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Custom measurement of retinal nerve fiber layer thickness using STRATUS OCT in normal eyes

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PURPOSE. To evaluate variability of retinal nerve fiber layer (RNFL) thickness measurements in normal eyes and their correlation with optic disc diameter by using two different scan options of the ultimate commercial optical coherence tomography (OCT) unit (STRATUS OCT™, Carl Zeiss Meditec, Inc., Dublin, CA).

METHODS. In this observational case series and instrument validation study 30 eyes of 30 normal subjects were enrolled. Each eye underwent optic disc vertical diameter measurement by means of both stereoscopic photography and planimetry and OCT; RNFL thickness measurements were performed using OCT. Three repetitions of two series of scans were performed. Each eye was scanned at two different options (RNFL thickness 3.4 and Nerve Head Circle). For each option descriptive statistics, analysis of variance, intraclass correlation coefficients (ICCs), and coefficients of variation (COVs) were calculated. To verify the correlation between the two methods of optic disc diameter assessment and to study the influence of optic disc diameter on RNFL measurement using the two different OCT options, Pearson's correlation coefficients were calculated.

RESULTS. Optic disc diameter length ranged from 1.47 to 2.04 mm (mean 1.709 mm, SD ± 0.147) with stereoscopic photographs, and from 1.47 to 2.02 mm (mean 1.703 mm, SD ± 0.143) with OCT (Pearson correlation coefficient 0.999, p<0.001). Mean RNFL thickness was 89.29 mm (SD ± 10.80 mm) using the RNFL thickness 3.4 scanning option and 89.88 mm (SD ± 1.72 mm) using the Nerve Head Circle protocol (Pearson correlation coefficient 0.065, p=0.734). The intersubject variance is higher using the RNFL thickness 3.4 option than using the NHC protocol (sum of square: 10147,60 vs. 257,41) (p<0.001); the intrasubject variance is very similar in the two groups (23,72 vs 23,60) (p=NS). The ICC is 99.89% when using the RNFL thickness 3.4 option, 95.62% with the NHC protocol (p=NS). COVs were 12.10% and 1.91% by using RNFL thickness 3.4 and Nerve Head Circle option, respectively. Pearson's correlation coefficient was 0.988 (p<0.001) when comparing optic disc diameter and RNFL thickness by using the RNFL thickness 3.4 option and -0.016 (p=0.932) when comparing optic disc diameter and RNFL thickness by using the Nerve Head Circle option.

CONCLUSIONS. These results suggest that both scan options give good RNFL thickness measurement reproducibility; the use of the Nerve Head Circle option leads to less interindividual variability and can minimize the effect of differences in optic disc diameter on RNFL thickness measurements in normal subjects. (Eur J Ophthalmol 2005; 15: 360-6)

Key Words. Optical coherence tomography, Optic disc, Retinal nerve fiber layer

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INTRODUCTION

Optical coherence tomography (OCT™) is a noncontact and noninvasive technology that allows cross-sectional imaging of the human retina using light. OCT™ allows direct measurement of retinal nerve fiber layer (RNFL) thickness by in vivo visualization of cross-sectional images of the retina and RNFL at histologic levels of resolution (approximately 10 µm) (1).

The results of our recent study of reliability of RNFL measurement using OCT™ programs for nerve head study state that the greatest amount of variability can be attributed to intersubject differences (2).

The recently developed Optical Coherence Tomographer (STRATUS OCT™, Carl Zeiss Meditec, Inc., Dublin, CA) gives the ophthalmologist the opportunity to customize scans and to tailor a single scan circle to examine RNFL thickness.

Custom scans can be useful to minimize interindividual variability due to different optic disc sizes and to develop a database of RNFL thickness, stratified by age, which helps the ophthalmologist to discriminate normal from early glaucomatous peripapillary RNFL (2).

In this study, we used the Stratus OCT™ to determine differences in variability of measurements and to show correlation between optic disc diameter and RNFL thickness measurements using two different scan protocols in normal eyes.

