Reproducibility of Nerve Fiber Layer Thickness Measurements by Use of Optical Coherence Tomography

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Objective: To evaluate the reproducibility of optical coherence tomograph (OCT) retinal nerve fiber layer (RNFL) measurements in normal and glaucomatous eyes by means of the commercially available OCT 2000 instrument (Humphrey Systems, Dublin, CA).

Design: Prospective instrument validation study.

Participants: One eye each from 10 normal subjects and 10 glaucoma patients.

Methods: Twenty subjects underwent a total of eight scanning sessions during two independent visits. In each session, five circular scans centered on the optic nerve head were performed. The first two sessions were performed by two experienced technicians. Followed by a 30-minute break, a third and a fourth session was completed by the same technicians. This sequence was duplicated on a second visit. Intrasession, intersession, intervisit, and interoperator reproducibility of quadrant and global RNFL measurements were calculated by use of a components of variance model.

Main Outcome Measures: RNFL thickness.

Results: The coefficient of variation for the mean RNFL thickness was significantly smaller ($P = 0.02$) in normal eyes (6.9%) than in glaucoma eyes (11.8%). The estimated root mean squared error based on the statistical model using three scans per patient was 5.8 and 8.0 μm for normal and glaucoma eyes, respectively. A components of variance model showed most of the variance (79%) to be due to differences between patients. Only a modest contribution to variability was found for session (1%), visit (5%), and operator (2%).

Conclusion: With the commercially available OCT, our results indicate that the RNFL measurements are reproducible for both normal and glaucomatous eyes. *Ophthalmology 2000;107:2278–2282 © 2000 by the American Academy of Ophthalmology.*

By use of retinal nerve fiber layer (RNFL) photographs, glaucomatous damage to the RNFL has been shown to precede functional loss by as much as 5 years.[1,2] Although simple and inexpensive, RNFL photographs require a clear media, a dilated pupil, a darkly pigmented fundus, a trained photographer, and, most important, an experienced observer. More so, RNFL photograph assessment is highly subjective, and diffuse RNFL loss in some eyes with glaucoma is particularly difficult to assess.

The optical coherence tomograph (OCT)[3–5] is one of several techniques for real-time quantitative and objective evaluation of the RNFL. OCT is a noninvasive, noncontact method that allows cross-sectional in vivo imaging of intraretinal layers.

OCT was initially developed to assess tissue morphology and thickness in vivo. This technology was initially designed for fiberoptic use.[6] Early use of this technology involved imaging of eyes and coronary arteries.[7] OCT imaging can detect and measure changes in tissue thickness with micron-scale sensitivity.[7,8] The anatomic layers within the retina can be imaged, and quantitative assessment of retinal and RNFL thickness is possible.[9] With a prototype instrument, OCT data were reported to correlate well with known properties of the human retina.[9] This technology has been applied to the evaluation of several ophthalmic conditions including macular holes,[10] diabetic retinopathy,[11] epiretinal membranes,[12] and glaucoma.[3–5] OCT was able to detect induced RNFL lesions in macaque monkeys.[13] Visual field loss and RNFL thickness, as measured in vivo by an OCT prototype, were well correlated in glaucomatous eyes.[14]

Previous studies have shown reproducibility of retinal thickness measurements in normal eyes[3,15] and glaucoma eyes[3] with an OCT prototype. In one study[15] a mean coefficient of variation in retinal thickness of less than 10% was found at locations 500 μm or more from fixation. Schuman et al[3] demonstrated adequate reproducibility in normal and glaucoma eyes with this early, noncommercial, prototype of the OCT. The intersubject standard deviations found for a 3.4-mm diameter scan, using internal fixation, were 13 and 21 μm, respectively, for normal and glaucoma eyes. Compared with the commercially available instrument, this earlier investigational prototype necessitated a much longer acquisition time (2.5 seconds as opposed to 1 second) and used a fiberoptic delivery system coupled to a slit lamp.

The goal of this study is to determine the reproducibility of RNFL measurements obtained with the first commercially available OCT (Humphrey-Zeiss Systems, Dublin, CA). An automated algorithm for edge detection and computing RNFL thickness, as well as a shorter acquisition time, characterize this device. We evaluated the intrasession, intersession, intervisit, and interoperator reproducibility for eyes of normal subjects and glaucoma patients.

