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Reproducibility of Retinal Nerve Fiber Layer and Macular Thickness Measurement by Spectral Domain Optical Coherence Tomography

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ABSTRACT **Ophthalmology in Russia. 2022;19(4):705–718**

Purpose: To assess the reproducibility of retinal nerve fiber layer (RNFL) and macular thickness by spectral domain optical coherence tomography (SD-OCT) when the same investigator does scan thrice in a span of one hour without reference to the previous scan, is able to get similar results or not, without using the repeat function. **Methods.** In this prospective observational study 200 subjects who fulfilled the inclusion & exclusion criteria were scanned 3 times as per pre-defined guidelines at 0 minutes, 30 minutes; 60 minutes on the same day, by the same investigator using SD-OCT for measurements of RNFL and macular thickness & observations were statistically analyzed & correlated. **Results.** In RNFL thickness, temporal sector shows the worst reproducibility as compared to other sectors. The RNFL thickness was greatest in superior quadrant and thinnest in temporal quadrant. Female values were significantly higher than males in RNFL superior and RNFL symmetry. For macular thickness, temporal sector (mid-zone) showed the worst reproducibility and in outer-zone, Inferior sector showed the worst reproducibility. It also shows that macular thickness was thinnest at the central zone (innermost 1 mm ring), thickest within the inner 3 mm ring and diminished peripherally. **Conclusion.** RNFL and macular thickness measurements by SD-OCT by the same observer at 0 minutes, 30 minutes and 60 minutes were very reproducible except in the sectors specifically mentioned. The greater the thickness of RNFL in any sector, the better will be the reproducibility in that sector. For macular thickness, temporal sector (mid-zone) showed the worst reproducibility & with an increase in age the macular thickness measurements decreases.

Keywords: macular thickness, reproducibility, retinal nerve fibre layer, spectral domain optical coherence tomography

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Воспроизводимость данных при измерении слоя нервных волокон сетчатки и толщины желтого пятна с помощью спектральной оптической когерентной томографии

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РЕЗЮМЕ **Офтальмология. 2022;19(4):705–718**

Цель: оценить воспроизводимость данных, касающихся слоя нервных волокон сетчатки (RNFL) и толщины макулы, с помощью оптической когерентной томографии SD-OCT, когда один и тот же исследователь выполняет сканирование трижды в течение одного часа без привязки к предыдущему сканированию. **Методы.** В этом проспективном обсервационном исследовании участвовали 200 человек, которые были просканированы трижды в соответствии с заранее определенными рекомендациями в 0, 30, 60 минут в один и тот же день одним исследователем с использованием SD-OCT для измерения RNFL и толщины макулы. Данные были статистически проанализированы и сопоставлены. **Результаты**. В толщине RNFL височный сектор показывает наихудшую воспроизводимость по сравнению с другими секторами. Толщина RNFL была наибольшей в верхнем квадранте и наименьшей в височном квадранте. Значения у женщин были значительно выше, чем у мужчин, в верхнем отделе CHBC, это касается и симметрии CHBC. В отношении толщины макулы височный сектор (средняя зона) показал наихудшую воспроизводимость. Во внешней зоне нижний сектор показал наихудшую воспроизводимость. Это также показывает, что толщина макулы была самой тонкой в центральной зоне (внутреннее кольцо 1 мм), самой большой в пределах внутреннего кольца, 3 мм, и уменьшалась на периферии. **Заключение.** Измерения CHBC и толщины желтого пятна с помощью SD-OCT одним и тем же наблюдателем через 0, 30 и 60 минут были высоковоспроизводимыми, за исключением отдельных упомянутых секторов. Чем больше толщина RNFL в любом секторе, тем лучше будет воспроизводимость в этом секторе. Для толщины макулы височный сектор (средняя зона) показал наихудшую воспроизводимость, и с возрастом измерения толщины макулы уменьшаются.

Ключевые слова: толщина макулы, воспроизводимость, слой нервных волокон сетчатки, оптическая когерентная томография спектральной области

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Конфликт интересов отсутствует

INTRODUCTION

Optical coherence tomography (OCT), first introduced by Huang et al. in 1991, has become an invaluable tool today for the early diagnosis and follow-up of cases of neuro-degenerative disorders like glaucoma, optic neuritis, multiple sclerosis etc. It allows for non-invasive, micrometer resolution of the cross-sectional images of the retinas in living human beings [1]. With this also increases the responsibility of the ophthalmologists to be sure of the subtle axonal loss results so that undue lifelong treatment is not initiated in suspected and duly diagnosed cases on the basis of the OCT results.

The basic principle of OCT is the measurement of the echo time delay of reflected infrared light with an interferometer and a low coherence light source. Though the axial resolution of the Stratus Time-Domain TD-OCT systems is reached at around 10 μm, may not be sufficient to detect early changes in the retinal nerve fibre layer (RNFL), because the peri-papillary RNFL thickness is less than 200 μm [2, 3].

