Jotmuil of Glaucoma 10:163-169
O 2(H)I Lippincoll Williams & Wilkins, Inc.

Influence of Scan Radius Correction for Ocular Magnification and Relationship Between Scan Radius With Retinal Nerve Fiber Layer Thickness Measured by Optical Coherence Tomography

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Purpose: To investigate how optical coherence tomography (OCT) modifies the preset scan parameters to correct the errors resulting from ocular magnification, the influence of examiner's final correction of those already modified parameters on retinal nerve fiber layer (RNFL) thickness measurements, the induced change on RNFL thickness measurements and RNFL estimated integrals ($RNFL_{cslimaled}$ $i_{,,legr}$ais) by adjusting the actual scan radius during RNFL examinations performed by OCT.

Methods: Thirty-five healthy patients underwent an RNFL examination by OCT four times using different scan radii. The first scan was performed with the preset circular scan diameter of 3.46 mm; the actual scan diameter was different, however, because it was modified by the OCT instrument. The second, third, and fourth scans were generated after readjusting the already modified scan diameter by the examiner to 3.46, 3.20, and 3.60 mm. The relationship of axial length and refractive error with the actual scan radius (with ocular magnification calculated by OCT), with the influence of the examiner's final correction on RNFL thickness measurements, with the relationship between scan radius with RNFL thickness measurements, and with RNFL were investigated.

Results: The actual scan diameter was found to be primarily determined by axial length (R = 0.97, *P* < 0.0001), but the influence of refractive error was small (R = -0.26, *P* = 0.067). Final correction of the actual scan radius by the examiner had a significant influence on RNFL thickness measurements *(P* = 0.025). RNFL thickness measurements obtained without correction of the actual scan radius for magnification were found to be inversely correlated with axial length (R = -0.54, *P* = 0.001), whereas no similar relationship was found when RNFL thickness measurements were obtained with correction (R = 0.21, *P* = 0.11). A reciprocal relationship between I/scan radius with RNFL thickness measurements (they tended to be thinner as scan radii were increased) was found (R = 0.41, *P* = 0.169), but $RNFL_{cslimated\ inlcgnils}$ areas were found to be independent of the scan radius *(P* = 0.521).

Conclusion: To increase the accuracy of RNFL thickness measurements, it will be appropriate for the examiner to manually correct the actual scan parameters to the desired or preset ones after their automatic modification performed by the OCT instrument. Keeping the actual scan radius constant for repeated exams is also recommended because RNFL thickness measurements were found to depend on scan size. Alternatively, $RNFL_{csliinalcd}$- $i_{,llcgRl}|_S$ could be used because they were found to be independent of the scan size.

Key Words: Axial length—Optical coherence tomography—Refractive error—Retinal nerve fiber layer thickness—Scan radius.

Received September 20, 20(H); accepted March 7, 2001

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Retinal nerve fiber layer (RNFL) thickness has been shown to be accurately measured by optical coherence tomography (OCT).[1-4] Axial and lateral resolutions of the OCT instrument have been reported to be approximately 10 xm and reproducibility has been reported to be between 11 and 25 xm.[2,5] Because of these advantages, OCT has been increasingly used for detection of new glaucoma cases and monitoring of established glaucoma cases.[5-10]

Some examiner-dependent issues of the OCT examination for RNFL investigation, such as the best clinically useful scan radius or diameter, have not been thoroughly studied. Because of the topographical anatomy of the human RNFL, the circumference and location of the scanning circle should be kept constant with respect to the optic nerve head over repeated measurements to decrease interobserver and intraobserver variability. The maintenance of a constant scan radius and circumference are also important for the development of normal databases.

When the patient's axial length and refractive error data were entered during an OCT examination, the preset scan radius was modified by the instrument. The aim of this automatic modification process was to cancel out the influence of magnification during the test. After modification of the preset parameters, the actual scan radius that was calculated by the software was displayed on the monitor.

This study was planned to investigate how the OCT instrument modifies the selected scan parameters (i.e., influence of factors such as axial length and refractive error on that modification process, the influence of corrections made by the operator to readjust the already modified scan radii to the preset or desired ones, the relationship between RNFL thickness measurements and the scan radius, and the usefulness of $RNFLT_{es'ilmilcd}$ i_n,eg,ais areas instead of RNFL thickness measurements as a measure of total nerve fiber count independent of the scan diameter).

