We investigated the interoperator reproducibility of peripapillary retinal nerve fiber layer (RNFL) and macular retinal thickness measurements using optical coherence tomography (OCT) in healthy Taiwanese eyes. In this study, OCT-3 was used by three trained and experienced operators to measure peripapillary RNFL and macular retinal thickness in a randomly chosen single eye from each normal subject. Mean thickness levels and the differences in thickness measurements among the three operators were calculated and compared. The eyes of 39 subjects (24 females and 15 males) were enrolled. The mean age of the subjects was 30.4±16.1 years (range, 11–46 years). The mean pupil diameter after pupillary dilation was 7.4±0.6 mm (range, 6–9 mm). Comparing peripapillary RNFL and macular retinal thickness measurements after pupillary dilation, there were no significant differences in: superior, inferior, temporal, and nasal peripapillary areas; 6 mm total macular volume and foveal thickness; and 1, 3 and 6 mm perifoveal areas among the three operators. In this study, OCT thickness measurements showed good interoperator reproducibility among three trained and experienced operators.

**Key Words:** macula, optical coherence tomography, reproducibility, retinal nerve fiber layer

Optical coherence tomography (OCT) allows cross-sectional *in vivo* imaging of intraretinal layers and measures retinal nerve fiber layer (RNFL) thickness by a noninvasive, noncontact, and transpupillary method [1]. It uses interferometry to provide thickness measurements, and is useful in the assessment of a variety of ophthalmic conditions, including glaucoma, diabetic retinopathy, and macular edema [1–6]. Although OCT can help in early diagnosis and follow-up of glaucoma and histologic analysis of macular retinal disease, the measuring variability among operators cannot be neglected. A higher specificity and lower variability by an experienced operator when using OCT would lead the technique to be more reliable with regard to the interpretation of thickness measurements. The purpose of this study was to evaluate interoperator reproducibility of peripapillary RNFL and macular retinal thickness measurements using OCT in healthy Taiwanese eyes.

**Patients and Methods**

Normal subjects with healthy eyes were enrolled in this prospective study. One randomly chosen eye from each subject was scanned by three trained and experienced operators, A, B, and C, on 3 separate
days. The eye was dilated with tropicamide 1% and phenylephrine 2.5% to achieve a minimum pupillary diameter of 6 mm and to obtain the same dilated pupil size in the same subject despite thickness measurements on different days.

Each subject had a best-corrected visual acuity of 16/20 or better, refractive error within 2 diopters of spherical equivalence, no diabetes, no other systemic diseases, no prior surgical history, central corneal thickness of 500–540 μm, intraocular pressure ≤ 20 mmHg (by Goldmann applanation tonometry), a cup-disc (C/D) ratio < 0.4, no asymmetry in C/D ratio between fellow eyes > 0.2, no peripapillary atrophy (e.g. scleral atrophy), and an intact neuroretinal rim without peripapillary hemorrhage, notches, localized pallor, or nerve fiber layer defect.

OCT (OCT-3: software version 4.0, OCT Model 3000, Stratus OCT, Carl Zeiss Meditec, Dublin, CA, USA) was performed using near-infrared, low-coherence illumination (820 nm) with a high tissue resolution of about 10 μm in a dark room. Three 360° circular scans with a diameter of 3.4 mm centered on the optic disc were performed. The peripapillary RNFL scan protocol consisted of three consecutive 360° circular scans with a diameter of 3.4 mm, each of which had 256 A-scans obtained in 0.64 seconds, taking a total time of 1.92 seconds to acquire the entire set of scans. Mean peripapillary RNFL thickness measurements after pupillary dilation were calculated and the whole circle and four sectors were obtained. The superior sector was defined as the arc from 45° to 135° and the inferior sector as from 225° to 315° in both eyes; the temporal sector as from 135° to 225° and 315° to 45°, and the nasal sector as from 315° to 45° and 135° to 225° in the right and left eyes, respectively. A grid was centered on the perifoveal area and divided the macula into nine subfields. The radius of the innermost circle corresponded to one-third of the disc size, approximately 500 μm. The radius of the two outer circles measured 1- and 2-disc diameters, respectively. The macula scan protocol comprised six consecutive 6 mm radial line scans centered on the fovea, each containing 128 A-scans taken in a single session of 1.92 seconds. Mean 6 mm total macular volume, foveal thickness, and perifoveal retinal thickness measurements after pupillary dilation were calculated.