Spectral-Domain SD-OCT also known as Fourier-Domain OCT or High Definition OCT, an improvement upon the Time-Domain OCT, is a spatially encoded frequency domain OCT system. As a result of the Fourier relation, the depth scan can be immediately converted to spectral information by Fourier transformation without movement of the reference arm. Thus, spectral domain OCT can provide much faster and more detailed structural information than any other available ophthalmic instrument [4]. It offers significant advantages in terms of a markedly improved image resolution (around 6μm), imaging speed, scan coverage and retinal segmentation algorithms over the conventional TD-OCT [5]. It also provides three-dimensional (3D) cubic data. When analyzing OCT scans, reproducibility of the results is a very important consideration for diagnosis and judging 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. The segmentation / reference marking of the retinal tissues 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 [6]. Thus first time OCT scanning is of paramount importance to establish a good baseline confidence. Assessment of reproducibility of RNFL and macular thickness by OCT is of paramount importance because reproducibility affects accuracy as well as the ability to monitor disease progression.

In this study on healthy subjects in Indian population, a relatively larger sample size is being taken to assess the reproducibility

of RNFL and macular thickness by Cirrus SD-OCT under the same conditions (same machine and investigator) without using the repeat function. The purpose of this study is to establish that whether the same investigator, when does the scanning thrice in a span of one hour, without reference to the previous scan, is able to get same or similar results or not.

METHODS

For this prospective, observational study, after calculating the minimum required sample size with 80 % power of study and 5 % level of significance, 200 subjects (400 eyes) of Indian origin with minimum 40 subjects of each gender were included who visited ophthalmology department of our tertiary care teaching hospital during the study period.

Inclusion criteria

- Age over 16 years and below 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 an 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.

Study methodology

Written informed consent was obtained from all subjects before inclusion in the study. An information sheet approved by the ethics committee for the purpose of the study was given to the participants to obtain their consent. It was then duly signed and dated by the researcher obtaining the consent as well as that of the witness. History was taken to rule out previous Retinal or Choroidal pathology and any other intraocular intervention.

Assessment of subjects was done regarding:

- Distance visual acuity;
- Refractive error;
- Slit lamp examination;
- Fundus examination;
- Goldmann applanation tonometry.

After pharmacological pupillary dilation and instillation of artificial tears, each subject was scanned 3 times (at 0 minutes, 30 minutes, 60 minutes) on the same day, by the same investigator using Cirrus spectral domain optical coherence tomography (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 & analysed for the study. All scans had an image quality factor of 50/100 or greater.

For RNFL thickness measurement (Fig. 2), the OPTIC DISC CUBE 200 × 200 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 hours and the other with four equal 90 degree sectors, each representing one quadrant. The chart displays RNFL thickness in micro-meters (um) and average RNFL thickness.

For macular thickness measurement (Fig. 3), MACULAR CUBE 512 × 128 protocols was used. According to Early Treatment Diabetic Retinopathy Study (ETDRS) map, macula is divided into 9 regions with 3 concentric rings measuring 1 mm (innermost ring), 3 mm (inner ring) and 6 mm in diameter (outer ring) centered on the fovea. The innermost 1 mm ring is the fovea (central zone) while the 3 mm inner ring (mid zone) and 6mm outer ring (outer zone) are further divided into four equal regions [7]. The patient must fixate on the target for 2.4 seconds for this type of acquisition. During the scan, 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.

Fig. 1. Optical coherence tomography (OCT)