Patients and Methods

Subjects

We examined one randomly selected eye from each of 20 subjects (10 glaucoma and 10 normal subjects). Mean age ($\pm$ standard deviation) was 44 $\pm$ 19 years and 67 $\pm$ 11 years for the normal and glaucoma groups, respectively. All subjects (both normal and glaucoma) underwent a complete eye examination, including medical and family history; visual acuity testing with refraction; intraocular pressure measurement; Humphrey Field Analyzer (Humphrey-Zeiss Systems, Dublin, CA) 24-2 full threshold standard achromatic perimetry; a complete slit-lamp examination, including gonioscopy; indirect ophthalmoscopy; stereoscopic optic nerve head photography; and nerve fiber layer photography. Informed consent was obtained from all participants, and the study was approved by the University of California, San Diego, Human Subjects Committee.

Inclusion criteria for normal subjects included a best-corrected visual acuity of 20/40 or better; a normal slit-lamp examination, including gonioscopy; normal Humphrey Field Analyzer visual field, as judged by the glaucoma hemifield test; intraocular pressure of 21 mmHg or less; normal appearing optic nerve heads; and no history of ocular surgery or laser treatment.

Glaucoma patients were defined on the basis of having either:

1. An abnormal Humphrey Field Analyzer 24-2 visual field defined as a glaucoma hemifield test outside normal limits, or corrected pattern standard deviation less than 5% of normal limits.
   or
2. Discs were classified as glaucomatous on the basis of a masked examination of stereophotographs by two glaucomatologists trained in reading stereophotographs. Clinical judgment was based on thinning or notching of the neuroretinal rim, asymmetry of cup disc ratio >0.2, excavation of the cup, or an RNFL defect.

Of the 10 glaucoma patients five met both the glaucomatous disc and field criteria, three met the disc but lacked the field criteria, whereas two met the field but lacked the disc criteria.

Exclusion criteria for both groups included a best-corrected visual acuity worse than 20/40, angle abnormalities on gonioscopy, other intraocular eye diseases, other diseases affecting the visual fields (pituitary lesions, demyelinating diseases, diabetes, human immunodeficiency virus positive, or acquired immune deficiency syndrome), or secondary causes of intraocular pressure increase (corticosteroid use, iridocyclitis, trauma) and any pathologic condition (including retinal) that could affect visual fields. A history of intraocular surgery, with the exception of uncomplicated cataract surgery, was considered the basis for exclusion for the normal group. Intraocular pressure was not used as an inclusion/exclusion criterion for the glaucoma group. Visual acuity in the normal group ranged from 20/20 to 20/25, and in the glaucoma group from 20/20 to 20/30.

The OCT Instrument

The OCT assesses retinal infrastructure by analyzing the temporal delay of back-scattered light, using low-coherence interferometry. The technology used by OCT is similar to ultrasonography, except that light rather than sound is used to perform the imaging. Low-coherence light is directed through a beam-splitter resulting in two beams, the signal beam directed at the tissue of interest and the reference beam directed at a reference mirror. Both amplitude and delay of light reflected from tissues are determined by an interferometer by adding the electromagnetic waves of the two reflected light beams. Because of the low coherence of the light source, interference of light reflected from the signal and reference beams occurs only when the delay of the reflections is nearly matched, resulting in high resolution. This instrument has a theoretical axial resolution of approximately 10 μm.[14,16]

In the commercially available OCT, RNFL is differentiated from other retinal layers using a thresholding algorithm.[16] The NFL is assumed to be correlated with the extent of the highly reflective layer at the vitreoretinal interface. Boundaries are located by searching for the first points on each scan where the reflectivities exceed a certain threshold (software version A4X1). Boundaries are thus identified when two thirds of the maximum reflectivity in each smoothed axial scan evaluated on a logarithmic scale is reached. NFL thickness is defined as the number of pixels between the anterior and posterior boundaries of the RNFL.[16]

In circle scan mode, the instrument generates RNFL thickness measurements at 100 points along a 360° circular path, at a preset diameter, resulting in measurement points spread out 3.6° apart. This information is presented graphically on a continuous XY plot, where X is retinal position (i.e., temporal, superior, nasal, inferior) and Y is RNFL thickness in microns. In addition, RNFL thickness is presented on two circular charts, one with 12 equal sectors, each representing 1 hour around the clock face, and the other with measurements in each of four quadrants (Fig 1). These charts display RNFL thickness numerically, in microns, for each region. A single mean RNFL thickness for the entire 360° scan also is displayed.

