

# Reproducibility of Optical Coherence Tomography Retinal Nerve Fiber Layer Thickness Measurements Before and After Pupil Dilation

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**Purpose:** To evaluate the intra- and interobserver reproducibility of peripapillary retinal nerve fiber layer (RNFL) thickness measurements before and after pupil dilation using spectral-domain optical coherence tomography (SD-OCT).

**Methods:** In this observational case series, 44 eyes of 44 healthy subjects were scanned by two trained operators on the same day, using Cirrus SD-OCT (Carl Zeiss Meditec, Dublin, CA, USA). Three scans were obtained before and after pupil dilation by each operator. Mean  $\pm$  standard deviation (SD) and coefficient of variation (CV) were used for description of results and variation of measurements respectively. Intraclass correlation coefficients (ICC) and Bland-Altman plots were used to evaluate validation and limits of agreement.

**Results:** Overall, 23 female and 21 male subjects with mean age of  $36.9 \pm 8.8$  (range, 20 to 50) years were enrolled. Mean RNFL thickness before pupil dilation was  $92.6 \pm 7.2$  (CV, 7.8%) and  $92.4 \pm 6.8$  (CV, 7.4%)  $\mu\text{m}$  by operator one and two, respectively. After pupil dilation, mean RNFL thickness was  $92.7 \pm 7.9$  (CV, 8.5%) and  $92.0 \pm 7.5$  (CV=8.2%)  $\mu\text{m}$  by observer one and two, respectively. ICCs ranged from 0.900 to 0.996. Mean absolute error of the two operators was less than  $4.1 \mu\text{m}$ . There were no significant differences in quadrant thicknesses before and after dilation. Interestingly, mean signal strength was not significantly affected by pupil dilation.

**Conclusion:** In normal subjects with clear media, peripapillary RNFL thickness measurements using Cirrus SD-OCT have high inter- and intraobserver reproducibility before and after pupil dilation. Pupil dilation may not be necessary in all subjects to obtain reproducible RNFL thickness measurements.

**Keywords:** Optical Coherence Tomography; Pupil Dilation; Retinal Nerve Fiber Layer; Reproducibility

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## INTRODUCTION

Optic nerve diseases, including glaucomatous optic neuropathy and neurological causes, need an objective, quantitative and sensitive

method to assess retinal nerve fiber layer (RNFL) thickness both for early diagnosis and monitoring disease progression. The importance of RNFL thickness measurements for early glaucoma diagnosis cannot be overstated; theoretically

RNFL thinning may be the earliest detectable structural change.

RNFL loss has been shown to precede functional loss in glaucoma by as much as five years.<sup>1</sup> RNFL thickness maps can potentially be used for a thorough evaluation of the RNFL in longitudinal monitoring of optic nerve disease; this issue is of extreme importance in the clinical management of a life-long disease such as glaucoma.<sup>2-9</sup> RNFL thickness measurement may also play a role in early detection of retinal toxicity of certain systemic medications.<sup>10</sup>

Optical coherence tomography (OCT), introduced in the mid-90s, is a valuable imaging technique for evaluation of the macula, optic nerve and RNFL. The latest commercially available generation of OCT devices, i.e. spectral-domain OCT (SD-OCT), is currently in clinical use.<sup>11</sup> Carl Zeiss Meditec, Dublin, CA, USA introduced an SD-OCT system, Cirrus, that offers faster scanning and better axial resolution than its time-domain OCT (TD-OCT) predecessor, Stratus.<sup>12</sup>

Repeatability and reproducibility of RNFL thickness measurements using TD- and SD-OCTs have been previously demonstrated.<sup>13-15</sup> Some studies have indicated that TD-OCT requires pupil dilation for acquisition of more reproducible RNFL thickness measurements.<sup>16-17</sup> In practice, RNFL analyses by TD-OCT are usually performed after dilating the pupil. According to the Cirrus SD-OCT manufacturer, images can be obtained with the device without pupil dilation, but there are few independent studies in the literature investigating the effects of pupil dilation on measurements obtained with Cirrus.<sup>18-19</sup>

## METHODS

This prospective cross-sectional study was performed on 44 healthy volunteers who accompanied patients at the ophthalmology clinics at Amiralmomenin Hospital, Rasht, Iran. Detailed consent forms were filled for each subject and the protocol was approved by the Ethics Committee of Guilan University of Medical Sciences.

Each subject underwent a complete ophthalmic examination including medical,

ocular and family history; best corrected visual acuity (BCVA), slit lamp biomicroscopy, intraocular pressure (IOP) measurement using Goldmann applanation tonometry (Model AT 900, Haag-Streit, Bern, Switzerland) and dilated fundus examination.