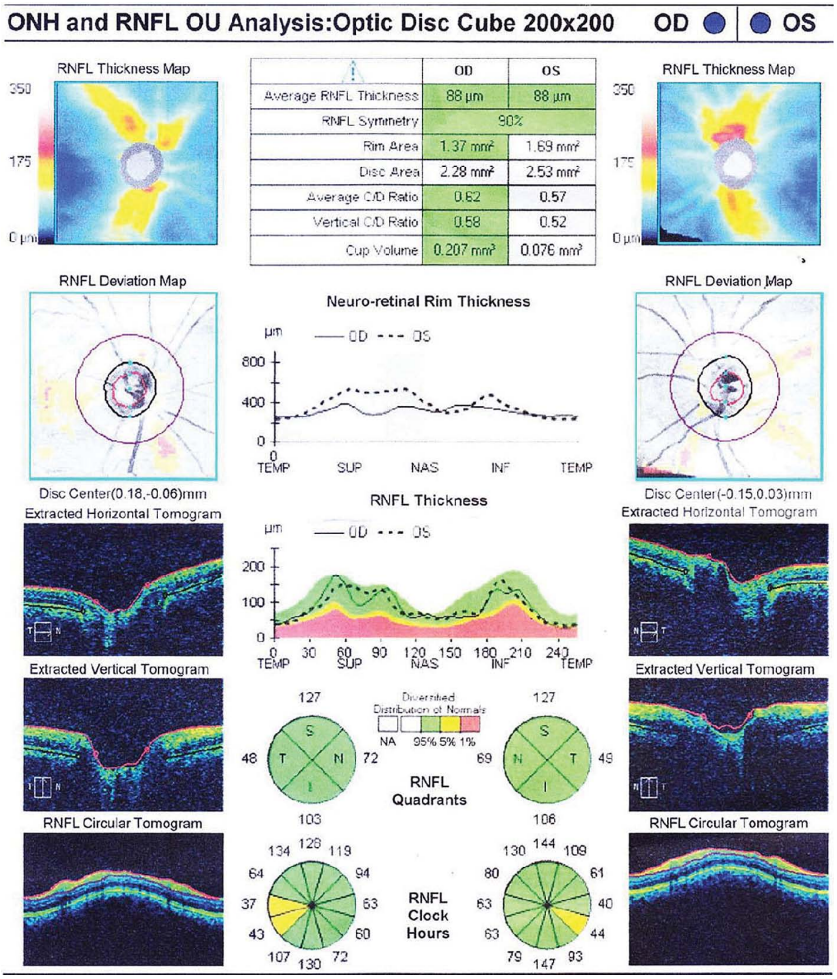

Fig. 2. ONH and RNFL analysis by OCT

Statistical Methods

Categorical variables will be presented in number and percentage (%) and continuous variables are presented as mean ± SD and median. Normality of data will be tested by Kolmogorov—Smirnov test. If the normality is rejected then non parametric test was used. Statistical tests were applied as follows:

1. Square root of the mean within subject variance was the common standard deviation of the repeated measurements.
2. Reliability analysis using a one-way random model was used to determine intra class correlation coefficient.
3. Pearson correlation coefficient (Spearman rank correlation coefficient for non parametric data) was used to correlate standard deviation of three measurements with RNFL thickness value.
4. Linear regression analysis was used to determine an association of mean macular thickness with age. Multi-variant analysis with age and gender as independent variables was performed to determine the variations in thickness measurements by gender when controlled for age and the associations of age with mean macular thickness, when controlled for gender.
5. Independent T-test Mann—Whitney test (for non-parametric data) was used to find out difference in thickness measurements by gender.
6. A p value of <0.05 was considered statistically significant.

The data was entered in MS Excel spread sheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

RESULTS

400 eyes of 200 healthy subjects (90 males, 110 females) were analysed. 40 eyes were excluded owing to the low signal strength of the images, and 14 eyes were excluded because of blinks during the scanning process. The mean subject age was 40.70 ± 12.53 years (range, 16 to 60 years). First reading has been labeled as baseline reading (Table 1 & Table 2).

Table 3 tabulates change in values of RNFL thickness measurements at 30 min and 60 min from baseline values of 0 min.

Table 1. Baseline values of RNFL thickness measurements

	Global	Symmetry	Superior	Inferior	Nasal	Temporal
Mean	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

* COV: Coefficient of variation.

Table 2. Baseline values of macular thickness measurements

	Central	Macula S	Macula I	Macula N	Macula T	Outer S	Outer I	Outer N	Outer T
Mean	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

Here COV given is the variation in values between the subjects at first reading.

Table 4 tabulates change in values of macular thickness measurements at 30 min and 60 min from baseline values of 0 min.

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

Table 6 demonstrates that in the RNFL thickness was greatest in superior quadrant and thinnest in temporal quadrant. It also shows that macular thickness was thinnest at the central zone (innermost 1 mm ring), thickest within the inner 3 mm ring and diminished peripherally.

Table 7 shows variation in RNFL and macular thickness.

Table 8 tabulates correlation between mean values and standard deviation of three repeated measurements. In Table 8 mean values of average RNFL, RNFL (nasal), RNFL (temporal) and central zone (macular thickness) had a significant negative correlation with standard deviation (that is with increase in mean values, standard deviation in the readings significantly decreases). It also shows mean values of outer-zone (superior), outer- zone (inferior) and