METHODS

We examined one randomly chosen eye of 30 healthy subjects (15 men, 15 women, 20 to 40 years of age, mean age 29.4 ± 2.5 years). The study was in adherence to the tenets of the Declaration of Helsinki, and informed consent was obtained from all patients.

Each subject received complete ophthalmologic evaluation, including medical, ocular, and family history; visual acuity testing with refraction; Humphrey Field Analyzer (Humphrey-Zeiss Systems, Dublin, CA) 30-2 full threshold standard achromatic perimetry; intraocular pressure (IOP) measurement; dilated slit-lamp stereo biomicroscopy; indirect ophthalmoscopy; and stereoscopic color optic nerve head photography and planimetry.

Criteria used to identify normal subjects were no history or evidence of glaucoma, retinal pathology, intraocular surgery, or laser therapy; best-corrected visual acuity (BCVA) of 20/40 or better; IOPs of 21 mmHg or lower; open angles by gonioscopy; no obvious pathology of the optic nerve or RNFL by stereoscopic slit-lamp biomicroscopy; absence of asymmetric ONH cupping (difference in vertical cup/disk ratio greater than 0.2 between the eyes in the presence of similar optic disk size); absence of increased cupping (vertical cup/disk ratio > 0.6) by stereoscopic optic nerve head photography; and normal Humphrey 30-2 visual field. Normal visual field test results were defined as those having no cluster of three or more adjacent points depressed more than 5 dB or of two adjacent points depressed more than 10 dB. Visual field criteria were based on the pattern deviation threshold values. Subjects were ineligible for inclusion into the study in case of any corneal, lens, or vitreous body abnormalities that could interfere with OCT™ image acquisition and peripapillary atrophy or tilted disk that could interfere with appropriate scan alignment.

Optic disc photography and planimetry

All participants had standard nonsimultaneous stereoscopic color ONH photographs at the same visit as the OCT™ imaging. All stereoscopic color fundus photographs of the optic nerve head were taken by the same trained ophthalmic photographer. Subjects’ pupils were dilated with 1% tropicamide. Photographs of the optic disc were taken using a Canon CF-60UVi fundus camera (Canon Inc., Japan) at the 30 degree setting, captured on 35-mm Kodak Ektachrome EPR 150 film (Eastman Kodak, Rochester, NY). Four sequential photographs of each eye were taken, with a lateral shift in camera position after two pictures, to obtain a stereo effect when the images are viewed stereoscopically.

The camera is not of a telecentric design, and the camera magnification for differing degrees of ametropia was calculated by photographing a target of known dimensions in a model eye set at varying degrees of ametropia (3). The disc slides were projected in a scale of 1 to 15. The outlines of the optic disc were plotted on paper and were analyzed morphometrically. To obtain values in absolute size units, that is, millimeter, keratometry readings, taken with a calibrated Javal-Schiotz keratometer, spectacle refraction, and ultrasound biometry, were used in order to correct for ocular magnification using the abbreviated axial length method (BRE2) (4). The border of the optic disc was identical with the inner side of the peripapillary scleral ring. The optic cup was defined on the basis of contour and not of pallor (5).

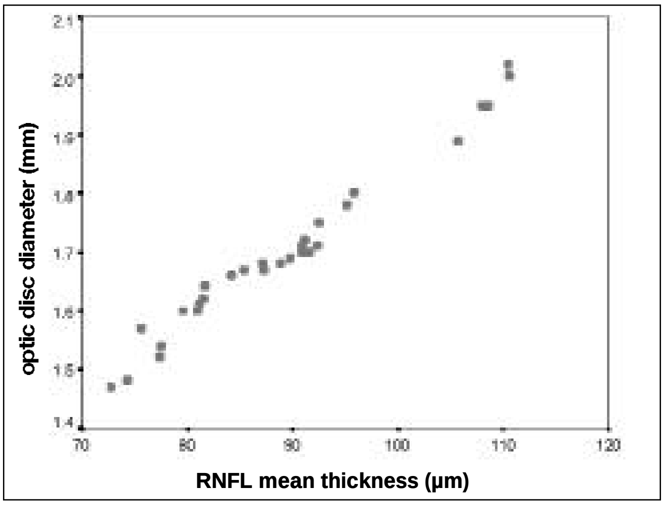

Fig. 1 - Scatterplot showing correlation between optic disc diameter and RNFL thickness as measured by means of RNFL thickness 3.4 protocol. A high correlation between the two variables is evident (Pearson's correlation coefficient = 0.988; p<0.001).