Exclusion criteria were a history of ocular trauma or intraocular surgery, family history of glaucoma, contraindications to pupil dilation, history of intolerance to mydriatics, use of photosensitizing agents during the past two weeks, BCVA < 20/20, presence of significant refractive errors (>6 diopters of spherical equivalent refraction or >3 diopters of astigmatism), IOP > 21 mmHg, any media opacification or abnormal slit lamp findings, occludable angle on gonioscopy, any vitreoretinal disorders, and abnormal optic disc appearance.

Before pupil dilation, two experienced operators measured peripapillary RNFL thickness of a randomly selected eye of each subject with Cirrus SD-OCT (software version 4.0, Model 400, Carl Zeiss Meditec, Inc. Dublin, CA, USA), respectively. The optic disc cube 200×200 was the scan acquisition protocol used for measuring peripapillary RNFL thickness and the procedure has previously been described by others.<sup>20</sup> In this protocol a 3.4 mm diameter circular scan centered on the optic disc is obtained. Cirrus SD-OCT presents RNFL thickness on two circular charts, one with 12 equal sectors each representing one clock hour and the other with four equal 90-degree sectors, each representing one quadrant. The chart displays RNFL thickness in micrometers (µm) and average RNFL thickness is also displayed.

In order to evaluate reproducibility of the measurements, both operators performed the scan three times consecutively for each subject within a ten minute interval between operators. The procedure was performed in random order to prevent any possible effect from fatigue. On the same day, pupillary dilation was achieved using two drops of 1% tropicamide instilled during a 5-minute interval. Half an hour following pupil dilation the same procedure was carried out in all subjects. Images with artifacts, missing parts, seemingly distorted anatomy or signal strength < 7 were excluded and/or retaken.

**Statistical Analysis**

Data was analysed using SPSS software (Version 16.0, SPSS Inc., Chicago, IL, USA). Mean ± standard deviation (SD) and coefficient of variation (CV) were used for description of results and variations of measurements respectively. Intraclass correlation coefficient (ICC) and Bland-Altman plots were used to evaluate validation and limits of agreement (LOA) between RNFL thickness measurements.

**RESULTS**

A total of 44 eyes of 44 volunteers including 23 female and 21 male subjects with mean age of 36.9±8.8 (range, 20 to 50) years were enrolled in the study.

Table 1 shows mean total and quadrantic RNFL thickness values obtained by each operator before and after pupil dilation, respectively. Mean difference ± SD and mean absolute error (MAE) between the two operators were 0.12±5.29 (95%CI, 0.20-0.44) µm and 3.89±3.58 (95% CI, 3.68-4.11) µm, respectively. There was a significant interobserver agreement (ICC=0.991, 95%CI, 0.990-0.992). Mean signal strength was not affected by pupil dilation (Table 2).

According to Bland-Altman analysis, the 95% LOA for overall RNFL thickness measurements between the two operators was -9.56 to 9.80 µm with a bias of 0.12±5.29 µm. Figure 1 demonstrates Bland-Altman plots for interobserver agreement for RNFL thickness measurements in individual quadrants. The

**Table 2.** Mean signal strengths (SS) obtained by the operators (before and after pupil dilation)\*

Operator	Mean SS ± SD (before pupil dilation)	Mean SS ± SD (after pupil dilation)
1	0.91 ± 0.08	0.92 ± 0.09
2	0.90 ± 0.10	0.90 ± 0.09

\* None of the differences were statistically significant

95%LOA was -11.19 to 10.68 µm in the superior quadrant, -7.91 to 8.08 µm in the temporal quadrant, -9.28 to 8.77 µm in the inferior quadrant, and -9.85 to 11.66 µm in the nasal quadrant. The Bland-Altman plot for overall RNFL thickness measurements before and after pupil dilation showed 95%LOA ranging from -9.28 to 10.15 µm with a bias of 0.52±5.50 µm.

Figure 2 shows Bland-Altman plots for average retinal thickness agreement in each quadrant, before and after pupil dilation. The 95%LOA was -9.66 to 11.80 µm in the superior quadrant, -8.11 to 8.94 µm in the temporal quadrant, -9.45 to 10.25 µm in the inferior quadrant, and -9.22 to 9.63 µm in the nasal quadrant.