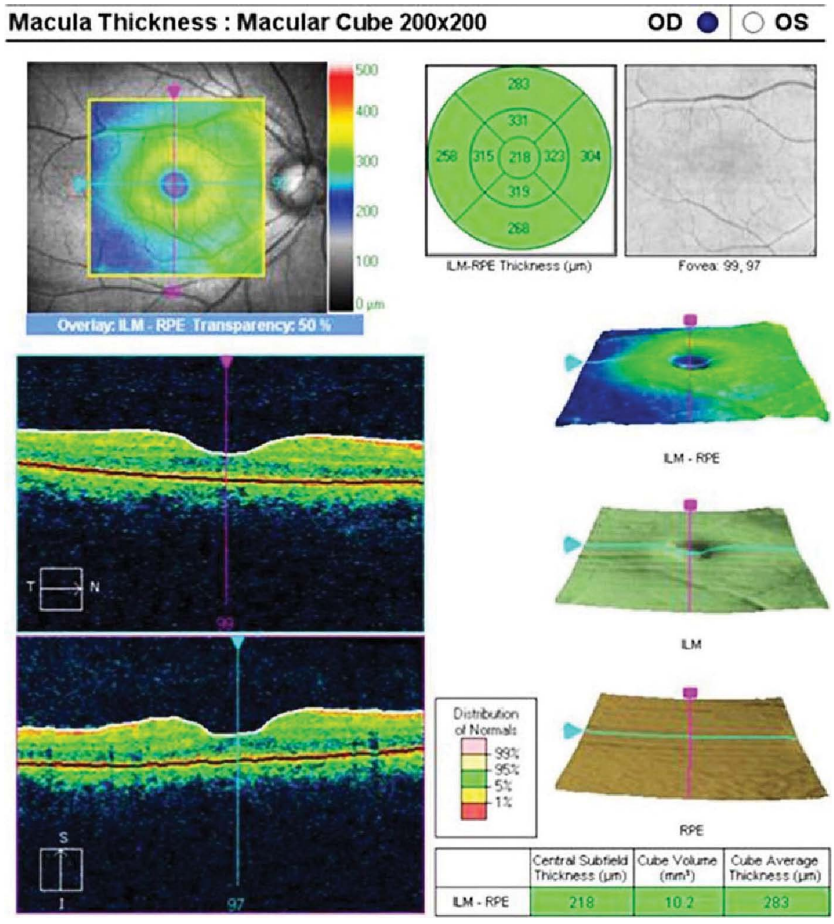

Fig. 3. Macula thickness analysis by OCT

Table 3. Change in values of RNFL thickness measurements at 30 min and 60 min from baseline values of 0 min

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

* Avg.: Average, † SD: Standard deviation.

Table 4. Change in values of macular thickness measurements at 30 min and 60 min from baseline values of 0 min

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

Table 5. Intra class correlation coefficient, coefficient of variation and test-retest variability of RNFL and Macular thickness measurements with the Cirrus HD-OCT in healthy eyes

	ICC	95 % CI		*P* value	Coefficient of variation (%)	Test retest variability
		Lower bound	Upper bound			
RNFL						
Avg RNFL	.991	.989	.992	<.001	1.01 ± 0.46	1.8 ± 0.81
RNFL symmetry	.993	.991	.994	<.001	1.18 ± 0.48	2.00 ± 0.81
RNFL Thickness S	.991	.989	.992	<.001	1.46 ± 0.79	3.49 ± 1.88
RNFL Thickness I	.995	.994	.996	<.001	1.25 ± 0.63	2.88 ± 1.48
RNFL Thickness N	.995	.994	.996	<.001	1.38 ± 0.84	1.9- ± 1.05
RNFL Thickness T	.987	.984	.989	<.001	2.38 ± 1.55	2.57 ± 1.53
Macula						
Central zone	.989	.987	.991	<.001	0.84 ± 0.42	3.89 ± 1.96
Mid zone						
Superior	.996	.995	.996	<.001	0.68 ± 0.41	4.15 ± 2.52
Inferior	.994	.993	.995	<.001	0.59 ± 0.44	3.70 ± 2.97

Table 5 (continued)

	ICC	95 % CI		*P* value	Coefficient of variation (%)	Test retest variability
		Lower bound	Upper bound			
Nasal	.999	.998	.999	<.001	0.49 ± 0.30	2.93 ± 1.99
Temporal	.991	.989	.992	<.001	0.9 ± 0.40	5.32 ± 2.54
Outer zone						
Superior	.993	.992	.994	<.001	0.40 ± 0.30	2.25 ± 1.71
Inferior	.932	.920	.943	<.001	1.10 ± 1.54	5.88 ± 8.31
Nasal	.984	.981	.987	<.001	0.64 ± 0.43	3.79 ± 2.69
Temporal	.993	.992	.994	<.001	0.57 ± 0.36	2.88 ± 1.84

* Avg.: Average, † ICC: Intra class correlation coefficient.

Table 6. Average value of RNFL and macular thickness

	Mean ± SD	Median	Min-Max	Inter quartile range
RNFL				
Avg. RNFL	90.28 ± 5.90	90.67	74.33–99.67	87.000–94.333
RNFL symmetry	84.73 ± 7.21	88.00	70.00–91.67	86.667–88.667
RNFL S	120.72 ± 11.76	123.00	98.67–145.67	108.000–126.000
RNFL I	115.95 ± 13.28	122.33	88.33–142.00	103.667–126.000
RNFL N	71.25 ± 8.71	72.67	51.67–84.00	65.000–77.333
RNFL T	55.22 ± 7.54	53.67	44.67–68.67	48.000–62.000
Macula				
Central zone	233.94 ± 12.05	238.00	212.00–251.33	223.667–246.000
Mid zone				
Superior	311.13 ± 20.95	315.00	280.67–343.33	284.500–324.000
Inferior	307.33 ± 17.30	303.00	282.67–341.33	286.833–318.667
Nasal	298.14 ± 27.55	304.33	239.33–340.67	281.000–315.667
Temporal	295.31 ± 17.83	284.67	274.33–331	277.333–308.333
Outer zone				
Superior	274.79 ± 10.02	274.00	259.67–299.67	269.667–279.167
Inferior	259.43 ± 11.26	258.67	247.67–314.67	250.333–260.333
Nasal	289.82 ± 10.61	288.00	279.00–314.67	281.333–291.000
Temporal	253.34 ± 12.05	249.50	236.67–278.67	244.000–264.000

* Avg.: Average, † SD: Standard deviation.