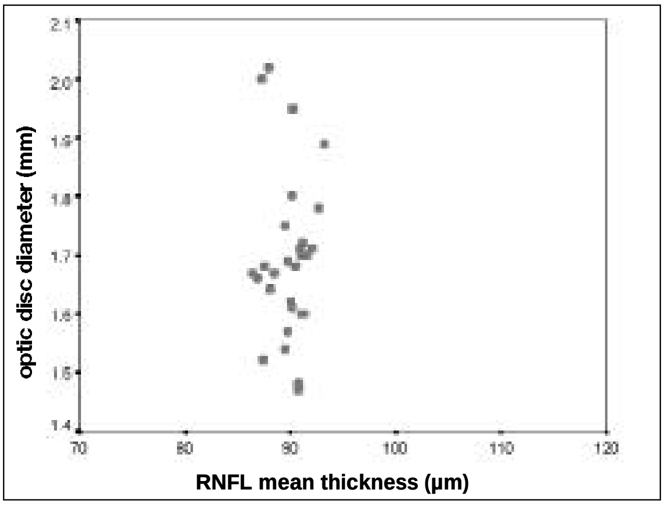

Fig. 2 - Scatterplot showing correlation between optic disc diameter and RNFL thickness as measured by means of Nerve Head Circle protocol. No correlation between the two variables is identifiable (Pearson's correlation coefficient = -0.016; p<0.932).

Optical coherence tomography technique

OCT™ was performed with a new generation Optical Coherence Tomographer (STRATUS OCT™, Carl Zeiss Meditec, Dublin, CA). Our sample was examined by using two different nerve head options (RNFL thickness 3.4 and Nerve Head Circle).

These two options result in similar outcome with the exception of the radius size. When one of the nerve head options is selected, an aiming circle is shown on the monitor. Both the position and the size of the aiming circle can be modified by the examiner. The operator has a video camera view of the scanning probe beam on the fundus and a computer monitor showing the acquired OCT™ image in real time. The examiner has to center the aiming circle on the optic nerve head (ONH) while the subject looks fixedly with the eye that is being studied (internal fixation technique). The centering technique depends on the examiner's ability to perform fine positioning of the aiming circle.

The nerve head programs perform a circular scan around the optic nerve head. Using the RNFL Thickness 3.4 option, the aiming circle diameter length is 1.7 mm and the scan diameter length is fixed at 3.4 mm. Using the Nerve Head Circle protocol, the aiming circle diameter has to be adjusted by the operator in order to measure the length of the optic disc vertical diameter. The aiming circle has to be modified in side and placed by the operator on the inner margin of the peripapillary scleral ring. When the shape of the optic disc does not conform perfectly to the aiming circle, only the superior and inferior margin have to be considered, in order to obtain the vertical diameter. At this point the length of the optic disc diameter can be read on the monitor.

Then, the scanning circle diameter can be adjusted by enlarging the aiming circle (optic disc) diameter by the desired value, which represents the distance from the optic disc edge to the scanned peripapillary retina. Theoretically, this permits performing RNFL thickness measurements in each subject at a prefixed distance from the optic disc edge, independently of the optic disc diameter.

In this study, when using the Nerve Head Circle option we performed RNFL thickness measurements 0.85 mm from the optic disc edge.

