

Reproducibility of Retinal Nerve Fiber Layer and Macular Thickness Measurements Using Spectral Domain Optical Coherence Tomography

Amit Sood¹, Rahul Omprakash Paliwal^{2*} , Rishu Yogesh Mishra³

Abstract

The objective of the research was to assess the reproducibility of retinal nerve fiber layer (RNFL) and macular thickness using spectral domain optical coherence tomography and to establish whether the same investigator can get the same or similar results when performing the scan thrice in an hour, without reference to the previous scan and the repeat function.

Materials and Methods. In this prospective observational study, 200 subjects who fulfilled the inclusion and exclusion criteria were scanned 3 times according to predefined guidelines at 0, 30 and 60 minutes on the same day, by the same investigator, using spectral domain optical coherence tomography for measurements of RNFL and macular thickness; observations were statistically analyzed and correlated.

Results. In RNFL thickness, the temporal sector showed the worst reproducibility as compared to other sectors. RNFL was the greatest in the superior quadrant and the thinnest in the temporal quadrant. For macular thickness, the temporal sector (mid zone) showed the worst reproducibility, while in the outer zone, the inferior sector showed the worst reproducibility; macular thickness was the thinnest at the central zone (innermost 1-mm ring), the thickest within the inner 3-mm ring and diminished peripherally.

Conclusions. RNFL and macular thickness measurements using spectral domain optical coherence tomography by the same observer at 0, 30 and 60 minutes were very reproducible, except for the sectors specifically mentioned. The greater the thickness of the RNFL in any sector the better was the reproducibility in that sector. For macular thickness, the temporal sector (mid zone) showed the worst reproducibility.

Keywords

Diagnostic Laboratory Technique; Reproducibility; Macular Thickness; Retinal Nerve Fiber Layer; Spectral Domain Optical Coherence Tomography

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Introduction

Optical coherence tomography (OCT), introduced by Huang *et al.* in 1991 for the first time, has become an invaluable tool for early diagnosis and follow-up of cases of neurodegenerative disorders like glaucoma, optic neuritis, multiple sclerosis, etc. [1–3]. It allows for non-invasive micrometer resolution of cross-sectional images of the retina in human beings [4]. Macular spectral domain optical coherence tomography (SD-OCT) system provides highly reproducible scan results for early detection of glaucoma [5]. It also increases the responsibility of ophthalmologists to be confident in minor axonal loss results so as not to initiate undue lifelong treatment in suspected and properly diagnosed cases based on OCT results.

The fundamental principle of OCT is to measure the echo time delay of reflected infrared light with an interferometer and a low-coherence light source. The axial resolution of the stratus time-domain OCT (TD-OCT) system is achieved at around 10 μm , which is insufficient to detect early changes in the retinal nerve fiber layer (RNFL), since peripapillary RNFL thickness is less than 200 μm [5, 6].

SD-OCT, known variously as high-definition OCT or Fourier-domain OCT, is a spatially encoded frequency domain OCT system having advantages and improvements over TD-OCT systems. The depth scan is immediately converted to spectral information by Fourier transformation without movement of the reference arm. Hence, SD-OCT provides fast and detailed structural information as

compared to other available ophthalmic equipment. It provides significantly improved scan coverage, image resolution (around 6 μm), imaging speed and retinal segmentation algorithms as compared to conventional TD-OCT system [5, 6]. In addition, it provides three-dimensional (3D) cubic data. When analyzing OCT scans, reproducibility of results is very important for diagnosis and assessing progression, regardless of the imaging instrument used. Though the systems are computerized and programmed to evaluate the scans automatically, yet the role of the investigator/operator is very significant. Retinal tissue segmentation/reference marking is pre-programmed (automatic segmentation), but the investigator needs to check it visually and make manual correction of the segment, if necessary. Single or multiple operators need to be careful in their assessment of the scans. The OCT software can identify previous scan locations (follow-up mode) and guide the OCT system to scan the same locations repeatedly during follow-up visits [7]. Thus, first time OCT scanning is of paramount importance to establish a good baseline confidence. Assessment of reproducibility of RNFL and macular thickness using OCT is of utmost importance as reproducibility affects both accuracy and the ability to monitor disease progression.

In this study on Indian healthy subjects, a relatively large sample size was taken to assess the reproducibility of RNFL and macular thickness using Cirrus SD-OCT under the same conditions (same machine and investigator) without using the repeat function.

