameter has been contested. Several studies have found that RNFL thickness measured at fixed diameter was positively correlated with optic disc area.^{19,20} The implication was that the number of nerve fibers in the RNFL depends on the disc area, and it might be possible to reduce the variation in the measured RNFL thickness if the scan diameter was adjusted according to disc diameter.

We offer an alternative hypothesis in that the number of nerve fibers in the RNFL is not dependent, to any significant degree, on the disc area in human eyes, and that the RNFL thickness, measured at a fixed OCT scan diameter, is not related to the *true* disc area. The correlation between the RNFL thickness and *apparent* disc area could be explained by the image magnification variation due to the variation of the axial length of the eye. We test our hypotheses by studying the relationships between OCT RNFL thickness, ONH area (by Heidelberg Retinal Tomograph II), and eye axial length.

MATERIALS AND METHODS

The cohort included all eligible normal participants in the Advanced Imaging for Glaucoma Study (AIGS), which is a multicenter,

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TABLE 1. Demographics and Measurement Statistics of Normal Participants

Parameter	Mean	SD	Minimum	Maximum
Age (y)	54.0	9.7	40.2	76.1
Axial length (mm)	23.7	0.8	21.9	25.5
Spherical equivalent (D)	−0.5	1.5	−5.5	3.6
Disc area (mm ²)	1.9	0.5	0.8	3.9
Disc diameter (mm)	1.5	0.2	1.0	2.2
RNFL (μm)	99.8	9.3	74.6	120.7

RNFL thickness, overall average.

prospective, longitudinal study to develop advanced imaging technologies to improve the detection and management of glaucoma. The Institutional Review Board at each participating center (University of Southern California Keck School of Medicine, University of Miami Miller School of Medicine, University of Pittsburgh School of Medicine) approved the study protocol, and all study procedures conformed to Health Insurance Portability and Accountability regulations and the Declaration of Helsinki for research involving human subjects.

Clinical Procedures and Definitions

After informed consent was obtained, participants underwent a complete ophthalmic examination by a glaucoma specialist including visual acuity, refraction, and slit-lamp evaluation. Two measurements of intraocular pressure (IOP) were obtained using the Goldmann applanation tonometry (Haag-Streit, Bern, Switzerland), which were then averaged to yield a single value for each eye. Visual field testing was performed using a field analyzer (Humphrey Automated; Carl Zeiss Meditec, Dublin, CA [Swedish interactive threshold algorithm Standard 24-2]). Following dilation, a detailed posterior-segment examination and stereo optic disc photography were performed. Axial length measurements were determined using the intraocular lens (IOL) optical biometer (IOL Master; Carl Zeiss Meditec, Dublin, CA) by an experienced ophthalmic technician.

Inclusion Criteria. Inclusion criteria included participants between the age of 40 and 79 years, the absence of ocular pathology on a detailed eye exam, IOP < 21 mm Hg, and normal visual field. The refractive errors were between +3.0 diopters (D) and −7.0 D spherical equivalent. Although the AIGS allowed enrollment of participants with previous uncomplicated cataract surgery, this subset was excluded from the current analysis because cataract surgery and IOL implantation removes the natural connection between axial eye length and refractive error. In addition, scans with signal strength score < 6 were excluded to avoid artifactually low RNFL thickness measurements due to inadequate signals. The complete inclusion and exclusion criteria are listed in the AIGS Manual of Procedures posted on www.AIGStudy.net.

Optic Disc Size Determination with Scanning Laser Ophthalmoscopy. A confocal scanning laser ophthalmoscope has been shown (The Heidelberg Retinal Tomograph II [HRT II]; Heidelberg Engineering GmbH, Dossenheim, Germany) to provide reproducible

measurements of the optic disc,²¹ and was used to measure disc area in this study. Using reflectance and height maps (HRT II), the disc boundaries were traced by an experienced, AIGS-certified ophthalmic photographer according to the procedure recommended by the manufacturer. Values from two high-quality scans were averaged. The focus setting, which provides information on the eye's refractive error, and the keratometry were recorded (HRT II). Based on this information, the software (HRT II) corrects the effect of magnification on the disc measurements. The HRT method had been found to correct for most magnification errors, to within a mean residual error of −1.3 to 2.0%, compared with an axial length derived standard.²² Thus, HRT-determined disc area should be much closer to the true value than the OCT-derived disc area, which was not corrected for magnification variation between eyes. The HRT-determined disc area measurement was used to calculate a disc diameter assuming a circular shape.

RNFL Thickness Measurements. Circumpapillary RNFL measurements were obtained using the standard “Fast RNFL” scan pattern. The scan records three consecutive circles around the optic disc, for a total of 768 axial scans recorded in 1.9 seconds. The circles have a nominal diameter of 3.46 mm (equivalent to 12°). Experienced photographers who were certified by the AIGS performed two separate, good quality scans (well-centered scans with signal strength ≥ 7). The overall average RNFL thickness measurements from the two scans were averaged to yield a single RNFL thickness value for each eye.