Table 7. Variation in RNFL and macular thickness

	Mean ± SD	Median	Min-Max	Inter quartile range
RNFL				
SD Avg RFNL	0.90 ± 0.40	1.00	0–2.31	0.577–1.000
SD RNFL symmetry	1.00 ± 0.40	1.00	0–2.52	0.577–1.155
SD RNFL S	1.74 ± 0.94	1.53	0–4.73	1.000–2.517
SD RNFL I	1.44 ± 0.74	1.16	0–3.51	1.000–2.000
SD RNFL N	0.95 ± 0.52	0.58	0–2.65	0.577–1.528
SD RNFL T	1.29 ± 0.76	1.00	0–4.93	1.000–1.732
Macula				
SD central zone	1.95 ± 0.98	1.73	0–6.08	1.528–2.517

Table 7 (continued)

	Mean ± SD	Median	Min-Max	Inter quartile range
Mid zone				
SD Superior	2.08 ± 1.26	2.00	0.58–6.66	1.000–2.646
SD Inferior	1.85 ± 1.48	1.53	0–8.08	1.000–2.082
SD Nasal	1.46 ± 1.00	1.53	0–6.11	1.000–1.528
SD Temporal	2.66 ± 1.27	2.52	1.00–7.23	1.732–3.215
Outer zone				
SD Superior	1.12 ± 0.86	1.00	0–5.13	0.577–1.528
SD Inferior	2.94 ± 4.15	1.53	0–15.88	0.577–2.082
SD Nasal	1.90 ± 1.35	1.53	0.58–7.55	1.000–2.082
SD Temporal	1.44 ± 0.92	1.00	0–5.13	0.789–1.732

***** Avg.: Average, † SD: Standard deviation.

Table 8. Correlation between mean values and standard deviation of three repeated measurements

	Spearman correlation coefficient	*P* value
RNFL		
Avg RNFL	-.172**	0.001
RNFL symmetry	-0.035	0.486
RNFL S	-0.074	0.139
RNFL I	0.043	0.389
RNFL N	-.516**	<.0005
RNFL T	.102*	0.041
Macula		
Central zone	-.321**	<.0005
Mid zone		
Superior	-0.316	<.0005
Inferior	0.218	<.0005
Nasal	0.282	<.0005
Temporal	0.140	0.005
Outer zone		
Superior	.236**	<.0005
Inferior	.695**	<.0005
Nasal	.548**	<.0005
Temporal	0.023	0.646

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

outer-zone (temporal) had a significant positive correlation with standard deviation (that is with increase in mean values, standard deviation in the readings significantly increases).

In Table 9 there was significant difference in values of males and females. Females' values were significantly lesser than male values in the following:

1) Average RNFL thickness;
2) RNFL inferior;
3) RNFL nasal;
4) RNFL temporal.

Females' values were significantly higher than males in RNFL superior and RNFL symmetry.

In Table 10 there is significant difference in values of males and females. Female values are significantly lesser than the male values.

Table 11 shows mean macular thickness was 272.95 ± 8.40 μm in females and 289.42 ± 12.32 μm in males.

Table 12 shows all the measurements except RNFL (nasal) were significantly associated with age as *P* value <0.05. It also shows negative beta coefficient that means with increase in age the value of measurements significantly decreases.

Table 13 shows multivariate regression analysis with age and gender as independent variables was performed to determine the variations in thickness measurements by gender

Table 9. Difference in gender values of RNFL thickness measurements with probability value

	Female (*N* = 220)	Male (*N* = 180)	*P* value
Avg RNFL			
Mean ± SD	87.90 ± 6.12	93.18 ± 4.04	<.0005
Median	87.33	93.33	
Min-Max	74.33–98.67	86.00–99.67	
Inter quartile range	87.000–91.333	87.667–97.500	
RNFL I			
Mean ± SD	111.62 ± 14.00	121.24 ± 10.11	<.0005
Median	103.67	122.33	
Min-Max	88.33–134.00	107.33–142.00	
Inter quartile range	101.333–126.667	108.000–124.000	
RNFL N			
Mean ± SD	67.77 ± 7.86	75.5 ± 7.77	<.0005
Median	72.33	78.33	
Min-Max	51.67–74.67	64.00–84.00	
Inter quartile range	64.333–73.333	66.333–81.667	
RNFL S			
Mean ± SD	122.29 ± 11.22	118.79 ± 12.14	<.0005
Median	125.33	123.00	
Min-Max	98.67–137.00	107.33–145.67	
Inter quartile range	120.167 — 126.333	108.000–123.333	
RNFL T			
Mean ± SD	51.87 ± 6.39	59.32 ± 6.78	<.0005
Median	48.67	61.83	
Min-Max	44.67–64.67	47.67–68.67	
Inter quartile range	47/000–57.000	49.667–64.500	
FL symmetry			
Mean ± SD	88.51 ± 1.00	80.11 ± 8.70	<.0005
Median	88.33	86.33	
Min-Max	87.00–91.67	70.00–90.00	
Inter quartile range	88.000–89.000	70.667–88.000	

* Avg.: Average, † SD: Standard deviation.