The objective of the research was to establish whether the same investigator can get the same or similar results when performing the scan thrice in an hour, without reference to the previous scan.

Materials and Methods

After calculating the minimum sample size required at 80% power and 5% significance level, 200 (400 eyes) subjects of Indian origin, at least 40 subjects of each gender, who were treated at the Ophthalmology Department of Mohan Eye Institute, a tertiary care teaching hospital in New Delhi, India, were included in this prospective observational study. The ophthalmology assessment of study subject was done from December 2014 to December 2015.

Inclusion Criteria

- age of 16 to under 60 years;
- no previous retinal or choroidal pathology;
- normal healthy eyes;
- subjects with a spherical equivalent between -5.0 diopters and +5.0 diopters with astigmatism less than 2 diopters (regular astigmatism).

Exclusion Criteria

- anterior segment dysgenesis;
- corneal scarring or opacities;
- proliferative or non-proliferative diabetic retinopathy;
- myopic refractive error of greater than 5.0 diopters;
- dilated pupil diameter of less than 2 mm.



Figure 1. Optical Coherence Tomography.

Study Methodology

Written informed consent was obtained from all subjects before inclusion in the study. The information sheet approved by the Ethics Committee was given to the participants to obtain their consent. It was then duly signed and dated by both the researcher who obtained the consent and the participant. History was taken to rule out previous retinal or choroidal pathology and any other intraocular intervention.

The subjects were assessed regarding:

- distance visual acuity;
- refractive error;
- slit lamp examination;
- fundus examination;
- Goldmann applanation tonometry.

After pharmacological dilation of the pupils with phenylephrine 5% + tropicamide 0.8% ophthalmic solution eye drops and instillation of artificial tears, each subject was scanned 3 times (at 0, 30, and 60 minutes) on the same day, by the same investigator, using the Cirrus SD-OCT 400 machine (Fig. 1). The scans of each individual subject were categorized independently as A (taken at 0 minutes), B (taken at 30 minutes), C (taken at 60 minutes). These scans were then correlated and analyzed for the study. All scans had an image quality factor of 50/100 or greater.

Fig. 2 shows printouts with measurements obtained from one of the patients taken from the optic nerve head (ONH) and RNFL areas, using one of the commercially available SD-OCT instruments, the Cirrus-OCT (Carl Zeiss Meditec Inc, Dublin, CA, USA) system [8]. For RNFL thickness measurements (Fig. 2), the Optic Disc Cube 200x200 scan acquisition protocol was used. 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° sectors, each representing one quadrant. The chart displays RNFL thickness in micro-meters (μm) and the average RNFL thickness [9, 10].

Fig. 3 shows printouts with measurements taken from one of the patients obtained for macular thickness measurement, using one of the commercially available SD-OCT instruments, the Cirrus-OCT (Carl Zeiss Meditec Inc, Dublin,

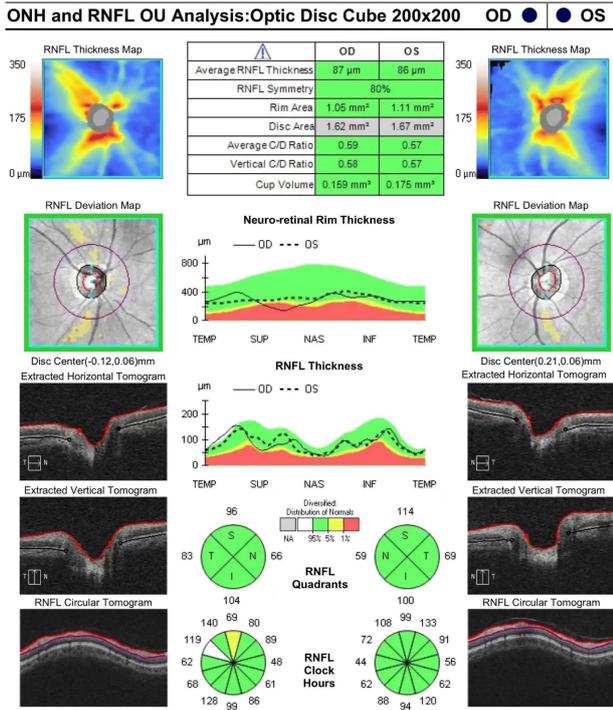


Figure 2. Optic nerve head and retinal nerve fiber layer analysis using optical coherence tomography.