Statistical Analysis

Both eyes of each subject were used for the analysis. To adjust for the correlation between the right and left eyes of each participant, the linear mixed models²³ was used in the statistical tests. Univariate and multivariate regression analyses were performed to identify independent associations between RNFL thickness measurements and other parameters. All analyses were conducted at a <0.05 significance level and used statistical analysis system programs (SAS System 8 programs; SAS Institute Inc., Cary, NC).

RESULTS

Demographics and Measurement Statistics

From the normal group of the AIGS, 196 eyes of 101 participants were found to satisfy the inclusion criteria for this study. The statistics on their demographics and eye measurements are listed in Table 1. The sex was 65.4% female and the ethnicity was 90.1% Caucasian.

Univariate Regression on RNFL Thickness

Univariate regression was performed between overall average RNFL thickness and several independent variables: age, axial length, spherical equivalent refractive error, disc area, sex, and ethnicity (Table 2). Age and axial length were found to have significant correlations with RNFL thickness, whereas sex almost reached statistical significance with RNFL thickness (*P*

TABLE 2. Univariate Regression of RNFL Thickness against Other Parameters

Parameter	Slope (μm/x)	SE of Slope	95% CI of Slope	R ²	P Value
Age (y)	−0.3	0.1	−0.5, −0.2	0.13	<0.0001
Axial length (mm)	−3.3	1.0	−5.2, −1.3	0.09	0.001
Spherical equivalent (D)	0.8	0.5	−0.2, 1.8	<0.01	0.109
Disc area (mm ²)	1.9	1.3	−0.8, 4.6	0.03	0.164
Disc diameter (mm)	4.7	3.3	−1.9, 11.3	0.03	0.164
Sex (female)	3.6	1.9	−0.1, 7.3	0.03	0.054
Ethnicity (Caucasian)	−2.9	3.0	−8.8, 3.1	0.008	0.337

FIGURE 1. Plot of RNFL thickness versus axial length. The solid line represents the linear regression fit.

= 0.054). The linear regression slope of RNFL thickness versus axial length was $-3.3 \mu\text{m}/\text{mm}$, with a 95% confidence interval of -5.2 to -1.3 (Fig. 1). This result agrees with the calculated value of $-4.2 \mu\text{m}/\text{mm}$ for the slope as predicted by our theoretical model of magnification effects (see Appendix, equation 12).

No correlation was found between RNFL thickness and disc area ($P = 0.164$). Our sample size was sufficient to detect a slope of $5 \mu\text{m}/\text{mm}^2$ (20% of the predicted magnification effect) at $P = 0.05$ significance level with more than 80% power. Furthermore, there was no significant correlation between disc area and axial length ($P = 0.8$).

Multivariate Regression Results

Multivariate regression analysis (Table 3) confirmed the results of the univariate regression. Age and axial length were again found to be significantly correlated with RNFL thickness, and the slopes were similar. The lack of association between RNFL and disc area was also confirmed.

Univariate Regression after Introducing Magnification Error on Disc Measurements

Since our disc size measurements have been corrected for magnification effects (HRT II software), we did not expect a correlation with RNFL thickness. However, reports of studies in previous literature have found such a correlation using disc size measurements that were not corrected for magnification variation. To re-create this artifactual correlation, we performed univariate regression of RNFL thickness on disc diameter and area, after reintroducing the magnification variation according to axial length variation (see Appendix,

TABLE 3. Multivariate Regression of RNFL Thickness against Other Parameters*

Parameter	Slope ($\mu\text{m}/x$)	SE	95% CI	P Value
Disc area (mm^2)	1.7	1.3	-0.9, 4.1	0.21
Age (y)	-0.3	0.1	-0.5, -0.2	0.0002
Sex (female)	2.8	1.6	-0.4, 6.1	0.08
Axial length (mm)	-3.1	0.9	-4.9, -1.3	0.001

* $R^2 = 0.25$.

TABLE 4. Univariate Regression of RNFL Thickness against Disc Parameters, after Reintroducing Magnification Variation into Disc Measurements

Parameter	Slope ($\mu\text{m}/x$)	SE	95% CI	R^2	P Value
Disc area (mm^2)	2.7	1.3	0.1, 5.3	0.06	0.04
Disc diameter (mm)	6.7	3.2	0.3, 13.1	0.06	0.04

equations 7 and 8). The results (Table 4) showed a significant correlation ($P = 0.04$) between RNFL thickness and both disc diameter and area.

Nerve Fiber Layer Thickness after Magnification Correction

We corrected for the effect of magnification by dividing the measured RNFL thickness by the magnification factor L_a/L (see Appendix, equations 6 and 10). The corrected RNFL thickness is equivalent to the RNFL thickness measured at a true scan diameter of 3.46 mm rather than a nominal scan diameter of 3.46 mm. The corrected RNFL thickness has a significant correlation with age, but not with other variables (Table 5). The corrected RNFL thickness no longer has a significant correlation with axial length. This again supports the argument that the correlation between RNFL thickness and axial length was due to the magnification effect, not a true anatomic correlation.