PATIENTS AND METHODS

Thirty-five healthy patients who were admitted to our eye clinic for the correction of their refractive errors were enrolled into this study. The following criteria were used for exclusion from the study: best-corrected visual acuity poorer than 20/20; macular or retinal disease interfering with fixation; media problems, such as cataracts and corneal or vitreus opacities; inadequate pupillary dilation (less than 5 mm); and definite or probable signs of glaucoma, such as intraocular pressure (IOP) greater than 17 mm Hg, glaucomatous optic disc changes (cup-to-disc ratio greater than or equal to 0.4 or asymmetric cup-to-disc ratios between fellow eyes greater than 0.2), or family history of glaucoma.

A routine ophthalmologic examination, including visual acuity testing, objective refraction with cycloplegia, biomicroscopic examination, IOP measurement by applanation tonometry, and a dilated fundus examination, especially of the optic disc, was performed in all patients before the RNFL thickness measurements. Axial length was also measured by A scan biometry before the OCT examination.

Twenty men and 15 women were enrolled into the study. Patient characteristics are shown in Table 1. The mean age was 42.6 ± 13.8 years (range, 18-68 years). The mean spherical equivalent of the refractive error was -1.38 ± 5.65 diopters (D) (range, -6.50 to +4.25 D). The mean axial length was 23.79 ±1.51 mm (range, 21.08-26.82 mm).

Retinal nerve fiber layer thickness measurements were done by the commercially available optical coherence tomography device developed by Humhprey Instruments, (San Leandro, CA). This instrument, which uses the principle of low coherence interferometry, has been reported to measure RNFLT directly with a high degree of accuracy. It uses a low coherent diode light with a wavelength of 810 nm as a light source for scanning. Because the OCT device has several clinical applications, such as retinal disease, glaucoma, and anterior segment disease, several scanning modes are integrated in its software. For glaucoma detection and monitoring, use of circular scanning modes is recommended. The optic nerve head is located at the center of those circles, and images are taken from 100 points along the circumference. RNFLT at each of those 100 points can be automatically calculated by special software. Then sector (30°), quadrant (90°), and whole retinal averages are automatically calculated and displayed.

In this study, before initiating the scanning process, a personal file for the patient was created and included file number, name, date of birth, refraction, and axial length for the two eyes separately. The patient's pupils were dilated, and the patient was properly seated in the examination chair and asked to fixate on an internal yellow-green light. Then circular scans around the optic disc were obtained.

An operator monitoring the patient's fundus image on

TABLE 1. *Patient characteristics*

Characteristic	Mean ± standard deviation	Range
Age (yrs)	42.6 ± 13.8	18 to 68
Refractive error (diopters)	-1.38 ±5.65	-6.50 to +4.25
Axial length (mm)	23.79 ± 1.51	21.08 to 26.82

a real-time infrared monitor could make fine adjustments using a control knob or a joystick to locate the optic nerve head exactly at the center of the circle selected for scanning. The time required to obtain a single scan was approximately 1 second, and during that time, the patient was asked not to blink and to maintain his or her gaze on the internal fixation light.

The scans were accepted for the study only if the signal-to-noise ratio was greater than or equal to 56 dB because our previous observations showed that lower ratios were associated with reduced reproducibility and precision (unpublished data). Because patients with media opacities and poorly dilated pupils were excluded from the study, scans with high signal-to-noise ratios could be achieved in all eyes with fine focusing of a low coherence light beam on the RNFL. In addition to sharp focusing, equal illumination in all segments of the fundus image and complete elimination of eye and head movements were also essential for obtaining images of superior quality.

For this study, the scanning process was repeated at least four times for both eyes of each patient by using a different scan radius, diameter, and circumference. Adjustments of the scan radius, diameter, and circumference were done in the following steps. For the first scan, one of the most commonly used preset circular scanning modes (i.e., optic nerve head R = 1.73 mm) was selected. In this mode, the preset scan radius was 1.73 mm, the diameter 3.46 mm, and th circumference 10.87 mm. However, actual projected scan radius, diameter, and circumference would be different from those preset values above because they were modified by the OCT instrument to reduce or eliminate the errors resulting from ocular magnification that occurred during the test. The actual projected scan radius would be automatically corrected by the OCT instrument and displayed on the monitor after the patient's axial length and refractive error data were entered. Therefore, because of that automatic modification, the first scan was obtained along different radii, diameters, and circumferences in each eye, despite the selection of the identical preset parameters.