CA, USA) system [8]. For macular thickness measurement (Fig. 3), the Macular Cube 512x128 protocol was used. According to the Early Treatment Diabetic Retinopathy Study (ETDRS) grid, the macula is divided into 9 regions with 3 concentric rings with diameters of 1 mm (innermost ring), 3 mm (inner ring) and 6 mm (outer ring) centered at the fovea. The 1-mm innermost ring is the fovea (central zone), while the 3-mm inner ring (mid zone) and 6-mm outer ring (outer zone) are further divided into four equal regions [8, 10]. For this type of acquisition, the patient must fixate on the target for 2.4 seconds. During scanning, the screen shows the operator an external view of the eye, a real-time fundus image, OCT images of the central crosshair, and the top and bottom B-scans. After capture, the "Review" screen provides qualitative information on the scan. If a subject blinks during the scan, the horizontal segments will appear black on the OCT image. If a subject loses fixation, saccades will be present where the blood vessels are not contiguous. If blinks or numerous artifacts are present, the operator clicks the "Try Again" button to return to the "Scan Acquisition" screen [8, 11].

Statistical Methods

The collected data were entered in a MS EXCEL spreadsheet and statistical analysis was done, using Statistical Package for the Social Sciences (SPSS) version 21.0.

Statistical tests were applied as follows:

1. Categorical variables were presented in numbers and percentages (%) and continuous variables were presented as mean \pm SD and median.
2. The square root of the mean within subject variance was the common standard deviation of the repeated measurements.

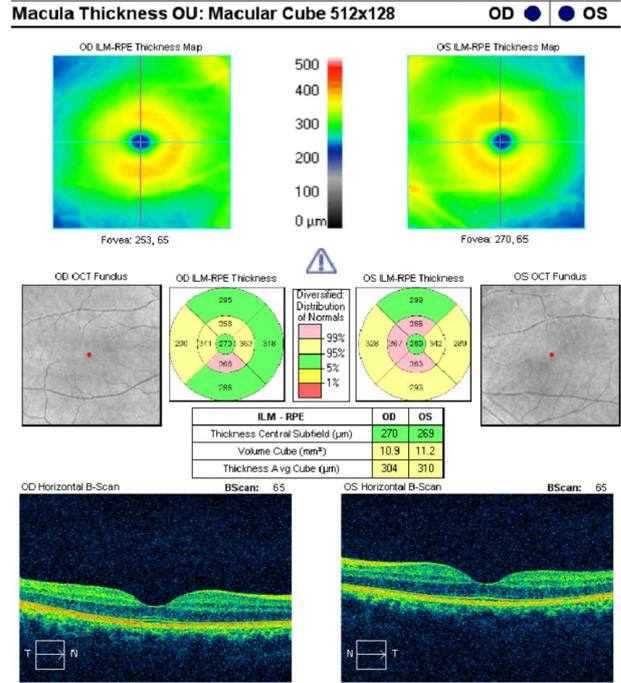


Figure 3. Macular thickness analysis using optical coherence tomography.

3. Reliability analysis using a one-way random model was used to determine the intraclass correlation coefficient (ICC).
4. The coefficient of variation (COV) and test-retest variability were calculated to quantify the repeatability.
5. A p-value of < 0.05 was considered statistically significant.

Results

There were analyzed 400 eyes of 200 (90 males, 110 females) healthy subjects. Forty eyes were excluded due to low signal strength of images, and 14 eyes were excluded due to blinks during scanning. The mean patients' age was 40.70 ± 12.53 years (from 16 to 60 years). The first reading was labeled as baseline reading (Table 1, 2).

Here, the COV is the variation in values between the subjects at first reading. Table 3 tabulates change in values of RNFL thickness measurements at 30 and 60 min as compared to the baseline values of 0 min.

Table 4 shows change in values of macular thickness measurements at 30 and 60 min as compared to the baseline values of 0 min.

Table 5 demonstrates that for RNFL thickness, the temporal sector showed the worst reproducibility as compared to other sectors. For macular thickness, the temporal sector (mid zone) showed the worst reproducibility, while in the outer zone, the inferior sector showed the worst reproducibility.

Table 1. Baseline values of retinal nerve fiber layer thickness measurements.