DISCUSSION

To optimize the detection of glaucomatous RNFL thinning, it is important to account for factors that can affect the measured RNFL thickness and allow for them by adjusting the RNFL thickness value or diagnostic threshold. So far, the following factors have been identified: (1) demographic factors such as age,^{4,24-33} race, and ethnicity^{24,32-34}; (2) larger optic disc size (diameter or area) was associated with thicker RNFL^{19,20,35}; (3) RNFL thickness decreases further away from optic disc margin^{36,37}; and (4) longer axial eye length and myopia were associated with thinner RNFL.^{38,39}

Demographic factors such as age and race could be dealt with separately from the other two, by the use of a diverse normative database that provides reference over a wide range of age and representative diversity of race and ethnicity. Factor (2) is an important consideration because the ONH size shows considerable variability between individuals, ranging between 0.8 and 6.00 mm^2 .⁴⁰⁻⁴⁴ It is not clear how to eliminate RNFL thickness variations related to ONH size, however. Different investigators have proposed performing OCT scans at either factor (1), fixed distance from the optic disc margin,^{37,45} or

TABLE 5. Univariate Regression of Magnification-Corrected RNFL Thickness against Other Parameters

Parameter	Slope ($\mu\text{m}/x$)	SE	95% CI	R^2	P Value
Age (y)	-0.3	0.1	-0.5, -0.2	0.13	<0.0001
Axial length (mm)	0.8	1.0	-1.1, 2.8	<0.01	0.40
Spherical equivalent (D)	-0.2	0.5	-1.2, 0.7	<0.01	0.62
Disc area (mm^2)	1.7	1.3	-0.9, 4.3	0.03	0.19
Disc diameter (mm)	4.3	3.2	-2.2, 10.7	0.04	0.19
Sex (female)	3.1	1.8	-0.5, 6.6	0.03	0.09
Ethnicity (Caucasian)	-3.8	2.9	-9.5, 1.8	0.02	0.18

factor (2), fixed multiples of optic disc diameter.^{20,46} Another alternative would be to perform OCT scanning at a fixed diameter, and then perform adjustment for optic disc size using a mathematical formula. Because factors (2), (3), and (4) are all affected by optical magnification of OCT scanning, it is difficult to know which scanning approach is best and which method of post-measurement adjustment to use. A quantitative theoretical framework that ties these factors together is needed.

To clarify the intertwined relationship between axial length, RNFL thickness, and disc size, we developed a mathematical model (Appendix) that started from simple reasonable assumptions. This model was used to resolve the question on whether the apparent relationship between RNFL thickness and disc size could be an artifact due to magnification (axial length) variation between eyes.

1. The cross-sectional area of each retinal nerve fiber and its associated glial tissue are approximately constant; therefore, the total RNFL cross-sectional area is approximately constant over scan circles near the ONH (see Appendix, equations 3 and 4).
2. Image magnification is inversely proportional to eye axial length (equation 6).
3. The true millimeter diameter of the OCT scan circle is inversely proportional to image magnification (equation 9).
4. The apparent disc diameter on an OCT image is inversely proportional to image magnification (equation 7).

Our model produced the following prediction that could be tested against results from our clinical study and other clinical studies:

1. Given a fixed nominal scan radius r_a (fixed in degrees of visual angle), the measured RNFL thickness is proportional to image magnification (equation 10).
2. The overall RNFL thickness over a circular scan is inverse proportional to the scan radius r (in millimeters, equation 5).
3. An artifactual correlation between RNFL thickness and disc diameter (or area) could be caused by magnification variation that is not corrected.

Several other groups of investigators had found that RNFL thickness was correlated with axial length and refractive error.^{37–39,47} The magnification effect was one possible explanation for this correlation. Our results showed that the slope of this correlation was close to the value predicted by the magnification effect (equation 12). Our model also provided a way to compensation for the effect of magnification (axial length) on RNFL thickness (equation 10). After accounting for the effect of magnification, RNFL thickness no longer depended on axial length or refractive error (Table 5). Patel et al.,³⁷ Kang et al.,³⁹ and Savini et al.⁴⁷ all found that mathematical magnification compensation removed the dependence of RNFL thickness on refractive error and axial length, in agreement with our results and reasoning. Rauscher et al.³⁸ thought that lower RNFL measurements recorded in myope could be due to differences in reflectivity in longer eyes or a predisposition to develop glaucoma in myopes. This explanation does not appear necessary since the magnification effect can entirely explain the lower RNFL measurement in myopes.

The true relationship between RNFL thickness and optic disc size is another issue that is confounded by magnification variation.