Table 10. Difference in gender values of macular thickness measurements with probability value

	Female (*N* = 220)	Male (*N* = 180)	*P* value
Central zone			
Mean ± SD	228.61 ± 10.64	240.45 ± 10.38	<.0005
Median	224.33	246ю00	
Min-Max	212.00–249.67	222.67–251.33	
Inter quartile range	222.333–238.000	223.667–246.333	
Mid zone			
Inferior			
Mean ± SD	297.21 ± 13.05	319.71 ± 13.38	<.0005
Median	297.33	318.67	
Min-Max	282.67–320.00	301.67–341.33	
Inter quartile range	285.667–310.667	303.000–337.000	

Table 10 (continued)

	Female (*N* = 220)	Male (*N* = 180)	*P* value
Nasal			
Mean ± SD	296.36 ± 15.12	300.32 ± 37.46	<.0005
Median	303.33	315.67	
Min-Max	280/00–318.67	239.33–340.67	
Inter quartile range	281.000–305.333	240.333–325.333	
Superior			
Mean ± SD	294.79 ± 12.36	331.11 ± 7.88	<.0005
Median	297.00	333.67	
Min-Max	280.67–315.67	322.33–343.33	
Inter quartile range	283.667–304.333	324.000–339.667	
Temporal			
Mean ± SD	285.63 ± 12.27	307.14 ± 16.39	<.0005
Median	283.33	308.83	
Min-Max	274.33–307.67	282.67–331.000	
Inter quartile range	276.333–295.667	284.000–323.667	
Outer zone			
Inferior			
Mean ± SD	252.29 ± 4.46	268.16 ± 10.90	<.0005
Median	250.33	268.83	
Min-Max	247.67–276.33	258.33–314.67	
Inter quartile range	249.667–255.667	259.000–277.833	
Nasal			
Mean ± SD	286.88 ± 7.72	293.41 ± 12.43	<.0005
Median	286.67	288.17	
Min-Max	279.00–312.67	281.33–314.67	
Inter quartile range	279.667–291.000	282.667–311.000	
Superior			
Mean ± SD	269.21 ± 7.03	281.62 ± 8.83	<.0005
Median	270.00	279.67	
Min-Max	259.67–294.67	274.00–299.67	
Inter quartile range	261.000–272.333	275.333–292.833	
Temporal			
Mean ± SD	245.56 ± 7.44	262.85 ± 9.52	<.0005
Median	244.00	264.83	
Min-Max	236.67–260.00	247.67–278.67	
Inter quartile range	239.000–246.000	250.000–268.333	

* Avg.: Average, † SD: Standard deviation.

Table 11. Variation in Macular thickness with gender

	Female (*N* = 220)	Male (*N* = 180)	*P* value
Mean macular thickness			
Mean ± SD	272.95 ± 8.40	289.42 ± 12.32	<.0005
Median	270.70	289.46	
Min-Max	265.00–288.19	272.30–307.78	
Inter quartile range	266.30–275.04	272.67–305.22	

Table 12. Univariate linear regression with age

	Unstandardized coefficients		*P* value	95.0 % confidence interval for B	
	B	Std. error		Lower bound	Upper bound
Avg RNFL	-.190	.022	<.0005	-.233	-.148
RNFL symmetry	-.059	.029	.041	-.115	-.002
RNFL S	-.121	.047	.010	-.213	-.030
RNFL I	-0.769	.037	<.0005	-0.841	-0.697
RNFL N	.007	.035	.839	-.061	.076
RNFL T	-.223	.028	<.0005	-.278	-.168

* Avg.: Average, † B: Scan taken at 30 minutes.

Table 13. Multivariate regression with age and gender

		Unstandardized coefficients		*P* value	95.0 % confidence interval for B	
		B	Std. error		Lower bound	Upper bound
Avg RNFL	Age	-.213	.018	<.0005	-.249	-.177
	Sex	5.811	.462	<.0005	4.903	6.719
RNFL symmetry	Age	-.027	.024	.261	-.073	.020
	Sex	-8.332	.594	<.0005	-9.500	-7.164
RNFL S	Age	-.109	.046	.020	-.200	-.017
	Sex	-3.237	1.169	.006	-5.535	-0.939
RNFL I	Age	-0.814	.029	<.0005	-0.870	-0.757
	Sex	11.615	0.720	<.0005	10.200	13.030
RNFL N	Age	-.023	.031	.463	-.085	.039
	Sex	7.784	.790	<.0005	6.230	9.338
RNFL T	Age	-.254	.023	<.0005	-.299	-.208
	Sex	8.075	.582	<.0005	6.931	9.218

* Avg.: Average, † B: Scan taken at 30 minutes.

when controlled for age and the associations of age with mean macular thickness, when controlled for gender. All the associations were coming significant except RNFL (nasal) with age, and RNFL symmetry with age.