	Global	Symmetry	Superior	Inferior	Nasal	Temporal
Mean (μm)	90.06	84.97	121.2	115.63	70.86	54.72
Standard deviation	5.9	7.75	12.01	12.96	8.8	7.57
COV (%)	6.55	9.12	9.91	11.21	12.42	13.83

Table 2. Baseline values of macular thickness measurements.

	Central	Mid Zone S	Mid Zone I	Mid Zone N	Mid Zone T	Outer Zone S	Outer Zone I	Outer Zone N	Outer Zone T
Mean (μm)	233.98	309.53	306.73	297.23	294.8	274.22	260.12	289.52	253.3
Standard deviation	11.75	21.43	16.22	27.43	15.15	9.48	13.14	8.98	11.34
COV (%)	5.02	6.92	5.29	9.23	5.14	3.46	5.05	3.10	4.48

Table 3. Changes in values of retinal nerve fiber layer thickness measurements at 30 and 60 min as compared to the baseline values of 0 min.

	Mean \pm SD	Median	Min-Max	Interquartile Range
Age	40.7 \pm 12.53	43	16 - 60	30 - 51
Avg. RNFL thickness 0 min	90.06 \pm 5.9	90	74 - 99	87 - 94
Avg. RNFL thickness 30 min	90.46 \pm 6.09	90	74 - 100	87 - 95
Avg. RNFL thickness 60 min	90.3 \pm 5.87	91	74 - 100	87 - 94
RNFL symmetry 0 min	84.97 \pm 7.75	89	68 - 93	86 - 90
RNFL symmetry 30 min	84.74 \pm 6.85	88	71 - 93	87 - 89
RNFL symmetry 60 min	84.48 \pm 7.17	87.5	70 - 91	87 - 89
RNFL thickness I 0 min	115.63 \pm 12.96	122	88 - 145	103 - 125
RNFL thickness I 30 min	116.27 \pm 13.68	122	87 - 142	102 - 127
RNFL thickness I 60 min	115.96 \pm 13.4	122.5	88 - 140	103 - 126
RNFL thickness N 0 min	70.86 \pm 8.8	72	51 - 83	66 - 77
RNFL thickness N 30 min	71.52 \pm 8.57	72	52 - 85	66 - 78
RNFL thickness N 60 min	71.38 \pm 8.88	73	52 - 84	65 - 77
RNFL thickness S 0 min	121.2 \pm 12.01	123	97 - 148	111 - 127
RNFL thickness S 30 min	120.43 \pm 11.78	123	100 - 144	107 - 126
RNFL thickness S 60 min	120.51 \pm 11.79	123	97 - 145	107 - 126
RNFL thickness T 0 min	54.72 \pm 7.57	54	46 - 70	48 - 61
RNFL thickness T 30 min	55.08 \pm 7.82	54	39 - 69	48 - 62
RNFL thickness T 60 min	55.86 \pm 7.47	54	46 - 69	48 - 63

Notes: Avg.: average; SD: standard deviation; RNFL thickness in micrometers (μm).

Discussion

Our sample size of 400 eyes of healthy subjects was reasonably large and statistically acceptable. Cirrus SD-OCT has 840-nm super luminescent diode as an optical source which acquires 27,000 A-scans/sec. Due to the high acquisition speed of Cirrus SD-OCT, image capture is possible at extremely low light exposures. The power incident of SD-OCT scan on the eye is less than 725 μm ; therefore, repeated measurements on normal healthy eyes is safe. This power incident is within the maximum permissible exposure limit for continuous exposure at that wavelength [12]. Cirrus SD-OCT shows probability code results for RNFL thickness using a white-green-yellow-red color code. For instance, when the thinnest 1% of normal age-matched population has similar RNFL thickness, the red code ('outside normal limits') is indicated. Yellow code represents 'suspect' ($1\% \leq \text{yellow} < 5\%$); green code represents 'normal' ($5\% \leq \text{green} \leq 95\%$); white code represents the thickest 5% of the population (white $> 95\%$). Theoretically, if

RNFL thickness measurements are stable, the probability code results will also be stable [8].

Reproducibility is a crucial reliability index in any OCT system as reproducibility of the measurements is crucial for monitoring disease and its early diagnosis [13, 14].