Our results support the hypothesis that the number of nerve fibers in the nerve fiber layer is not dependent on the optic disc size. The RNFL thickness measured by OCT was not

correlated with the HRT-determined optic disc area, which is magnification corrected. Previously, histologic studies have found that there are more nerve fibers in monkey eyes with larger optic disc heads.⁴⁸ Human histologic studies were equivocal. Two studies found no correlation between axon count and disc size or scleral canal area.^{36,49} Another study found a small correlation between nerve fiber count and optic disc area, but the correlation ($R^2 = 0.14$) was much smaller than the correlation between nerve fiber count with retrobulbar optic nerve cross-sectional area ($R^2 = 0.67$).⁵⁰ Overall, the evidence supports that, in humans, the optic disc area is only weakly associated with the number of nerve fibers that pass through it. A recent study by Mansoori et al.⁵¹ found no correlation between spectral domain OCT-derived RNFL thickness and optic disc size, and the authors suggested that the number and distribution of optic nerve fibers within the RNFL is somewhat independent of optic disc size.

If the total RNFL cross-sectional area is not dependent on optic disc size, then what accounts for the apparent relationship between the average RNFL thickness and disc size found by a number of investigators?^{19,20,35} Our results suggest that this was due to magnification variation related to axial length variation within the human population. When the disc area was corrected for magnification variation, there was no correlation with RNFL thickness noted. When the magnification variation was reintroduced, a significant correlation was found between RNFL thickness and apparent disc area. These evidences support magnification as the link between RNFL thickness and apparent disc area (or diameter). Previous investigators did not take the magnification variation into account. Savini et al.¹⁹ used the OCT to measure optic disc size without making correction for axial length or magnification. Budenz et al.³⁵ corrected the magnification differences between fundus cameras, but did not account for the magnification variation due to axial length variation. Thus, the link between RNFL thickness and apparent disc size that was found by Savini and colleagues and Budenz and colleagues was probably due to the magnification artifact rather than a true anatomic correlation.

Our derivation of the $1/r$ dependence of RNFL thickness (equation 5) using Gauss's flux theorem (equations 1 and 2) is valid only for the overall average thickness, but not for quadrant or other sector averages (see Appendix). Because the course of nerve fibers shifts temporally as they radiate out from the optic disc, the temporal quadrant gains at the expense of the nasal quadrant at greater scan radii. Thus the temporal quadrant RNFL thickness can be expected to decrease more slowly than $1/r$, whereas the nasal quadrant decreases more quickly. This deviation also affects the associated magnification correction formula (equation 10). Kang and colleagues found that the effect of axial length variation on RNFL thickness differs across quadrants. This is to be expected from the asymmetric divergence of the retinal nerve fibers. An important corollary of Gauss's flux theorem when applied to the RNFL is that the integral of RNFL thickness over a complete scan circle is the total RNFL cross-sectional area, a conserved quantity. This not only provides a simple $1/r$ dependence but also means that the overall average RNFL thickness is not affected by decentration of the scan circle due to eye motion or variation in disc morphology or RNFL distribution. Thus, the overall average RNFL thickness has a special advantage relative to quadrant and sector averages for the purpose of detecting glaucoma by comparison with a normative database or comparison of multiple measurements made over time.

Because of the apparent correlation between RNFL thickness and apparent disc size, some investigators^{20,46} have advocated that the scan circle diameter should be a fixed multiple of the disc diameter or the disc diameter plus a constant. Our results do not support varying the diameter of the scan circle. According to

equation 5, the measured RNFL thickness is inversely related to the radius (or diameter) of the scan circle. Therefore increasing the scan diameter for a larger disc would artifactually decrease the measured RNFL thickness. Indeed, this effect was found by Savini et al.⁴⁶ and Carpineto et al.²⁰ when they tried to vary the scan diameter. Although it is possible to compensate for the use of differing scan diameters in different eyes by the use of a mathematical model, this adds an additional level of complexity; moreover, the compensation is strictly valid only for overall RNFL thickness, not sector or quadrant RNFL thickness.

A more accurate way of minimizing RNFL thickness variation in the normal reference population would be to scale the nominal scan radii by the magnification factor. This way, the actual millimeter scan radius can be made constant. However, this still requires the use of a second instrument to provide the necessary axial length data prior to the OCT scan. A simpler and more flexible method would be to scan at a constant nominal radius (what is being done now) and correct for the magnification effect of axial length variation afterward. This correction is small for most people but more important for high myopes or hyperopes.

A limitation of this study is that we did not directly show the $1/r$ dependence of overall RNFL thickness in individual eyes. Skaf et al.⁴⁵ and Patel et al.³⁷ analyzed RNFL thickness at various distances from the disc margin. They both found that RNFL became thinner with greater distance from the disc margin, but this relationship was clearly not linear when plotted over a 1.2- or 1.4-mm distance. By examining the appearance of their plots, this nonlinear relationship could be consistent with $1/r$, but unfortunately they did not perform any fit with $1/r$. With higher speed Fourier-domain (also known as spectral or spectral-domain) OCT, it is now possible to map RNFL thickness over a wide area and make measurements over a range of analytic circles or ellipses in postanalysis.^{37,39} We plan to analyze the Fourier-domain OCT results in the AIG study database and report the results in a later publication.