OCT Measurements

Subjects underwent eight OCT scanning sessions. Five circular scans of 3.4-mm diameter centered on the optic disc, of good quality (as judged by an experienced observer), were obtained and stored for each session. The diameter of the circular scan was fixed at 3.4 mm (1.7 mm radius), on the basis of a previous study performed on the prototype instrument.[3] Schuman et al[3] arrived at this optimal diameter because it is large enough to avoid overlap with the optic nerve head in nearly all eyes and yet allow measurement in an area with thicker RNFL than expected with a 4.5-mm diameter circle. In addition, reproducibility was found to
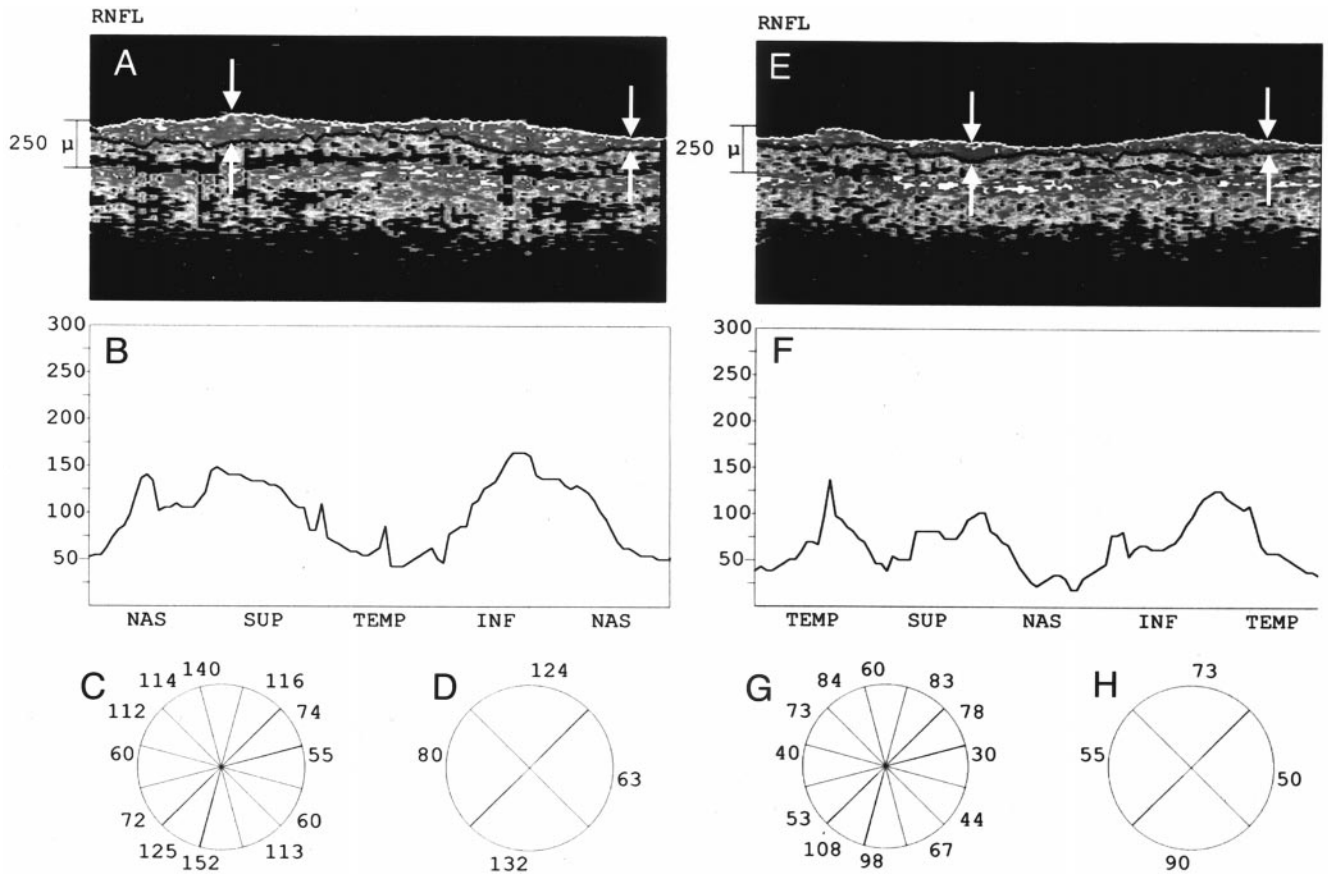
Figure 1. Representative optical coherence tomography (OCT) retinal nerve fiber layer (RNFL) scan results from a normal subject (left) and a glaucoma patient (right). **A, E:** gray scale of OCT cross-sectional retinal image, arrows delineate the RNFL. **B, F:** graphical representation of RNFL thickness along the circular scan. **C, G:** RNFL thickness (μm) in each of 12 equal sectors, each representing 1 hour around the clock face. **D, H:** RNFL thickness (μm) in each of four quadrants.