In our study, the baseline values of the mean RNFL thickness at 0 min were as follows: 90.06 μm - the global RNFL thickness; 84.97 μm - the RNFL thickness symmetry; 121.2 μm in the superior quadrant; 115.63 μm in the inferior quadrant; 70.86 μm in the nasal quadrant; 54.72 μm in the temporal quadrant. Table 5 shows the inter-session ICC, COV, and test-retest variability of three scans performed on a single day for the RNFL and macular measurements, respectively. For the average RNFL thickness measurements, the ICC was 0.991, the COV was 1.01%, and the test-retest variability was 1.8 μm . For the RNFL symmetry, the ICC was 0.993, the COV was 1.18%, and the test-retest variability was 2.00 μm . For quadrants, the ICC ranged from 0.991 (superior quadrant) to 0.995 (inferior quadrant) and 0.995 (nasal quadrant) to 0.987 (tem-

Table 4. Changes in values of macular thickness measurements at 30 and 60 min as compared to the baseline values of 0 min.

	Mean ± SD	Median	Min - Max	Interquartile Range
Central Zone				
0 min	233.98 ± 11.75	236	210 - 253	225 - 245
30 min	233.86 ± 12.17	238	211 - 251	223 - 246
60 min	233.97 ± 12.61	237	212 - 254	222 - 247
Mid Zone				
Inferior 0 min	306.73 ± 16.22	303	283 - 338	287 - 318
Inferior 30 min	308.24 ± 18.12	305	283 - 350	289.5 - 320
Inferior 60 min	307.04 ± 17.78	302	280 - 343	288 - 318
Nasal 0 min	297.23 ± 27.43	305	237 - 337	280 - 315
Nasal 30 min	298.46 ± 27.75	304	240 - 345	281 - 316
Nasal 60 min	298.73 ± 27.56	304	241 - 340	282 - 317
Superior 0 min	309.53 ± 21.43	314.5	278 - 343	283.5 - 323
Superior 30 min	311.5 ± 20.99	314	281 - 347	288 - 324
Superior 60 min	312.36 ± 20.61	317	282 - 347	286.5 - 325
Temporal 0 min	294.8 ± 15.15	287	276 - 325	281 - 306
Temporal 30 min	295.92 ± 19.23	286	272 - 335	275.5 - 310
Temporal 60 min	295.2 ± 19.25	285	272 - 337	276.5 - 310
Outer Zone				
Inferior 0 min	260.12 ± 13.14	257	248 - 309	250 - 259
Inferior 30 min	259.01 ± 11.17	260	246 - 318	251 - 260
Inferior 60 min	259.15 ± 11.58	258	247 - 317	250.5 - 261
Nasal 0 min	289.52 ± 8.98	287	279 - 312	283 - 292
Nasal 30 min	289.85 ± 11.86	287	278 - 319	281 - 291
Nasal 60 min	290.1 ± 11.27	288	278 - 317	281 - 292
Superior 0 min	274.22 ± 9.48	274	259 - 297	269 - 278
Superior 30 min	275.17 ± 10.04	274	261 - 301	270 - 279
Superior 60 min	274.99 ± 10.67	274	259 - 302	270 - 279.5
Temporal 0 min	253.3 ± 11.34	253	237 - 278	243 - 263
Temporal 30 min	253.32 ± 12.65	250	235 - 280	244 - 264
Temporal 60 min	253.38 ± 12.38	247	236 - 280	244 - 265

Notes: Macular thickness in micrometers (µm); SD: standard deviation.

Table 5. Intraclass correlation coefficient, coefficient of variation and test - retest variability of retinal nerve fiber layer and macular thickness measurements using the Cirrus spectral domain optical coherence tomography in healthy eyes.