**DISCUSSION**

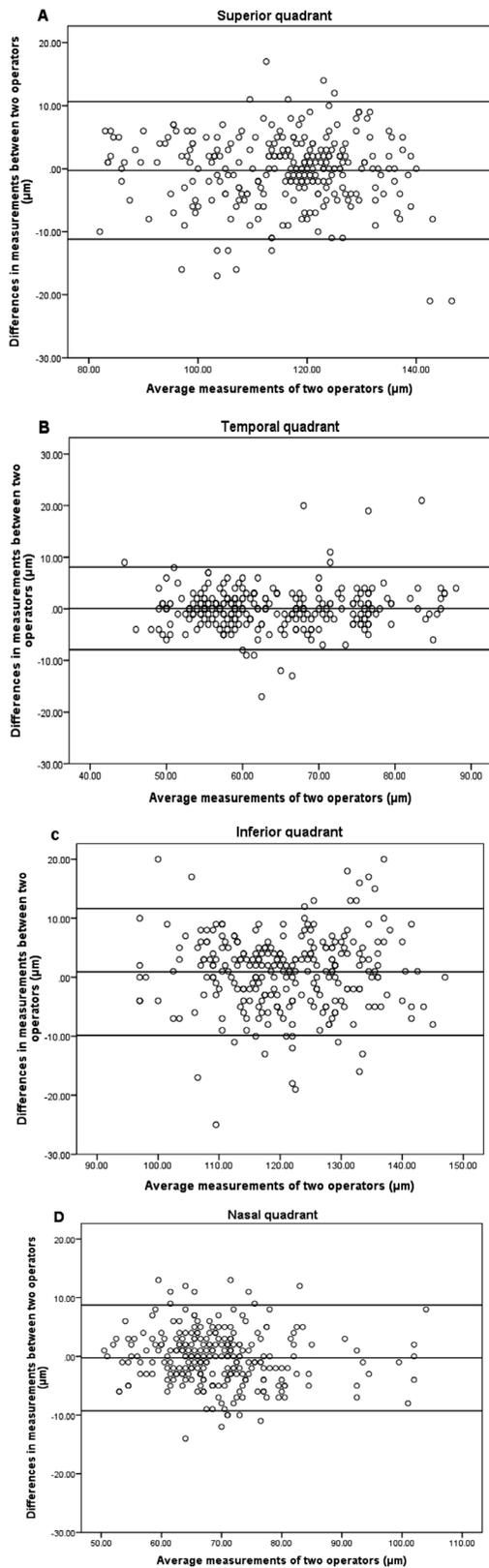
In the current study we observed good intra- and interobserver reproducibility for peripapillary RNFL thickness measurements in each quadrant using Cirrus SD-OCT which is in agreement with other reports.<sup>14,20,21</sup> In the current study, the highest reproducible quadrant was found to be the temporal one, although the differences were not statistically significant.

**Table 1.** Mean values of three RNFL thickness measurements before and after pupil dilation\*

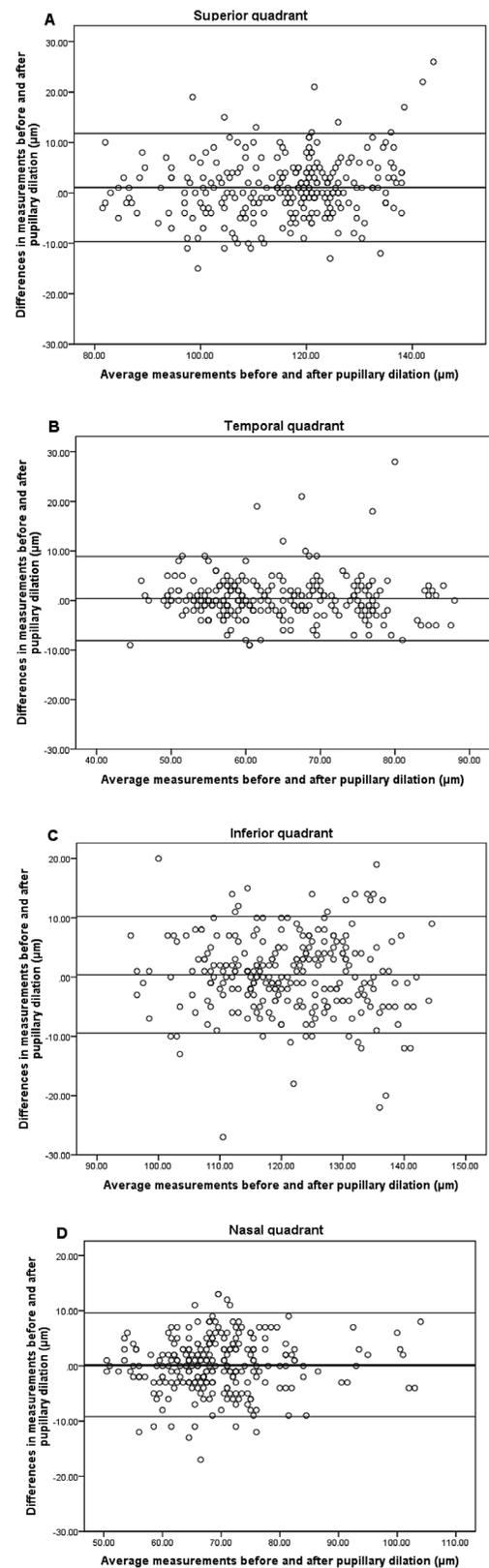
	Mean thickness ± SD (CV) Before pupil dilation (µm)		Mean thickness ± SD (CV) After pupil dilation (µm)	
	Operator 1	Operator 2	Operator 1	Operator 2
Average	92.6 ± 7.2 (7.8%)	92.7 ± 7.9 (8.5%)	92.4 ± 6.8 (7.4%)	92.0 ± 7.5 (8.2%)
Temporal quadrant	64.1 ± 6.2 (9.7%)	63.9 ± 6.2 (9.8%)	63.6 ± 6.1 (9.6%)	63.6 ± 6.2 (9.8%)
Superior quadrant	115.6 ± 9.2 (8.0%)	116.2 ± 9.5 (8.2%)	114.9 ± 9.5 (8.3%)	114.8 ± 9.3 (8.1%)
Nasal quadrant	69.3 ± 6.8 (9.75%)	69.9 ± 6.7 (9.5%)	69.5 ± 6.5 (9.4%)	69.4 ± 6.7 (9.7%)
Inferior quadrant	121.4 ± 10.8 (8.9%)	120.9 ± 10.7 (8.8%)	121.4 ± 11.0 (9.1%)	120.1 ± 11.1 (9.2%)