Before OCT™ examination, axial length measurement was performed with a calibrated I^3 system ABD (Innovative Imaging Inc., Sacramento, CA) using a tonometer mounted, hard tipped probe, taking the mean of five high quality readings for each subject. Refractive correction (spherical equivalent) and axial length values were entered in the OCT™ Edit Patient dialog box. As specified in the STRATUS OCT™ user manual, "This is for record-keeping only. It has no impact on the angle and magnification of scan patterns when projected into the eye during scan-

TABLE I - OPTIC DISC DIAMETER AND MEAN NERVE FIBER LAYER THICKNESS BY NERVE HEAD SCAN OPTION*

Subject no.	Optic disc diameter (mm)		Mean RNFL thickness (μm) option	
	Stereoscopic photography and planimetry	OCT™	RNFL thickness (3.4)	Nerve Head Circle
1	1.71	1.70	90.92	91.00
2	1.68	1.68	88.83	90.50
3	1.72	1.72	91.08	91.17
4	1.76	1.75	92.42	89.42
5	1.90	1.89	105.65	93.17
6	2.04	2.02	110.50	87.92
7	2.02	2.00	110.60	87.33
8	1.96	1.95	108.58	90.17
9	1.54	1.54	77.50	89.50
10	1.48	1.48	74.27	90.78
11	1.60	1.60	80.93	91.21
12	1.67	1.66	84.17	86.83
13	1.52	1.52	77.30	87.46
14	1.47	1.47	72.67	90.80
15	1.72	1.71	90.83	90.90
16	1.70	1.70	91.50	91.50
17	1.96	1.95	108.00	90.09
18	1.70	1.70	91.00	91.00
19	1.71	1.71	92.33	92.08
20	1.70	1.69	89.67	89.74
21	1.67	1.67	87.33	88.42
22	1.68	1.67	85.33	86.33
23	1.68	1.68	87.17	87.58
24	1.79	1.78	95.17	92.63
25	1.81	1.80	95.75	90.05
26	1.58	1.57	75.50	89.67
27	1.62	1.61	81.08	90.14
28	1.62	1.62	81.42	89.93
29	1.61	1.60	79.60	91.06
30	1.65	1.64	81.63	88.03
Mean ± SD	**1.709 ± 0.147†**	**1.702 ± 0.143†**	**89.29 ±10.80‡**	**89.88 ± 1.72‡**

*Based on 90 measurements of 30 eyes for each option.
†Pearson's correlation coefficient 0.999, p<0.001.
‡Pearson's correlation coefficient 0.065, p=0.734.
RNFL = Retinal nerve fiber layer; OCT™ = Optical coherence tomography

TABLE II - RESULTS OF ANALYSIS OF VARIANCE, INTRACLASS CORRELATION COEFFICIENT, AND COEFFICIENT OF VARIATION BETWEEN THE TWO SCAN OPTIONS

Scan option	Analysis of variance Sum of square	Intrasubject variance	ICC (%)	COV (%)
RNFL thickness 3.4	10147.60	23.72	99.89	12.10
Nerve Head Circle	257.41 p<0.001	23.60 p=NS	95.62 p=NS	1.91

ICC = Intraclass correlation coefficient
COV = Coefficient of variation
NS= Not significant

ning" (STRATUS OCT™ user manual, Carl Zeiss Meditec Inc., Dubin, CA, 2002, chap 2, page 6).

Each eye of each subject was dilated with 1% tropicamide before recording the images. Only one randomly chosen eye of each individual underwent OCT™ measurements of RNFL thickness. Each eye was scanned at two different options (RNFL thickness 3.4 and Nerve Head Circle) in a random order to prevent bias by fatigue effect.

Three repetitions of two series of scans were performed. All scans were performed by the same experienced examiner using near infrared illumination (840 nn) to minimize patient discomfort.

The quality of the scans was assessed prior to the analysis and poor quality scans were rejected. An experienced examiner evaluated each scan individually to ensure adequate technical quality for analysis. Only good quality OCT™ data as judged by the appearance of the NFL pictures were used for further analysis. Images with artifacts, missing parts, or showing seemingly distorted anatomy were excluded. A signal-to-noise ratio (SNR) higher than 35 and 100% quality A-scan were chosen as quality assurance cut-off.

Three series of good quality scans for each option were obtained. For each option the thickness was determined by averaging the three measurements. Mean RNFL thickness measurements, based on three individual scans, were used in the analysis. For each scan, the global RNFL thickness was determined from 768 points around the disc.