In addition to the correlation between axial length and RNFL thickness, we also found age to be correlated with RNFL thickness in our study. Thinner RNFL thickness measurements were found in the eyes of older participants. Our results confirm data from previous histology and clinical cross-sectional studies. Increasing age is associated with decreasing RNFL thickness, as shown by histology,^{26–28} and measured by scanning laser polarimetry,^{29–31} as well as OCT.^{4,24,52} Contrary results showing the lack of difference in RNFL thickness between young and old individuals has also been demonstrated previously by histology.⁵³ However, the great preponderance of data supports an age-related attenuation of RNFL thickness.

To summarize, we found that overall RNFL thickness, as measured by OCT scans of constant diameter, does not depend on the true disc size. Therefore, there is no need to vary the scan diameter between eyes according to disc size. To minimize the variation in measured RNFL thickness in normal eyes, one may wish to correct for the effects of axial length variation and age-related attenuation.

APPENDIX. MATHEMATICAL MODEL OF MAGNIFICATION EFFECT ON RNFL THICKNESS

Relationship between RNFL Thickness and Scan Radius

Retinal nerve fibers originate from cell bodies in the ganglion cell layer, traverse the RNFL, and exit the eye at the ONH.^{54,55}

FIGURE A1. Electric field analogy of RNFL. Ganglion cell bodies are represented by unit positive charges, the nerve fibers by electric field lines, and the ONH by an aggregate negative charge equal to the sum of all the positive charges. The circular tomographic scan line L transects all of the nerve fibers because it encloses the ONH, just as the integral of electric field lines passing through an enclosing surface is equal to the enclosed electric charges according to Gauss's equation.

This source-and-sink system is a two-dimensional analogy to the charge-and-flux system of electric field theory. The ganglion cells are analogous to unit positive charges, the nerve fibers are analogous to electric field flux lines, and the ONH is analogous to a large aggregate of negative charges (Fig. A1). We can borrow from Gauss's law concerning the electric field, which in its integral form states the surface integral of electric field flux is equal to the electric charge enclosed by the surface.⁵⁶

$$Q = \oint_S \epsilon E \cdot ds \quad (1)$$

where Q is the sum electric charge enclosed in surface S , ϵ is the electric constant, E is the electric field, and ds is the differential surface area.

Gauss's law of electric field is one physical application of Gauss's flux theorem. The same mathematical theorem can be applied to the retinal nerve fiber layer. The equivalent equation for nerve fibers is written by reducing Gauss's equation from three dimensions to two dimensions.

$$N = \oint_L n \cdot dl \quad (2)$$

where N is the total number of retinal nerve fibers enclosed in line L (Fig. A1), n is the nerve fiber number density per length, and dl is the differential line length.

To convert from nerve fibers number to RNFL thickness, we multiply equation 2 by cross-sectional area a to obtain equation 3. The area a is the average cross-section of a nerve fiber plus its associated glial tissue.

$$A = \oint_L t \cdot dl \quad (3)$$

where $A = Na$ is the total cross-sectional area of the RNFL in an eye and $t = a$ is the RNFL thickness.

In a circular scan of the RNFL, the integral of equation 3 can be simply evaluated:

$$A = 2\pi rT \quad (4)$$

where r is the radius of the scan circle and T is the RNFL thickness averaged over the scan circle. We rewrite equation 4 to show how the average RNFL thickness depends on scan radius.

$$T = A/(2\pi r) \quad (5)$$

The theoretical result shows that the average RNFL thickness should be inversely proportional to the radius of the scan circle in an OCT circumpapillary scan. The number of nerve fibers transected by the scan circle is diminished by the number of ganglion cells enclosed inside the circle (in Fig. A1 this equivalent to having some positive charges inside the enclosing circle would partially cancel the negative charge of the ONH). However, if the scan circle is close to the optic disc edge, then the number of ganglion cells enclosed can be considered to be negligibly small.

Note that equation 4 is true for only total RNFL cross-sectional area and, therefore, equation 5 is true only for the overall average RNFL thickness. If one measures the cross-sectional area of RNFL over a quadractic arc, there is no guarantee that the result would be $1/4$ of the total RNFL cross-sectional area, and the average RNFL thickness over a quadrant is not guaranteed to obey the $1/r$ trend. In the eye, the RNFL distribution is known to be nonuniform: as nerve fibers radiate out from the disc, they shift away from the nasal quadrant toward the temporal quadrant because the macula is temporal to the disc. This asymmetric divergence should make temporal RNFL thickness decrease more slowly than $1/r$ and nasal RNFL thickness decrease more rapidly than $1/r$.