By using an adjustment knob on the control panel of the instrument, the actual projected scan radius, already modified by the OCT software, was then adjusted (corrected) by the examiner to 1.73 mm, and a second scan was generated. Therefore, in all patients, the second scans were obtained along the identical and exact scan actual radius of 1.73 mm, diameter of 3.46 mm, and circumference of 10.87 mm. The examiner adjusted (corrected) the actual projected scan radius to 1.60 mm in each patient, and then the third scans were generated by using the identical actual scan radius of 1.60 mm, diameter of 3.20 mm, and corresponding scan circumference of 10.05 mm. Final scans were generated by using the identical actual projected scan radius of 1.85 mm, diameter of 3.70 mm, and scan circumference of 11.62 mm for each patient after adjustment (correction) made by the examiner.

Example

For the particular eye of a patient whose axial length was 26 mm and refraction was -6 D, the sequence of scans would be as follows: first scan with actual projected radius of 1.82 mm, second scan with actual projected radius of 1.73 mm, third scan with actual projected radius of 1.60 mm, and fourth scan with actual projected radius of 1.80 mm. To decrease intermeasurement variability, the average of the thickness values obtained on each of the 100 points along the whole circumference of the circular scans (whole retinal averages) was used for data analysis and comparison in the current study. In the previous studies, the use of quadrant (90°) and sector (30°) averages was shown to be associated with considerably more variation and thus low reproducibility.[2,5]

Data and Statistical Analysis

To calculate the modification of the preset scan radius by the OCT instrument, the difference between actual projected scan radius and preset radius (1.73 mm) was calculated for each eye: $\text{radius}_{difference}$ (mm) = radius_{actual} (mm) − 1.73 (radius_{preset}).

The relationship of axial length and refractive error with the actual projected scan radius (calculated by the OCT instrument and displayed on its monitor) was investigated by using single and multiple linear regression analysis. Then average RNFL thickness measurements obtained from the first (using the preset values, no correction by the examiner) and second (correction by the examiner and actual projected scan radius adjusted to 1.73 mm) scans were compared in each of the eyes with a paired-samples / test to find out the influence of actual scan radius correction by the examiner on RNFL thickness measurements. The differences in RNFL thickness measurements between those two scans were calculated for each eye, and their correlation with axial length and refractive error was also determined.

To investigate the influence of scan radius change on RNFL thickness measurements, the average RNFL thickness measurements obtained from the second, third, and fourth scans were compared for each eye by using a repeated-measures analysis of variance.

Two assumptions were made: In the peripapillary area, there is no change in the total number of nerve fibers, and the RNFL spreads uniformly in each direction. It was then predicted that a reciprocal relationship should exist between RNFL thickness and I/scan radius. To test this model, a correlation analysis was performed between RNFL thickness measurements and 1/corresponding scan radii.

Finally, average RNFL thickness measurements obtained from the second, third, and fourth scans taken from each eye were multiplied with their corresponding scan circumferences to estimate the integral values of the total RNFL area: $RNFLT_{estimiUcd\ inlcgrals}$ (xm^2) = $RNFLT_{avcrapc}$ **(XM)** x scan circumference (XM).

Then three different products calculated for the second, third, and fourth scans were compared by using a repeated-measures analysts of variance. All the statistical tests were performed by using the computer program SPSS for Windows Release 7.0 (SPSS Inc., Chicago, IL).

RESULTS

Modification of the Preset Scan Radius by the OCT Instrument, Influence of Axial Length, and Refractive Error

Despite the selection of the preset radius of 1.73 mm, the actual projected scan radius that was calculated by the OCT instrument and displayed on the monitor was found to be between 1.51 and 1.87 mm (1.68 ± 0.09 mm) for the study eyes. The mean difference between those actual projected scan radii with the preset value of 1.73 mm was found to be 0.05 ± 0.09 mm. The actual projected scan radius was found to have statistically significant positive correlation with axial length (correlation coefficient R = 0.97, R^2 = 0.94, and $P < 0.0001$). However, the relationship between refractive error and the actual projected scan radius was weak and there was no statistically significant correlation (correlation coefficient R= -0.26, R^2 = 0.07, and P = 0.067).