RNFL; retinal nerve fiber layer; SD, standard deviation; CV, coefficient of variation

\* None of the comparisons were statistically significant



**Figure 1.** Bland-Altman plots of quadrantic retinal nerve fiber layer (RNFL) thickness values by two operators: (A) superior, (B) temporal, (C) inferior, and (D) nasal quadrants.



**Figure 2.** Bland-Altman plots of quadrantic retinal nerve fiber layer (RNFL) thickness values before and after pupil dilation: (A) superior, (B) temporal, (C) inferior, and (D) nasal quadrants.

Good reproducibility is most likely due to the fact that Cirrus SD-OCT does not require manual scan centration on the optic disc as long as the peripapillary region is included in the optic disc cube. The scan registration process performed by the Cirrus algorithm is fully automated, reducing the likelihood of operator error. Conversely, TD-OCT requires the operator to choose the location of the circle, and previous studies have already shown that this procedure may affect the reproducibility of RNFL thickness measurements.<sup>20</sup>

OCT is typically performed following pupil dilation, since an image acquired through a narrow pupil may be truncated on the ends, or signal may be weak, leading to poor image quality. Hee et al stated that optical alignment is sensitive to pupil diameter and that there is reduction in the field of view.<sup>16</sup> Smith et al demonstrated that reproducibility of RNFL measurements was not satisfactory in 23.7% of undilated eyes.<sup>17</sup> However, other studies demonstrated that pupil dilation does not affect RNFL thickness measurements.<sup>22,23</sup> All of the above-mentioned studies have been performed using TD-OCTs. There are few studies performed with SD-OCT. In a study in healthy subjects, Savini et al showed that pupil dilation did not affect RNFL thickness measurements obtained by Cirrus SD-OCT.<sup>18</sup> In another study using Cirrus-SD-OCT, Massa et al revealed that RNFL measurements in healthy individuals and glaucoma patients were not influenced by pupil size.<sup>19</sup> These two studies did not address interobserver reproducibility.

Our study showed that pupil dilation had no significant influence on peripapillary RNFL thickness measurements obtained by Cirrus SD-OCT. In comparison to the above mentioned reports, we studied both intra- and interobserver reproducibility and observed significant reproducibility with Cirrus OCT for RNFL measurements. Mean values and signal strength did not change before and after dilation.

Our study is limited by the use of healthy volunteers rather than glaucoma patients. The other drawback is the age of our subjects (ranging from 20 to 50 years) with a relatively larger pupil size, a condition which may not be present in

older persons or glaucoma patients.<sup>22,23</sup> Finally, it is not clear whether in the presence of media opacity the same results will be reproduced. Therefore, our results may not be generalizable to older subjects, especially those with glaucoma or cataract.

In summary, our study showed that in healthy subjects with clear media, peripapillary RNFL thickness measurements using Cirrus-SD-OCT have high inter- and intraobserver reproducibility before and after pupil dilation. Therefore, pupil dilation may not be necessary in all subjects to obtain reproducible RNFL thickness measurements.

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### Conflicts of Interest

None.

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