Statistical analysis

All data were analyzed using the statistical package of SPSS 10.0 for Windows (SPSS Inc., Chicago, IL).

We examined both left and right eyes; left eyes were considered as mirror images of right eyes. Descriptive statistics were analyzed for each option (RNFL Thickness 3.4 and Nerve Head Circle). The coefficient of variation (COV = 100 x [standard deviation/mean] %) was used to estimate variability of the two options. To assess measurement reliability analysis of variance and intraclass correlation coefficient were used. To compare optic disc vertical diameter measurements using the two different techniques (planimetry and OCT™) and to verify the influence of optic disc diameter on RNFL measurement using the two different OCT™ options, Pearson's correlation coefficients were calculated.

RESULTS

OCT™ was well tolerated by all subjects. Using the two different options no difference in image quality was detected. Optic disc diameter length ranged from 1.47 to 2.04 mm (mean 1.709 mm, SD ± 0.147) when evaluated with stereoscopic photographs, and from 1.47 to 2.02 mm (mean 1.703 mm, SD ± 0.143) when measured by OCT™ (Pearson correlation coefficient 0.999, p<0.001). Mean RNFL thickness was 89.29 mm (SD ± 10.80 mm) using the RNFL thickness 3.4 scanning option and 89.88 mm (SD ± 1.72 mm) using the Nerve Head Circle protocol (Pearson correlation coefficient 0.065, p=0.734) (Tab. I). The analysis of variance shows that the intersubject variance is higher using the RNFL thickness 3.4 option than using the NHC protocol (sum of square: 10147,60 vs 257,41) (p<0.001); the intrasubject variance is very similar in the two groups (23,72 vs 23,60) (p= not significant). The intraclass correlation coefficient is 99.89% when using the RNFL thickness 3.4 option, 95.62% with the NHC protocol (p= not significant). Coefficients of variation were 12.10% and 1.91% by using RNFL thickness 3.4 and Nerve Head Circle option, respectively (Tab. II). Pearson's correlation coefficient was 0.988 (p<0.01) when comparing optic disc diameter and RNFL thickness by using the RNFL thickness 3.4 option and -0.016 (p=0.932) when comparing optic disc diameter and RNFL thickness by using the Nerve Head Circle option. Figures 1 and 2 show correlations between the optic disc diameter and RNFL thickness measurements performed by means of each of the two protocols.

DISCUSSION

OCT™ is a high resolution technique that can create cross-sectional images of the NFL. OCT™ is the optical analog of ultrasound B-scan, providing images with much higher resolution in both the axial and lateral dimensions (8 to10 µm and 20 µm, respectively, using the STRATUS OCT™) (6). No reference plane is required to calculate NFL thickness because OCT™ provides an absolute cross-sectional measurement of retinal substructure, from which the NFL is calculated (1). OCT™ has been shown to quantitatively correlate with visual field and NFL appearance (7). In addition, OCT™ is able to reproducibly identify diffuse as well as focal RNFL defects that can occur in glaucoma (8).

In our previous study of reliability of RNFL thickness OCT™ measurements (2) we hypothesized that RNFL thickness measurements could be largely influenced by interindividual variability of the absolute size of the optic nerve head. Therefore, we suggested to verify the use of scanning circles whose radius was longer than the aiming circle radius (r) following the formula R = r+x (x is the number of millimeters), in order to measure NFL thickness at the same distance from the optic disc edge, independently of optic disc size.

In this study we performed optic disc size measurements using both optic disc stereophotography and planimetry, corrected for ocular and camera magnification, and OCT™. Planimetry using a stereoscopic viewing system permits reliable evaluation of optic disc vertical diameter. High levels of agreement for planimetry have been described elsewhere (6, 9-11).