Simple Regression Equations

Simple regression equations are as follows: $raclius_{dirierencc}$ (mm) = -1.483 + 0.061 x axial length (mm): radius.actual (mm) = 0.247 + 0.061 x axial length (mm); $radius_{dirrcrencc}$ (mm) = -0.072 - 0.0039 x refractive error (D); and $radius_{actual}$ (mm) = 1.658 - 0.0039 x refractive error (D).

Multiple Regression Equations

Multiple regression equations are as follows: $radius_{difference}$ (mm) = -1.468 + 0.060 x axial length (mm) - 0.0032 x refractive error (D); and radius.actual (mm) = 0.262 + 0.060 x axial length (mm) - 0.0032 x refractive error (D).

It was concluded that the actual projected scan radius was dependent on axial length but relatively independent of refractive error, because refractive error was not found to have any statistical correlation with the actual scan radius in the simple regression model. If it were included in the multiple regression model, the R value of the model would decrease from 0.97 to 0.96 and R^2 would decrease from 0.94 to 0.92.

Influence of the Adjustment of Projected Scan Radius By the Examiner on RNFL Thickness Measurements

This was done by comparing RNFL thickness measurements obtained in the first and second scans. The average RNFL thickness was 98.58 ± 15.66 xm in the first scan and 95.09 ± 9.06 xm in the second scan. Because of the use of different actual scan parameters in each eye, the measurements obtained in the first scans showed a larger scatter and greater standard deviation as compared with the ones of second scans, which were obtained along the fixed actual scan circumference of 10.87 mm. The difference of RNFL thickness measurements in those two scans was found to be statistically significant (P = 0.025) and inversely correlated with axial length (correlation coefficient R = -0.63, R^2 = 0.40, $P < 0.0001$). Also, RNFL thickness measurements obtained from the first scan (without correction or adjustment of the actual projected scan radius by the examiner) showed a significant inverse correlation with axial length (correlation coefficient R = -0.54, R^2 = 0.29, P = 0.001). In other words, if no final correction (adjustment) of the actual projected scan radius was made by the examiner, thinner RNFL thickness measurements would be found for the longer eyes and thicker RNFL thickness measurements for the shorter ones. No statistically significant correlation was found between RNFL thickness measurements with axial length if appropriate correction of the actual projected scan radius was made (correlation coefficient R = -0.21, R^2 = 0.04. P = 0.11).

Influence of Scan Radius Change on RNFL Thickness Measurements and RNFLT Estimated Integral Areas

Average of RNFL thickness measurements obtained from the first, second, third, and fourth scans were shown in Table 2. The influence of the scan radius change on

TABLE 2. *Average (RNFLT) measurements obtained in four consecutive seans using different radii*

Radius	Average RNFLT (xm)	Range (xm)
Variable (average, 1.68 mm; range, 1.51-1.87 mm)	98.58 ± 15.56	63-124
1.60 mm	103.29 ±9.38	85-123
1.73 mm	95.09 ± 9.06	77-113
1.85 mm	88.60 ±9.05	69-102

the RNFL thickness measurements was statistically significant by a repeated-measures analysis of variance *(P <* t).0001). It could be easily seen that average RNFL thickness decreased as the scan radius increased. A strong and statistically significant correlation vvas found betvveen RNFL thickness and 1/scan radius (correlation coefficient R = 0.411, R* = 0.169, *P* < 0.0001). The average $RNFLT_{eslimated\ inlegral}$ areas for the second, third, and fourth seans are shovvn in Table 3. Those three $RNFLT_{estimated\ integral}$ areas were close to each other, and no statistically significant difference betvveen those vvas found by a repeated-measures analysis of variance *(P* - 0.521).