1) In RNFL (superior) and RNFL symmetry female values were significantly higher than males after adjusting for age.

2) Female values were significantly lesser than male values after adjusting for age in the following:

Average RNFL:

· RNFL (inferior),

· RNFL (nasal),

· RNFL (temporal).

3) Average RNFL, RNFL (superior), RNFL (inferior) and RNFL (temporal) significantly decreases with increase in age after adjusting for gender.

Table 14 shows all the macular thickness measurements were significantly associated with age as *P* value <0.05. It also shows negative beta coefficient that means with increase in age the value of macular thickness measurements significantly decreases.

Table 15 shows multivariate regression analysis with age and gender as independent variables was performed to determine the variations in macular thickness measurements by gender when controlled for age and the associations of age with mean macular thickness when controlled for gender. All the associations came out to be significant.

DISCUSSION

Our sample size of 400 eyes of healthy subjects is reasonably large and statistically acceptable with Cirrus HD-OCT. Cirrus HD-OCT, with an 840-nm super luminescent diode as an optical source, acquires 27 000 A-scans/s. Since the acquisition speed of spectral domain OCTs such as the Cirrus HD-OCT is high, images can be taken at extremely low light exposures. Repeated measurements on normal healthy eyes may be safe because the power incident of the scan on the eye is limited to less than 725 μw, which is within the American National Standards Institute maximum permissible exposure limit for continuous exposure at that wavelength [8]. The Cirrus HD-OCT shows probability code results of RNFL thickness using a white-green-yellow-red colour code. For instance, when the thinnest 1 % of a normal age-matched population has a similar RNFL thickness, the red code ("outside normal limits") is indicated. Yellow code represents "suspect" (1 % ≤ yellow < 5 %), green code represents "normal" (5 % ≤ green ≤ 95 %), and white code represents the thickest 5 % of the population (white > 95 %). Theoretically, if the

Table 14. Univariate linear regression with age for Macular thickness

	Unstandardized coefficients		*P* value	95.0 % confidence interval for B	
	B	Std. error		Lower bound	Upper bound
Central zone	-.540	.040	<.0005	-.618	-.461
Mid zone					
Superior	-.450	.081	<.0005	-.609	-.291
Inferior	-.795	.057	<.0005	-.906	-.684
Nasal	-1.585	.076	<.0005	-1.735	-1.435
Temporal	-.825	.058	<.0005	-.940	-.711
Outer zone					
Superior	-.209	.039	<.0005	-.285	-.133
Inferior	-.312	.042	<.0005	-.395	-.229
Nasal	-.656	.027	<.0005	-.709	-.603
Temporal	-.371	.044	<.0005	-.459	-.284

* Std. Error: Standard Error, † B: Scan taken at 30 minutes.

Table 15. Multivariate regression with age and gender for macular thickness

		Unstandardized coefficients		*P* value	95.0 % confidence interval for B	
		B	Std. error		Lower bound	Upper bound
Central zone	Age	-.591	.030	<.0005	-.650	-.532
	Sex	13.288	.758	<.0005	11.798	14.778
Mid zone						
Superior	Age	-.596	.030	<.0005	-.656	-.537
	Sex	37.782	.760	<.0005	36.289	39.276
Inferior	Age	-.890	.029	<.0005	-.947	-.834
	Sex	24.673	.719	<.0005	23.259	26.088
Nasal	Age	-1.616	.075	<.0005	-1.764	-1.468
	Sex	7.921	1.891	<.0005	4.203	11.640
Temporal	Age	-.917	.034	<.0005	-.985	-.850
	Sex	23.752	.860	<.0005	22.061	25.443
Outer zone						
Superior	Age	-.260	.029	<.0005	-.316	-.203
	Sex	13.047	.728	<.0005	11.616	14.477
Inferior	Age	-.377	.026	<.0005	-.429	-.326
	Sex	16.794	.657	<.0005	15.501	18.086
Nasal	Age	-.688	.021	<.0005	-.730	-.645
	Sex	8.212	.540	<.0005	7.150	9.275
Temporal	Age	-.443	.026	<.0005	-.493	-.392
	Sex	18.381	.645	<.0005	17.113	19.650

* Std. Error: Standard Error, † B: Scan taken at 30 minutes.

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

Reproducibility is a crucial reliability index in any OCT system as reproducibility of the measurements is very important for diagnosis of disease and monitoring of disease progression. In our study for average RNFL thickness, the ICC (Intra class correlation coefficient) value is 0.99 and the test-retest variability is 1.8 μm. For quadrants, the ICC values of the Cirrus HD-OCT ranged from 0.991 superior to 0.995 inferior and 0.995 nasal to 0.987 temporal. CV's (coefficient of variation) were 1.46 % superior, 1.25 % inferior, 1.38 % nasal and 2.38 % temporal. Therefore, temporal sector has the least reliability as compared to other sectors.