	ICC	95% Confidence Interval		P - value	COV (%)	Test - Retest Variability
		Lower Bound	Upper Bound			
RNFL						
Average RNFL	0.991	0.989	0.992	<0.001	1.01 ± 0.46	1.80 ± 0.81
RNFL symmetry	0.993	0.991	0.994	<0.001	1.18 ± 0.48	2.00 ± 0.81
RNFL thickness S	0.991	0.989	0.992	<0.001	1.46 ± 0.79	3.49 ± 1.88
RNFL thickness I	0.995	0.994	0.996	<0.001	1.25 ± 0.63	2.88 ± 1.48
RNFL thickness N	0.995	0.994	0.996	<0.001	1.38 ± 0.84	1.90 ± 1.05
RNFL thickness T	0.987	0.984	0.989	<0.001	2.38 ± 1.55	2.57 ± 1.53
Macula						
Central Zone	0.989	0.987	0.991	<0.001	0.84 ± 0.42	3.89 ± 1.96
Mid Zone						
Superior	0.996	0.995	0.996	<0.001	0.68 ± 0.41	4.15 ± 2.52
Inferior	0.994	0.993	0.995	<0.001	0.59 ± 0.44	3.70 ± 2.97
Nasal	0.999	0.998	0.999	<0.001	0.49 ± 0.30	2.93 ± 1.99
Temporal	0.991	0.989	0.992	<0.001	0.90 ± 0.40	5.32 ± 2.54
Outer Zone						
Superior	0.993	0.992	0.994	<0.001	0.40 ± 0.30	2.25 ± 1.71
Inferior	0.932	0.920	0.943	<0.001	1.10 ± 1.54	5.88 ± 8.31
Nasal	0.984	0.981	0.987	<0.001	0.64 ± 0.43	3.79 ± 2.69
Temporal	0.993	0.992	0.994	<0.001	0.57 ± 0.36	2.88 ± 1.84

poral quadrant). The COVs were 1.46% (superior quadrant), 1.25% (inferior quadrant), 1.38% (nasal quadrant) and 2.38% (temporal quadrant). The test-retest variability ranged from 3.49 μm (superior quadrant) to 2.88 μm (inferior quadrant) and 1.90 μm (nasal quadrant) to 2.57 μm (temporal quadrant). Therefore, our study showed that in RNFL thickness, the temporal sector showed the worst reproducibility as compared to other sectors. RNFL thickness measurements using SD-OCT by the same observer on the same day at 0, 30 and 60 minutes were very reproducible in normal healthy eyes; except for the sectors specifically mentioned.

Samin Hong *et al.* conducted a similar study in 2010 using Cirrus SD-OCT and have found that the reproducibility of RNFL thickness in healthy eyes was excellent (the ICC was 0.970, the COV was 2.38%, the test-retest variability was 4.5 μm) as compared to the previous reports for Stratus TD-OCT, which is in line with our study results. However, the variability was relatively higher in the nasal area, which is inconsistent with our study, and may be attributed to the factors associated with study sample size, geographic and demographic variations [15].

D. Cabrera DeBuc *et al.* scanned RNFL thickness using SD-OCT for 3 sessions with a 30-second rest in between to study the inter-session repeatability and identify the pitfalls affecting the reliabilities. They have found that the values of the coefficient of repeatability (CR) $< 5 \mu\text{m}$ and the COV of 5% were revealed in the outer layers only. The values of the COV were not significantly different in the unregistered scanning session. They have concluded that the rotations in the unregistered scanning sessions did not cause significant change in repeatability. In our study, we have found the highest COV value in the temporal sector ($2.38 \pm 1.55 \%$); the superior sector had the highest test-retest variability ($3.49 \pm 1.88 \mu\text{m}$); the values of the COV were not significantly different ($p < 0.05$) in the three scanning sessions, suggesting very good reproducibility and repeatability. These results can play a superior role in arguing the stability of SD-OCT results [16].

In our study, the ICC values of Cirrus SD-OCT for quadrants ranged from 0.98 to 0.99. In previous studies conducted by Sull AC *et al.*, Chan A *et al.*, using Stratus TD-OCT, they ranged from 0.67 to 0.97. These variations between the two systems were more prominent in the nasal sector as the nasal ICC values of Stratus TD-OCT (from 0.67 to 0.88) were lower than those of Cirrus SD-OCT (0.99), and the nasal test-retest variability of Stratus TD-OCT (16.0 μm) was much larger than that of Cirrus SD-OCT (1.9 μm) [17, 18]. Stratus TD-OCT selects the inner segment/outer segment junction as the outer retinal boundary for macular thickness measurements, whereas the SD-OCT system selects the retinal pigment epithelium as the outer retinal boundary for thickness measurements, thus, leading to an increase in macular thickness reported with these OCT systems as compared to the TD-OCT systems [19].

Chen X *et al.* compared the repeatability and reproducibility of the axial and lateral retinal measurements, using handheld OCT systems and a tabletop OCT system.