DISCUSSION

Although OCT technology allows high-quality cross-sectionai images of the retina in vivo, quantitative measurement of RNFL thickness and contour stili pose some technical difficulties. Some researchers have pointed out that OCT seans vvere closely matched vvith retinal histology, vvhereas others have failed to show such a correspondence.[1-3,11-14] Also, despite the increasing use of OCT for detection of new glaucoma cases and follow-up of established cases, disproportionate results betvveen OCT and other structural tests, such as red-free nerve fiber layer photography, nerve fiber analyzer (NFA), and Heidelberg Retina Tomograph (HRT), could occasionally.be detected in some instances.[8]

The following issues could be suggested as potential sources of the discrepancy betvveen RNFL thickness measurements obtained by OCT vvith those found in histologic measurements in the same retinal locations: inadequate resolution power and light source,[15] limited number of test points,[5,15] directional reflectance property of RNFL[15-17] (deviation from perpendicular orientation of only 8° vvould cause RNFL signal to decrease by a factor of 10), instrument's softvvare which delineates the borders of RNFL and calculating RNFL thickness,[15] inadequate pupillary dilation, media opacities, ocular magnification,[18] natural variability of axon count, inadequate histologic data on topographic anatomy of human RNFL,[13,14] and patient age. Because of these difficulties, normal databases could not be developed. Because the causes are diverse, solutions must be different for each of these technieal issues. Resolution povver, light source, number of test points, and softvvare improvements have been recently addressed in a second-generation OCT device.[19]

TABLE-3. *Average* $(RNFLT_{estimated\ integral})$ *values for the repeated seans*

Radius	Average $(RNFLT_{estimated\ integral})$ (xm2)	Range (xm2)
1.60 mm or 1,6(X) u.m	1,038,020 ±94,232	854,250-1,236,150
1.73 mm or 1,730 u.m	1,034,2<X)± 99,700	836,990-1,228,310
1.85 mm or 1,850 jj.m	1,030,300 ± 105.260	801,780-1,185.240

More clinical studies are needed to understand the influence of pupil size, media opacities, and patient age on RNFL thickness measurements, and histologic studies are necessary for improving our understanding of normal topographical anatomy of the RNFL and axon count of patients vvith and without glaucoma. Conversely, improving the accuracy of RNFL thickness measurements can also be possible vvithout any need for modifying the softvvare or hardware of the currently available instrument by simply taking the measurements along identical cireles in every circumstance and trying to keep the actual scan parameters constant. To our knowledge, there has been no published study investigating the patient-related factors influencing the actual scan size projected onto the peripapillary RNFL by the OCT instrument. The effects of magnification during optic nerve head topographic measurements vvere published, and appropriate softvvare for its correction vvas integrated into optic nerve head analysers such as HRT.[20,21] In a study in vvhich various methods for correction of ocular magnification vvere compared, it vvas found that axial length vvas the most important parameter, and keratometry and refraetive error vvere not found to be as accurate as axial length.[20] In another study, the magnification characteristics of various fundus imaging systems vvere investigated and the researehers found that there vvere tvvo different groups of instruments. In most of them, the magnification vvas found to depend only on the axial length (telecentric systems), vvhereas the magnification in the remaining instruments vvas found to be somewhat influenced by the refraetive error also (nontelecentric systems).[22] In the telecentric systems, any emergent ray of light from the fundus becomes parallel to the optical axis after refraction by the camera's imaging system.[22]

During an OCT examination, the selected preset scan radius is automatically modified by the instrument's softvvare and the calculated actual scan radius is displayed on the monitor. This modification of the preset scan radius is assumed to be performed to overcome the magnification produced by the patient's eye. However, the automalic correction of ocular magnification by the OCT instrument does not mean that adjustment of the scan radius has been completed. A final correction should be made to reach the desired scan parameters because the actual scan radius has been already changed by the instrument. The OCT instrument does not automatically make that final correction and the examiner herself must make it by using a control knob, not by changing the preset parameters.