Fundus Image Magnification and Axial Length

It has been shown that the magnification of fundus imaging is inversely proportional to the axial length of the eye being imaged.⁵⁷ The transverse dimension of a fundus feature measured by a fundus imaging system (fundus camera, OCT scanner, or scanning laser ophthalmoscope) is proportional to the angle θ subtended by rays from the edges of the object. The angle θ is equal to the physical dimension x of the object divided by the distance from the fundus to the nodal point of the eye. Thus the longer the eye is, the smaller θ is by proportion. Thus for the “Fast RNFL” scan pattern, it would be more accurate to say that the scan diameter is 12° rather than 3.46 mm, because the angular specification is not dependent on axial length variation. A simple equation to describe this is:

$$M = L_a/L \quad (6)$$

where M is the relative magnification factor (its value being 1 for the average eye), L is the axial length, and L_a is the axial length of the average eye.

Magnification and Apparent Disc Size

If a fundus imaging system does not adjust for axial length variation, then the measured optic disc diameter will be proportional to the magnification factor.

$$D_m = M \cdot D \quad (7)$$

where D_m is the measured disc diameter and D is the actual disc diameter.

The disc area is proportional to the square of the disc diameter, so the magnification effect is squared as well.

$$A_m = D_m^2/4 = M^2 A \quad (8)$$

where A_m is the measured disc area and A is the actual disc area.

Magnification and Average RNFL Thickness

Scan patterns for OCT are specified in terms of angles and displayed as lengths, assuming an average axial length. Thus the Fast RNFL scan pattern has a fixed angular diameter, and the millimeter diameter depends on the axial length of the eye, having the nominal value of 3.46 mm only for an eye of exact average dimensions. The same scan pattern would project to a larger physical dimension on the fundus for a longer eye. The relationship between the actual radius of the fast RNFL scan pattern, relative to the nominal value, is expressed by the following equation:

$$r = r_a/M \quad (9)$$

where r is the actual OCT scan radius and r_a is the nominal OCT scan radius, which for the Fast RNFL scan is 1.73 mm. The nominal scan radius was specified for the average eye.

Combining equations 5 and 9, we obtain:

$$T = MA/(2\pi r_a) \quad (10)$$

A linear regression analysis of RNFL thickness against axial length should find correlation due to magnification having a slope that can be found by differentiating T against L .

$$dT/dL = -(L_a/L^2)A/(2r_a) \quad (11)$$

where d is the differential operator. Evaluation of this expression near the average axial length of a normal population gives the following approximate value:

$$dT/dL \approx -T_a/L_a \quad (12)$$

where T_a is the average RNFL thickness of the average eye.

We expect a positive correlation between the average RNFL thickness and apparent disc size because both are proportional to the magnification factor and inversely related to the axial length (equations 6–12). This correlation should disappear if axial length variation is taken into account in multivariate analysis.