be significantly better at the 3.4-mm diameter than at the 2.9-mm diameter. Images were judged to be of sufficient quality on the basis of the subjective evaluation of an experienced operator. Within each session, the instrument alignment and controls were not changed, unless as part of the image acquisition process. However, at the end of each session the chin rest, joy-stick, focus, intensity, and contrast controls were randomly changed, so all alignment and fine-tuning adjustments had to be re-initiated at the start of the next session. For each subject, RNFL thickness was assessed in four retinal regions: temporal (316°–45° on unit circle), superior (46°–135°), nasal (136°–225°), and inferior (226°–315°). Average RNFL thickness (0°–359°) was also assessed.

Two experienced technicians performed two consecutive sessions. At the completion of the second session the patient rested for at least 30 minutes. The third and fourth sessions were performed in a similar fashion. Subjects were scanned on two visits, spaced 1 to 4 weeks apart (an average of 8 ± 6 days apart). The examiners' order during the first and second and again during the third and fourth sessions was determined by use of a random number generated table. Subjects received a maximum of 40 scans, in eight sessions, split between two visits. Of the 20 subjects, three subjects missed the second visit and five subjects missed half a visit (participated in six of the eight sessions).

Image acquisition was as follows: pupils were dilated to ≥5 mm; a patch was placed over the nontested eye to improve fixation; artificial tears were used for the tested eye at the onset of the

first and third sessions; and landmark and repeat scan features were used to facilitate placement of the scan circle in the same location. The scanned images consist of 100 A-scans of information along the 3.4-mm scan circle, obtained during 1 second. RNFL thickness is quantified by an automated computer algorithm that identifies the anterior and posterior borders of the RNFL, and the data are summarized by clock hours, quadrants, and overall.[16,17]

Statistical Analysis

A random effects analysis of variance model was used to estimate variance components for patient, operator, visit (nested under patient), session (nested under visit), patient by operator interaction, and residual (scan to scan) variation by means of restricted maximum likelihood. Splus version 3.4 (MathSoft, Inc., Seattle, WA) was used for these calculations and for testing the statistical significance of these effects. Estimated root mean squared errors and coefficient of variation (root mean squared errors divided by RNFL thickness) were both calculated using the components of variance model on the basis of three images for randomly chosen observer, session, and visit. This root mean squared error estimates the standard deviation of the RNFL thickness measurement as it would be obtained in a hypothetical reproducibility study in which each patient was seen in separate visits by a randomly chosen observer for one session using three images.

Table 1. Components of Variance Model for the Mean Retinal Nerve Fiber Layer Thickness Containing the Six Components Listed in the Left Column for the Glaucoma and Normal Groups

Components of the Model	Normal Eyes (n = 10)			Glaucomatous Eyes (n = 10)		
	Variance Components (μm^2)	P Value	% of Variance	Variance Components	P Value	% of Variance
Patient	239.5	<0.001	79	328.9	<0.001	72
Operator	0.0	0.212	0	0.0	0.800	0
Patient by operator	0.0	0.987	0	28.5	0.300	6
Visit	16.7	0.035	6	28.8	0.047	6
Session	3.1	<0.001	1	2.1	0.013	0.5
Residual (scan to scan)	42.4	NA	14	70.1	NA	15
Total	301.7	NA	100	458.4	NA	100

NA = not applicable.

Results

Figure 1 shows typical individual OCT printouts of one normal subject and one glaucoma patient. At the bottom of each scan, RNFL thickness, in micrometers, is presented in both quadrants and clock hours.