Reproducibility was defined as consistency of measurement during interoperator administration of a test. Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA). Analysis of variance was used to compare the mean peripapillary RNFL and macular retinal thickness measurements among the three operators. A p value <0.05 was considered to be statistically significant.

RESULTS

The healthy eyes of 39 normal Taiwanese subjects (24 females and 15 males) were examined. Their mean age was 30.4±16.1 years (range, 11–46 years). The mean age for females and males was 31.2±19.9 years (range, 11–46 years) and 30.1±15.2 years (range, 13–44 years), respectively. Mean pupil diameter after dilation was 7.4±0.6 mm (range, 6–9 mm).

Mean RNFL thickness was 129.2±18.2 μm for superior, 145.9±24.5 μm for inferior, 73.4±15.7 μm for temporal, and 85.3±18.8 μm for nasal peripapillary areas; mean 6 mm total macular volume was 6.6±0.2 mm³ and mean foveal thickness was 171.4±9.5 μm; mean retinal thickness was 192.4±17.2 μm in 1 mm perifoveal, 252.3±7.1 μm in 3 mm perifoveal, and 228.7±10.1 μm in 6 mm perifoveal areas as measured by operator A.

Mean RNFL thickness was 135.1±16.1 μm for superior, 142.2±21.1 μm for inferior, 77.1±16.2 μm for temporal, and 76.9±12.7 μm for nasal peripapillary areas; mean 6 mm total macular volume was 6.6±0.3 mm³ and mean foveal thickness was 168.4±8.1 μm; and mean retinal thickness was 191.8±19.9 μm in 1 mm perifoveal, 252.7±10.6 μm in 3 mm perifoveal, and 227.8±10.6 μm in 6 mm perifoveal areas as measured by operator B.

Mean RNFL thickness was 132.7±17.6 μm for superior, 143.6±22.5 μm for inferior, 75.6±16.0 μm for temporal, and 81.1±16.7 μm for nasal peripapillary areas; mean 6 mm total macular volume was 6.6±0.3 mm³ and mean foveal thickness was 169.7±8.6 μm; and mean retinal thickness was 192.3±16.8 μm in 1 mm perifoveal, 252.0±9.2 μm in perifoveal, and 228.2±10.0 μm in 6 mm perifoveal areas as measured by operator C.

Comparing the thickness measurements among the three operators, there were no significant differences in superior (p=0.268), inferior (p=0.822), temporal (p=0.533), and nasal (p=0.198) peripapillary areas; total macular volume (p=0.874) and foveal thickness (p=0.933); nor in 1 mm (p=0.988), 3 mm...
Reproducibility of OCT measurements

<table>
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<th>Table. Thickness measurements*</th>
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<td><strong>After pupillary dilation</strong></td>
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<td><strong>Peripapillary areas (μm)</strong></td>
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*Data presented as mean ± standard deviation.

(p = 0.986), and 6 mm (p = 0.922) perifoveal retinal areas (Table).

**DISCUSSION**

OCT-3 is a diagnostic computerized technique used for quantitative and objective in vivo investigation of RNFL thickness [1]. Using OCT-3, each tissue plane produces a reflection which results in a different and measurable interference depending on the traversed distance. When compared to ultrasound, such as B-scan, OCT-3 has the inherent advantage of being able to obtain a significantly higher spatial resolution by using a much shorter wavelength of light, and is thus able to produce high-resolution cross-sectional images of the retina [7]. The imaging technology visualizes and measures anatomic layers of the retina and assesses RNFL [1,8,9]. The images are produced by scanning the optical beam in the transverse direction to produce a two- or three-dimensional image of tissue reflection and scattering [10].