In this study for quadrants, the ICC values of the Cirrus HD-OCT ranged from 0.98 to 0.99, whereas previous studies by other authors [9, 10], using Stratus TD-OCT ranged from 0.67 to 0.97. The differences between the two systems become more prominent in the nasal sector because the nasal

ICC values of the Stratus TD-OCT (range, 0.67 to 0.88) were lower than those of the Cirrus HD-OCT (0.99), and the nasal test-retest variability of the Stratus TD-OCT (16.0 μm) was much larger than that of the Cirrus HD-OCT (1.9 μm).

For macular thickness central zone, ICC was 0.989, CV was 0.84 %, and test-retest variability was 3.89 μm. For mid-zone, ICC ranged from 0.996 superior to 0.994 inferior and 0.999 nasal to 0.991 temporal. CV's were 0.68 % superior, 0.59 % inferior, 0.49 % nasal and 0.90 % temporal. Test-retest variability ranged from 4.15 superior to 3.70 inferior μm and 2.93 nasal to 5.32 temporal μm. For outer-zone, ICC ranged from 0.993 superior to 0.932 inferior and 0.984 nasal to 0.993 temporal. CV's were 0.40 % superior, 1.10 % inferior, 0.64 % nasal and 0.57 % temporal. Test-retest variability ranged from 2.25 superior to 5.88 inferior μm and 3.79 nasal to 2.88 temporal μm. Therefore our study shows that in RNFL thickness, temporal sector shows the worst reproducibility as compared to other sectors which is not in agreement with the study conducted by Samin Hong et al. in 2010 [4]. For macular thickness, Temporal sector (mid-zone) showed the worst reproducibility and in Outer-zone, Inferior sector showed the worst reproducibility.

In this study, our results show a mean macular thickness of 272.95 ± 8.40 μm (females) and 289.42 ± 12.32 μm (males) and mean macular thickness of central zone (foveal thickness) of 228.61 ± 10.64 μm (females) and 240.45 ± 10.38 μm (males). Sull AC et al. in 2010 conducted a study that has shown a decrease in macular thickness with age [9]. A.C. Sull et al. in 2010 [9], A. Chan et al. in 2006 [10], S. Grover et al. in 2009 [11], have shown no association of macular thickness with age and / or gender, suggesting that studies comparing macular thickness measurements should carefully control for age-based and gender-based variations [12, 13]. Our results also showed an association with age, that is decrease in macular thickness with age, which is in agreement with the study conducted by Duan XR et al. in 2010 [14]. It also shows that male gender to be associated with greater macular thickness (central zone) and mean macular thickness as compared to female gender in healthy eyes which is in agreement with the study conducted by Mehreen Adhi et al. in 2012 [7]. Thus, demographic variations may be important parameters when comparing macular thickness measurements, and diagnosing and monitoring macular pathologies. The literature shows that of the commercially available OCT system, a documented variability in macular thickness measurements which has been demonstrated by A.C. Sull et al. in 2010 [9], J.E. Legarreta et al. in 2008 [15], C.K. Leung et al. in 2008 [16] and A. Giani et al. in 2008 [17]. Stratus TD-OCT selects the inner segment / outer segment junction as the outer retinal boundary for macular thickness measurements, spectral domain OCT system select RPE as the outer retinal boundary for thickness measurements, thus leading to an increase in macular thickness reported with these OCT systems, when compared with the TD-OCT systems.

The present study concludes that:

1. RNFL and macular thickness measurements by SD-OCT by the same observer on the same day at 0 minutes, 30 minutes and 60 minutes were very reproducible in normal healthy eyes; except in the sectors specifically mentioned.
2. The RNFL thickness in temporal sector had worst reproducibility as compared to other sectors.
3. The average mean thickness values of RNFL, that of nasal and temporal sectors had a significant negative correlation with standard deviation (that is with an increase in thickness values, standard deviation in the readings significantly decreases).
4. Superior and inferior RNFL sectors showed no correlation with standard deviation. In other words, the greater the thickness of RNFL in any sector, the better will be the reproducibility in that sector.
5. For macular thickness, temporal sector (mid-zone) showed the worst reproducibility and that in outer-zone, Inferior sector showed the worst reproducibility.
6. An association of macular thickness with age that is, with increase in age the macular thickness measurements decreases.
7. The male gender was associated with greater macular thickness (central zone) and mean macular thickness as compared to female gender in healthy eyes.

LIMITATIONS

Our study has showed that in RNFL thickness evaluation, temporal sector had worst reproducibility, which is thin naturally as compared to other sectors. Therefore, the question arises whether in diseases such as glaucoma and neurodegenerative disorders where RNFL thinning occurs, will the reproducibility be good or not? This suggests that reproducibility in diseased states needs to be evaluated separately by any future investigator.

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