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References

- Huang D, Swanson EA, Lin CP, et al. Optical coherence tomography. *Science*. 1991;254:1178-1181.
- Fechtner RD, Weinreb RN. Mechanisms of optic nerve damage in primary open angle glaucoma. *Surv Ophthalmol*. 1994;39:23-42.
- Deleon-Ortega JE, Arthur SN, McGwin G Jr, Xie A, Monheit BE, Girkin CA. Discrimination between glaucomatous and non-glaucomatous eyes using quantitative imaging devices and subjective optic nerve head assessment. *Invest Ophthalmol Vis Sci*. 2006;47:3374-3380.
- 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.
- Schuman JS, Pedut-Kloizman T, Hertzmark E, et al. Reproducibility of nerve fiber layer thickness measurements using optical coherence tomography. *Ophthalmology*. 1996;103:1889-1898.
- Blumenthal EZ, Williams JM, Weinreb RN, Girkin CA, Berry CC, Zangwill LM. Reproducibility of nerve fiber layer thickness measurements by use of optical coherence tomography. *Ophthalmology*. 2000;107:2278-2282.
- Panulescu LA, Schuman JS, Price LL, et al. Reproducibility of nerve fiber thickness, macular thickness, and optic nerve head measurements using Stratus OCT. *Invest Ophthalmol Vis Sci*. 2004;45:1716-1724.
- Carpineto P, Ciancaglini M, Zuppari E, Falconio G, Doronzo E, Mastropasqua L. Reliability of nerve fiber layer thickness measurements using optical coherence tomography in normal and glaucomatous eyes. *Ophthalmology*. 2003;110:190-195.
- Sommer A, Pollack I, Maumenee A. Optic disc parameters and onset of glaucomatous field loss. I. Methods and progressive changes in disc morphology. *Arch Ophthalmol*. 1979;97:1444-1448.
- Pederson J, Anderson D. The mode of progressive disc cupping in ocular hypertension and glaucoma. *Arch Ophthalmol*. 1980;98:490-495.
- Sommer A, Quigley HA, Robin AL, Miller NR, Katz J, Arkel S. Evaluation of nerve fiber layer assessment. *Arch Ophthalmol*. 1984;102:1766-1771.
- Sommer A, Katz J, Quigley HA, et al. Clinically detectable nerve fiber atrophy precedes the onset of glaucomatous field loss. *Arch Ophthalmol*. 1991;109:77-83.
- Quigley HA, Katz J, Derick RJ, Gilbert D, Sommer A. An evaluation of optic disc and nerve fiber layer examinations in monitoring progression of early glaucoma damage. *Ophthalmology*. 1992;99:19-28.
- Zeyen TG, Caprioli J. Progression of disc and field damage in early glaucoma. *Arch Ophthalmol*. 1993;111:62-65.
- Quigley HA, Enger C, Katz J, Sommer A, Scott R, Gilbert R. Risk factors for the development of glaucomatous visual field loss in ocular hypertension. *Arch Ophthalmol*. 1994;112:644-649.
- Sommer A, Miller NR, Pollack I, Maumenee AE, George T. The nerve fiber layer in the diagnosis of glaucoma. *Arch Ophthalmol*. 1977;95:2149-2156.
- Chauhan BC, McCormick TA, Nicolela MT, LeBlanc RP. Optic disc and visual field changes in a prospective longitudinal study of patients with glaucoma: comparison of scanning laser tomography with conventional perimetry and optic disc photography. *Arch Ophthalmol*. 2001;119:1492-1499.
- Kass MA, Heuer DK, Higginbotham EJ, et al. The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. *Arch Ophthalmol*. 2002;120:701-713; discussion 829-830.
- Savini G, Zanini M, Carelli V, Sadun AA, Ross-Cisneros FN, Barboni P. Correlation between retinal nerve fiber layer thickness and optic nerve head size: an optical coherence tomography. *Br J Ophthalmol*. 2005;89:489-492.
- Carpineto P, Ciancaglini M, Aharrh-Gnama A, Cirone D, Mastropasqua L. Custom measurement of retinal nerve fiber layer thickness using STRATUS OCT in normal eyes. *Eur J Ophthalmol*. 2005;15:360-366.
- Miglior S, Albé E, Guareschi M, Rossetti L, Orzalesi N. Intraobserver and interobserver reproducibility in the evaluation of optic disc stereometric parameters by Heidelberg Retina Tomograph. *Ophthalmology*. 2002;109:1072-1077.
- Garway-Heath DF, Rudnicka AR, Lowe T, Foster PJ, Fitzke FW, Hitchings RA. Measurement of optic disc size: equivalence of methods to correct for ocular magnification. *Br J Ophthalmol*. 1998;82:643-649.
- Laird NM, Ware JH. Random-effects models for longitudinal data. *Biometrics*. 1982;38:963-974.
- Varma R, Bazzaz S, Lai M. Optical tomography-measured retinal nerve fiber layer thickness in normal Latinos. *Invest Ophthalmol Vis Sci*. 2003;44:3369-3373.
- Parikh RS, Parikh SR, Sekhar GC, Prabakaran S, Babu JG, Thomas R. Normal age-related decay of retinal nerve fiber layer thickness. *Ophthalmology*. 2007;114:921-926.
- Quigley HA, Dunkelberger GR, Green WR. Retinal ganglion cell atrophy correlated with automated perimetry in human eyes with glaucoma. *Am J Ophthalmol*. 1989;107:453-464.
- Balazsi AG, Rootman J, Drance SM, Schulzer M, Douglas GR. The effect of age on the nerve fiber population of the human optic nerve. *Am J Ophthalmol*. 1984;97:760-766.
- Johnson BM, Miao M, Sadun AA. Age-related decline of human optic nerve axon populations. *Age*. 1987;10:5-9.
- Chi QM, Tomita G, Inazumi K, Hayakawa T, Ido T, Kitazawa Y. Evaluation of the effect of aging on the retinal nerve fiber layer thickness using scanning laser polarimetry. *J Glaucoma*. 1995;4:406-413.
- Tjon-Fo-Sang MJ, de Vries J, Lemij HG. Measurement by nerve fiber analyzer of retinal nerve fiber layer thickness in normal subjects and patients with ocular hypertension. *Am J Ophthalmol*. 1996;122:220-227.
- Poinoosawmy D, Fontana L, Wu JX, Fitzke FW, Hitchings RA. Variation of nerve fiber layer thickness measurements with age and ethnicity by scanning laser polarimetry. *Br J Ophthalmol*. 1997;81:350-354.
- Girkin CA, McGwin G Jr, Sinai MJ, et al. Variation in optic nerve and macular structure with age and race with spectral-domain optical coherence tomography. *Ophthalmology*. 2011;118:2403-2408.
- Budenz DL, Anderson DR, Varma R, et al. Determinants of normal retinal nerve fiber layer thickness measured by Stratus OCT. *Ophthalmology*. 2007;114:1046-1052.
- Girkin CA, Sample PA, Liebmann JM, et al. African Descent and Glaucoma Evaluation Study (ADAGES): II. Ancestry differences in optic disc, retinal nerve fiber layer, and macular structure in healthy subjects. *Arch Ophthalmol*. 2010;128:541-550.
- Budenz DL, Anderson DR, Varma R, et al. Determinants of normal retinal nerve fiber layer thickness measured by Stratus OCT. *Ophthalmology*. 2007;114:1046-1052.
- Varma R, Skaf M, Barron E. Retinal nerve fiber layer thickness in normal human eyes. *Ophthalmology*. 1996;103:2114-2119.
- Patel NB, Luo X, Wheat JL, Harwerth RS. Retinal nerve fiber layer assessment: area versus thickness measurements from elliptical scans centered on the optic nerve. *Invest Ophthalmol Vis Sci*. 2011;52:2477-2489.
- Rauscher FM, Sekhon N, Feuer WJ, Budenz DL. Myopia affects retinal nerve fiber layer measurements as determined by