In the current study, the first investigated issue was how the OCT instrument could calculate the ocular magnification and modify the preset scan parameters. To answer this, we used statistical methods to find out the influence of some patient-related parameters, such as axial length and refractive error, because no information was given by the manufacturer in the instruction manual. It was found that axial length was the primary determinant of the actual scan size projected on the RNFL, whereas the influence of refractive error was small and could be negligible. It was found that for each 1-mm increase in axial length, the actual projected scan radius increased approximately 0.06 mm or 3.5% (e.g., from 1.73 to 1.79 mm). Whereas large changes in refractive error, for example ± 10 D would result in only a 0.03-mm (±2%) change in actual scan diameter. Because the magnification of the OCT instrument highly depended on axial length, it could be classified as a telecentric imaging device.

In our study, RNFL thickness measurements that were obtained after final correction made by the examiner were found to be statistically different from those found by merely entering the preset scan radius without correction of the scan radius by the examiner. If final correction was not performed by the examiner, the RNFL thickness would be found to be thinner than it was in long eyes, probably because scans were generated at much longer distances from the optic disc, and thicker in short eyes because of the proximity of the scanning circles to the optic disc. The amount of measurement error was found to be statistically correlated with axial length data. This will ultimately lead to increased incidence of glaucoma diagnosis (because of a thinner RNFL) in myopic eyes with longer axiallength and decreased incidence (because of a thicker RNFL) in hyperopia patients with short axial lengths during OCT examinations. This is an important finding and implies that final correction of the actual projected scan radius, already modified by the instrument, should be done in every patient and especially in the studies investigating the relationship of RNFL thickness measurements with axial length or refractive error. Although not investigated in the current study, estimates of sensitivity, specificity, and areas under the receiver operating characteristic (ROC) curve for differentiating between eyes with and without glaucoma may be improved by obtaining RNFL thickness measurements after appropriate correction of actual scan radius.

The optimal scan radius or circumference along which RNFL thickness measurements should be performed has not been adequately investigated and is still unknown. Schumann et al.[2] recommended the use of a 3.45-mm circumference because they pointed out that it was associated with better reproducibility. In our study, RNFL thickness was found to become thinner as the scan radius and circumference were increased. This was evident also after final correction of the actual scan radius by the examiner. A reciprocal relationship was found between the RNFLT and scan radius. RNFL thickness measurements were found to be proportional to I/scan radius. This was not an unexpected result because it was assumed that there was no change in the total number of nerve fibers in the peripapillary region and that the RNFL spread uniformly in each direction. Because of the dependence of RNFL thickness measurements to the scan radius and thus circumference as shown in the current study, separate nomograms must be developed for each particular scan radius or other measures independent of and not influenced by the scan radius must be found.

Retinal nerve fiber layer total area could be such a parameter. It was assumed to give a more meaningful estimation of the total amount of retinal nerve fiber bundles, whereas RNFL thickness showed only the density. We knew from histologic studies that RNFL thickness and density decrease as the distance from optic nerve head increases.[13,14] Also, especially in cases of high myopia and tilted discs, RNFL thickness may show localized differences that do not usually imply an abnormality and RNFL total area can be in the normal range.

In the current study, RNFL total area could be only roughly estimated by multiplying the average RNFL thickness by its corresponding scan diameter along which it was obtained. Because it was only an estimation, we termed it $RNFL_{estimated\ integrals}$ Although we used $RNFL_{estimated\ integrals}$ instead of the true RNFL total area, we found that three different $RNFL_{estimated\ integrals}$ calculated for three consecutive scans (by using different scan radii) taken from the same eye were found to be statistically similar. Because $RNFL_{estimated\ integrals}$ were found to be independent of the scan radius and were relatively constant for a particular eye, it was concluded that their use as a measure of total number of nerve fiber bundles seemed to be justified. Precise calculation of RNFLA automatically vvith a special softvvare integrated into the OCT instrument wiil be more ideal and undoubtedly will improve our estimation of calculating the total number of surviving RNFL bundles and is thus strongly recommended.

In conclusion, we think that it vvould be possible to increase the accuracy and precision of RNFL thickness measurements vvith the current OCT instrument and softvvare by simply trying to keep the scan parameters constant in all patients. In order to get rid of the magnification produced by the patient's eye, readjustment of the already OCT instrument-modified scan parameters by the examiner is found to be necessary. Also, the use of other measures independent of scan size, such as $RNFL_{cstimated\ integrals}$ is also recommended because RNFL thickness measurements vvere found to be largely dependent on scan radius.

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