Some authors reported the following foveal retinal thickness values of the normal population after pupillary dilation of 153 ± 15 μm [11], 147 ± 17 μm [3], 152 ± 21 μm [12], 154 ± 13 μm [13], 152 ± 17 μm [14], and 142 ± 18 μm [15]. Mok et al reported that the peripapillary RNFL measurements at the superior, inferior, temporal, and nasal areas were 145 ± 24 μm, 154 ± 26 μm, 98 ± 32 μm, and 87 ± 16 μm, respectively [7]. Gürses-Özden et al reported reproducibility of thickness measurements after pupillary dilation using OCT [16]. They reported mean RNFL of 119.7 ± 23.9 μm, 121.5 ± 22.4 μm, 74.7 ± 15.1 μm, and 75.8 ± 23.9 μm in the superior, inferior, temporal, and nasal peripapillary areas, respectively, and mean total macular volume and foveal thickness of 7.2 ± 0.3 mm³ and 184.1 ± 25.7 μm, respectively, as measured by operator 1; and mean RNFL of 127.9 ± 25.7 μm, 115.1 ± 21.2 μm, 78.3 ± 18.2 μm, and 70.0 ± 24.9 μm in superior, inferior, temporal, and nasal peripapillary areas, respectively, and mean total macular volume and foveal thickness of 7.2 ± 0.3 mm³ and 183.9 ± 23.8 μm, respectively, as measured by operator 2. There was no significant difference in thickness measurements between operators 1 and 2, and interoperator reproducibility was high while using OCT [16]. The differences among Mok et al’s, Gürses-Özden et al’s, and our data may be related to race. However, the mean thickness measurements were slightly different between Gürses-Özden et al’s and our data and between Gürses-Özden et al’s and Mok et al’s data. The key areas in peripapillary RNFL thickness measurements are the superior and inferior areas. In Gürses-Özden et al’s data, despite there being no significant difference, the mean inferior RNFL thickness was slightly larger than the superior thickness as measured by operator 1, and superior thickness was larger than inferior thickness as measured by operator 2. Then, in Mok et al’s and our data, the mean inferior RNFL thickness was larger than superior thickness. We propose that a well-trained and experienced operator is important when using OCT.

Our results showed that there were no significant differences in thickness measurements in peripapillary areas, total macular volume, foveal thickness, and perifoveal areas after pupillary dilation among three operators A, B, and C. Some ophthalmologists suspected the reproducibility of OCT. Our results demonstrated...
good interoperator reproducibility among our three trained and experienced operators using OCT in healthy Taiwanese eyes. However, our study investigated healthy eyes rather than patients’ eyes. Some patients with poor cooperation or ocular diseases find it hard to maintain visual fixation during OCT measurement. In future, OCT reproducibility should be tested among trained and experienced operators in subjects with ocular diseases.

REFERENCES

用光學同軸眼底斷層掃描儀測量視網膜
神經纖維層及黃斑部視網膜厚度
測量值的再現性

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探討用光學同軸眼底斷層掃描儀測量正常眼視神經乳頭周圍視網膜神經纖維層及黃斑部視網膜厚度的測量值，並評估測量值在不同檢查人員間的再現性。每位正常受
檢者任選一眼，由三位具經驗的檢查者用光學同軸眼底斷層掃描儀測量受檢眼視網
膜神經纖維層及黃斑部視網膜厚度，並分析比較平均厚度值及三位檢查者檢查結果
的差異性。研究中 39 位受檢者共 39 眼，受檢者 24 位女性，15 位男性，平均
年齡 30.4 歲。檢查前瞳孔散大後的平均瞳孔直徑為 7.4 公厘。比較瞳孔散大後的
厚度測量值，無論視神經乳頭周圍視網膜神經纖維層或黃斑部視網膜厚度，在三位
具經驗檢查者間，並無顯著差異。用光學同軸眼底斷層掃描儀測量正常眼視神經
乳頭周圍視網膜神經纖維層及黃斑部視網膜厚度，在檢查者之間其測量值有好的再
現性。

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