- optical coherence tomography. *J Glaucoma*. 2009;18:501-505.
39. Kang SH, Hong SW, Im SK, Lee SH, Ahn MD. Effect of myopia on the thickness of the retinal nerve fiber layer measured by Cirrus HD optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2010;51:4075-4083.
 40. Bengtsson B. The variation and covariation of cup and disc diameters. *Acta Ophthalmol*. 1976;54:804-818.
 41. Britton RJ, Drance SM, Schulzer MD, Douglas GR, Mawson DK. The area of the neuroretinal rim of the optic nerve in normal eyes. *Am J Ophthalmol*. 1987;103:497-504.
 42. Tomita G, Takamoto T, Schwartz B. Glaucoma like discs without increased intraocular pressure or visual field loss. *Am J Ophthalmol*. 1989;108:496-505.
 43. Jonas JB, Gusek GC, Naumann GOH. Optic disc, cup and neuroretinal rim size, configuration, and correlations in normal eyes. *Invest Ophthalmol Vis Sci*. 1988;29:1151-1158.
 44. Varma R, Tielsch JM, Quigley HA, et al. Race-, age-, gender-, and refractive error-related differences in the normal optic disc. *Arch Ophthalmol*. 1994;112:1068-1076.
 45. Skaf M, Bernardes AB, Cardillo JA, et al. Retinal nerve fibre layer thickness profile in normal eyes using third-generation optical coherence tomography. *Eye*. 2006;20:431-439.
 46. Savini G, Barboni P, Carbonelli M, Zanini M. The effect of scan diameter on retinal nerve fiber layer thickness measurement using Stratus optical coherence tomography. *Arch Ophthalmol*. 2007;125:901-905.
 47. Savini G, Barboni P, Parisi V, Carbonelli M. The influence of axial length on retinal nerve fibre layer thickness and optic-disc size measurements by spectral-domain OCT. *Br J Ophthalmol*. 2011;96:57-61.
 48. Quigley HA, Coleman AL, Dorman-Pease ME. Larger optic nerve heads have more nerve fibers in normal monkey eyes. *Arch Ophthalmol*. 1991;109:1441-1443.
 49. Mikelberg FS, Yidegiligne HM, White VA, Schulzer M. Relation between optic nerve axon number and axon diameter to scleral canal area. *Ophthalmology*. 1991;98:60-63.
 50. Jonas JB, Schmidt AM, Müller-Bergh JA, Schlötzer-Schrehardt UM, Naumann GO. Human optic nerve fiber count and optic disc size. *Invest Ophthalmol Vis Sci*. 1992;33:2012-2018.
 51. Mansoori T, Viswanath K, Balakrishna N. Correlation between peripapillary retinal nerve fiber layer thickness and optic nerve head parameters using spectral domain optical coherence tomography. *J Glaucoma*. 2010;9:604-608.
 52. Parikh RS, Parikh SR, Sekhar GC, Prabakaran S, Babu JG, Thomas R. Normal age-related decay of retinal nerve fiber layer thickness. *Ophthalmology*. 2007;114:921-926.
 53. Repka MX, Quigley HA. The effect of age on normal human optic nerve fiber number and diameter. *Ophthalmology*. 1989;96:26-32.
 54. Airaksinen PJ, Doro S, Veijola J. Conformal geometry of the retinal nerve fiber layer. *Proc Natl Acad Sci USA*. 2008;105:19690-19695.
 55. Jansonius NM, Nevalainen J, Selig B, et al. A mathematical description of nerve fiber bundle trajectories and their variability in the human retina. *Vision Res*. 2009;49:2157-2163.
 56. Hecht E. *Optics*. 4th ed. Reading, MA: Addison-Wesley; 2001.
 57. Bayraktar S, Bayraktar Z, Yilmaz OF. Influence of scan radius correction for ocular magnification and relationship between scan radius with retinal nerve fiber layer thickness measured by optical coherence tomography. *J Glaucoma*. 2001;10:163-169.