To correct the magnification of images that result from the optics of the eye we used the abbreviated axial length method (BRE2) (4) that differs little from the more detailed calculations that use keratometry, ametropia, ACD, and lens thickness in addition (method BRE1) (4), and is appreciably more accurate than the methods that use keratometry and ametropia alone (12). The use of spherical error to correct for magnification, although an accepted methodology, is generally less accurate than formulae that use axial length. The consequence of using spherical error is that the vertical diameter of the larger discs can be underestimated whereas the vertical diameter of smaller discs can be overestimated (12).

It is clear however that different imaging methods may provide different estimates of optic disc size. The potential differences in estimated disc size derived from different examination methods should be taken into consideration when transferring data generated in this study to clinical practice or other population studies.

The ultimate OCT™ device (STRATUS OCT™) gives the opportunity to measure RNFL thickness using an unalterable scanning diameter of 3.4 mm or building custom scans around the optic disc adjusting for the optic disc diameter. The latter option can be thought to minimize the importance of different disc sizes in larger scale studies on both normal and glaucomatous eyes.

In this study, when using the Nerve Head Circle option, RNFL thickness measurements were performed 0.85 mm from the optic disc edge. In this way, one may suppose that only subjects having their nerve head diameter different from 1.7 mm will have different measurements using the two different options.

This study was designed to verify the hypothesis that a custom program for RNFL thickness measurement could provide less variable results than the traditional option (3.4 mm) that uses a fixed scan circle, independently of the optic disc diameter. We also wished to determine whether the differences between RNFL thickness measurements obtained with the two options were linked to differences in optic disc diameter.

We found that both scan options resulted in similar mean RNFL thickness measurements and that most of the optic disc diameter measurements are quite close to 1.70 mm., but, as shown in Table I, all subjects whose optic disk diameter differs more largely from the average measure show evident difference in RNFL measurements using the two different options (Cases 5 to 11, 13, 14, 17, 26, 29). This finding reflects the well-known inverse relationship between the distance from the disc margin and RNFL thickness (2, 13).

Both scan options showed high measurement reproducibility. Variability of measurements was significantly smaller when using the Nerve Head Circle option that permits obtaining RNFL thickness measurements at a fixed distance from the optic disc margin, independently of the optic disc size. Using the RNFL thickness (3.4) option the NFL is measured closer to disc border in large discs than in small and crowded ones (2). This leads to a larger intersubject variability than when using a custom option that permits measurement of RNFL thickness always at the same distance from the optic disc edge.

In this study, the standard deviation of measurements obtained with the STRATUS OCT™ device was 1 to 10 mm for overall RNFL thickness, thus confirming previous studies (2, 6-8, 13-18).

Mean RNFL thickness measurements obtained in this study both with the traditional option or with the custom program were quite different from those obtained by investigators who used previous OCT™ devices, both prototype or commercially available OCT™ units (OCT™ version 1 and 2) (2, 6-8, 13-18). In particular, the results of our RNFL thickness measurements were smaller than those obtained by Jones et al (15), who examined 15 normal subjects with a mean age of 30.8 years (SD ± 10.9 years) and found a mean RNFL thickness of 127.87 ± 9.81 mm using a 1.74 mm radius scan of the previous OCT™ version (Humphrey OCT™ Model 2000). Less recent studies (7, 16), in which a prototype or the first commercial OCT™ device (OCT™ version 1) were used, also showed mean RNFL measurements thicker than those obtained by STRATUS OCT™.

Although further studies may be needed to confirm our suppositions, some interesting points emerged from our study. The STRATUS OCT™ gives us the opportunity to customize scans to measure RNFL thickness at the same distance from the optic disc edge in every subject, independently of the optic disc diameter. In presence of small or large optic discs the use of the traditional option (RNFL thickness 3.4) is likely to introduce a larger amount of variability in RNFL thickness measurements, only partially linked to a real interindividual difference. Therefore, one may think that using custom scans less variable results can be obtained. The high correlation between optic disc diameter and RNFL thickness measurements obtained using the 3.4 option confirms our hypothesis. Finally, if the results of our investigation are confirmed by further large scale studies, this will lead to the need for a new normative database of NFL thickness, stratified not only by age but also by optic disc diameter, which helps the ophthalmologist to discriminate normal from early glaucomatous peripapillary NFL.

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