Variance components were estimated using the random effects analysis of variance (ANOVA). The components of the model included patient, operator, patient by operator, visit, session, and residuals. The model was tested for each group separately (Table 1). Seventy-nine percent of the variance in the model was attributed to differences between subjects. Five percent and 1% of the variance were attributed to intervisit and intersession variability, respectively. Glaucoma patients were found to be significantly more variable than normal subjects ($P = 0.03$).

The P values in Table 1 test whether the corresponding variance component differs from zero. Thus, $P = 0.013$ for the "between session" component for glaucomatous eyes informs us that the variance component represents statistically significant session-to-session variation, but the estimated value is small, accounting for 0.5% of the total variation.

The estimated root mean squared error for overall RNFL thickness on the basis of the components of variance model, using three scans per patient, were 5.8 and 9.1 μm for normal and glaucoma eyes, respectively (Table 2). The root mean squared error for

Table 2. Estimated Root Mean Squared Errors and Coefficient of Variation Both Calculated Using the Mean of Three Images for Randomly Chosen Observer, Session, and Visit.

Quadrant	Normal Eyes n = 10		Glaucomatous Eyes n = 10	
	RMS Error (microns)	CV	RMS Error (microns)	CV
Temporal	7.12	0.11	9.52	0.17
Superior	8.93	0.09	11.63	0.13
Nasal	11.08	0.18	14.97	0.28
Inferior	8.59	0.08	10.82	0.12
Mean of four quadrants	5.83	0.07	9.10	0.13

Data are presented for each quadrant separately and as the mean of four quadrants.

CV = coefficient of variation; RMS = root mean squared.

normal subjects in the superior and inferior quadrant was 8.9 and 8.6 μm, respectively. The root mean squared error for glaucoma patients in the superior and inferior region was 11.6 and 10.8 μm, respectively. The coefficient of variation for the mean RNFL thickness was significantly smaller ($P = 0.02$) in normal eyes (7%) than glaucoma eyes (13%). The coefficient of variation was larger in the temporal and nasal quadrants than in the superior and inferior quadrants.

Overall, reproducibility for the OCT in a clinical setting showed the root mean squared error of the estimate of mean RNFL thickness of three scans to be 5.8 and 9.1 μm for normal and glaucoma eyes, respectively, and 7.0 μm when both groups were combined.

Discussion

Reproducibility for the OCT showed the standard error of the components of variance model estimate of mean RNFL thickness of three scans to be 7.0 μm. These data are comparable to the published report on the reproducibility of the prototype OCT, with standard deviations of 13 and 21 μm for normal and glaucoma eyes, respectively.[3] Similarly, we found the glaucoma patients to be significantly more variable than the normal subjects. However, age differences between the groups preclude us from being able to attribute this difference solely to the different diagnosis.

Regional reproducibility data (Table 2) show the nasal quadrant to be the least reproducible (highest root mean squared error) and the temporal to be the most in both normal and glaucoma subjects. When incorporating the RNFL thickness into a reproducibility calculation, the coefficient of variation for the superior and inferior quadrants is smaller than for the nasal and temporal quadrants. This is due to the smaller mean RNFL thickness values in the nasal and temporal quadrants.

It is possible that some of the variability encountered may be attributed to the relatively small number of sampled points (100 A scans) in each OCT acquisition. This may be a greater source of variability in glaucoma patients than normal subjects because of the focal nature of some glaucomatous RNFL defects. Gurses-Ozden et al[18] recently demonstrated that increasing the number of A scans per

acquisition fourfold significantly reduced the coefficient of variation in those quadrants with corresponding visual field defects.

A relatively small amount of variability was attributed to intersession (1%) and intervisit (5%) imaging. In addition, only 2% of the variability was attributed to interoperator variability. This is a reassuring finding for a diagnostic instrument, because comparisons of measurements taken for the same patient over a period of time may be compared even when measurements are obtained by different experienced operators.

RNFL measurements obtained in this reproducibility study are consistent with known properties of the RNFL and with patterns observed in glaucoma patients.[4,5] They illustrate known properties of RNFL thickness (Fig 1), with thicker superior and inferior nerve fiber bundles in both normal and glaucomatous eyes.

In summary, the OCT acquired data were in agreement with the known properties of the RNFL. A mixed effects model showed the only two significant components of variability to be patient and residuals, thus implying that intersession, intervisit and interoperator variability combined accounted for less than 10% of total variability. These results indicate that the reproducibility of OCT is adequate for assessing long-term follow-up of patients for progression of glaucomatous optic neuropathy or RNFL damage.

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