Three OCT systems were used: handheld Leica Envisu, investigational handheld swept-source OCT (UC3), and Heidelberg Spectralis tabletop system. All three OCT systems have been found to have good repeatability and reproducibility with the COV less than 8.5% for the RNFL thickness [20]. Our results have shown the COV $< 1.5 \%$ for the average RNFL thickness in the test-retest measurements and high reproducibility for the SD-OCT system.

In our study, for macular thickness central zone, the ICC was 0.989, the COV was 0.84%, the test-retest variability was 3.89 μm . For the mid zone, the ICC ranged from 0.996 (superior sector) to 0.994 (inferior sector) and 0.999 (nasal sector) to 0.991 (temporal sector). The COVs were 0.68% (superior sector), 0.59% (inferior sector), 0.49% (nasal sector) and 0.90% (temporal sector). The test-retest variability ranged from 4.15 μm (superior sector) to 3.70 μm (inferior sector) and 2.93 μm (nasal sector) to 5.32 μm (temporal sector). For the outer zone, the ICC ranged from 0.993 (superior sector) to 0.932 (inferior sector) and 0.984 (nasal sector) to 0.993 (temporal sector). The COVs were 0.40% (superior sector), 1.10% (inferior sector), 0.64% (nasal sector) and 0.57% (temporal sector). The test-retest variability ranged from 2.25 μm (superior sector) to 5.88 μm (inferior sector) and 3.79 μm (nasal sector) to 2.88 μm (temporal sector).

Therefore, our study showed that for macular thickness, in the mid zone, the temporal sector showed the worst reproducibility and in the outer zone, the inferior sector showed the worst reproducibility.

Demographic variations can be important parameters when comparing macular thickness measurements and diagnosing and monitoring macular pathologies. Natung T *et al.* (2016) studied macular thickness in healthy Indian eyes, using Zeiss SD-OCT. The mean central subfield thickness of all subjects was $240.40 \pm 18.26 \mu\text{m}$. Overall, the nasal quadrant was the thickest followed by the superior, inferior, and temporal subfields [21]. We have found the similar results in the outer zone with extremely high level of the ICC (0.932-0.993) in the repeated measurement test.

According to García-Franco R *et al.*, who studied Mexican healthy population at the age of 18-70 years in 2020 using Huvitz OCT, normal macular thickness values in the foveal macular region of the subjects studied were thinner than values reported in other populations. Using the ETDRS grid, the mean central subfield thickness was $227.4 \pm 18.9 \mu\text{m}$; macular thickness was thicker in the inner ring than in the outer ring [22]. The results of our study are in an agreement with the data obtained by García-Franco R *et al.* except for the nasal region where the average macular thickness values were similar in the test-retest measurements. However, the range of values in the nasal outer zone was wider than that in the inner one. These differences were probably caused by study sample size, demographic and geographic factors.

Wang KL *et al.* evaluated the variability and reproducibility of central foveal thickness measurements using handheld SD-OCT in supine infants versus conventional adult tabletop SD-OCT imaging. The authors have found that handheld SD-OCT was a reproducible instrument to

measure foveal thicknesses in supine infants [23]. The results obtained in our study have shown a high level of SD-OCT result reproducibility in adult patients. According to our results, SD-OCT is a high-precision clinical laboratory instrument for diagnosing pathological conditions in adult and pediatric patients.

Conclusions

1. RNFL and macular thickness measurements using SD-OCT by the same observer on the same day at 0, 30 and 60 minutes were very reproducible in normal healthy eyes; except for the sectors specifically mentioned.
2. RNFL thickness in the temporal sector had the worst reproducibility as compared to other sectors.
3. For macular thickness, the temporal sector (mid zone) showed the worst reproducibility, while in the outer zone, the inferior sector showed the worst reproducibility.

Limitations

Our study has shown that in RNFL thickness evaluation, the temporal sector had the worst reproducibility, which was naturally thin as compared to other sectors. Therefore, the following question arises: "Will the reproducibility be good in diseases such as glaucoma and neurodegenerative disorders in which RNFL thinning occurs?" This suggests that reproducibility in diseased states needs to be evaluated separately.

Ethical Statement

This research study on human subjects was conducted in accordance with the ethical standards of the institutional and national research committees and according to the WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects. The research study protocol was approved by the Institutional Ethics Committee - Letter No. MEI/IEC/2014/71 Dated 02/12/2014.

Informed Consent

Written informed consent was obtained from all the subjects before inclusion in the study.

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Conflict of Interest

The authors declare that no conflicts exist.

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