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Effect of Refractive Correction Error on Retinal Nerve Fiber Layer Thickness: A Spectralis Optical Coherence Tomography Study

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Background: Identifying and assessing retinal nerve fiber layer defects are important for diagnosing and managing glaucoma. We aimed to investigate the effect of refractive correction error on retinal nerve fiber layer (RNFL) thickness measured with Spectralis spectral-domain optical coherence tomography (SD-OCT).

Material/Methods: We included 68 participants: 32 healthy (normal) and 36 glaucoma patients. RNFL thickness was measured using Spectralis SD-OCT circular scan. Measurements were made with a refractive correction of the spherical equivalent (SE), the SE+2.00D and the SE–2.00D.

Results: Average RNFL thickness was significantly higher in the normal group (105.88±10.47 μm) than in the glaucoma group (67.67±17.27 μm, $P<0.001$). In the normal group, +2.00D of refractive correction error significantly affected measurements of average ($P<0.001$) and inferior quadrant ($P=0.037$) RNFL thickness. In the glaucoma group, +2.00D of refractive correction error significantly increased average ($P<0.001$) and individual quadrant (superior: $P=0.016$; temporal: $P=0.004$; inferior: $P=0.008$; nasal: $P=0.003$) RNFL measurements compared with those made with the proper refractive correction. However, –2.00D of refractive correction error did not significantly affect RNFL thickness measurements in either group.

Conclusions: Positive defocus error significantly affects RNFL thickness measurements made by the Spectralis SD-OCT. Negative defocus error did not affect RNFL measurement examined. Careful correction of refractive error is necessary to obtain accurate baseline and follow-up RNFL thickness measurements in healthy and glaucomatous eyes.

MeSH Keywords: **Glaucoma • Refractive Errors • Retina • Tomography, Optical Coherence**

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Background

Glaucoma is a progressive optic neuropathy that can result in irreversible blindness [1,2]. The loss of visual function from glaucoma is directly caused by structural damage to retinal ganglion cells and their axons, resulting in retinal nerve fiber layer (RNFL) loss [3,4]. An RNFL defect precedes measurable optic nerve head damage and visual field loss [5,6]. Therefore, identifying and assessing retinal nerve fiber layer defects are important for diagnosing and managing glaucoma [7].

Optical coherence tomography (OCT) is an interferometric imaging modality designed to provide high-quality cross-sectional images of biological tissues *in vivo* [8,9]. Since its introduction, it has become widely used to objectively and quantitatively evaluate RNFL thickness changes in eyes with glaucoma [10–14]. The recent development of spectral-domain (SD) OCT has increased image acquisition speed (more than 100-fold) and axial resolution (from 10 μm to <5 μm). However, even with these impressive advancements, recent studies have shown that SD-OCT imaging artifacts remain common in clinical practice [15–18]. Patient-dependent factors, including dry eye [19], cataract [20–22], eye movements [23], head tilt [16,17], and even vitreous floaters [24,25], could affect scan quality and produce imaging artifacts. Furthermore, operator-dependent factors such as OCT lens opacities [15] and device-dependent factors such as inaccurate retinal segmentation [26] could also cause imaging artifacts. Production of some imaging artifacts could be decreased and avoided by careful observation of the live image, standardizing operation of the device, and application of artificial tears [15–17,19,23–25], but others still are difficult to overcome [20–22]. Imaging artifacts lead to incorrect comparisons to the normative database and affect glaucoma diagnoses. Therefore, it is important to identify the various OCT imaging artifacts and to critically evaluate examination results.

In routine clinical practice, OCT images are sometimes obtained in subjects who have not had their refractive error properly corrected; whether refractive correction error causes SD-OCT imaging artifacts is unknown. Therefore, this study investigates the effect of refractive correction error on RNFL thickness measurements made with the Spectralis SD-OCT system in healthy and glaucomatous eyes.

Material and Methods

Study subjects and examinations

All study participants were recruited at the Department of Ophthalmology at the First Hospital of China Medical University between September and December 2015. All study conduct adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants after they received an explanation of the nature of the study and risks/benefits of participation. The study was approved by the Ethics Committee of the First Hospital of China Medical University.

Subjects were included in the study if all of the following were true: at least 18 years of age, spherical equivalent (SE) was between –3.00D and +3.00D, cylindrical refractive error was <1.50D, and best-corrected visual acuity (BCVA) was at least 20/40. Subjects were excluded from participation if they had a history of ocular trauma or surgery, active corneal disease, media opacity (mild cataract acceptable), or other retinal and/or nonglaucomatous optic nerve disease (e.g., diabetic retinopathy and ischemic optic neuropathy).

Participants included normal individuals (normal group) and glaucoma patients (glaucoma group). Normal individuals had no significant ocular disease (mild cataract acceptable), intraocular pressure (IOP) ≤21 mm Hg, reliable and normal visual field (VF) test results (pattern standard deviation >5% and glaucoma hemifield test within normal limits [27]), and a normal fundus examination. Glaucoma patients included those with any form of primary glaucoma, as determined using the criteria of the European Glaucoma Society [28]. One eye was randomly selected as the study eye in the normal group using a random number generator statistical table. In the glaucoma group, the more damaged eye was selected as the study eye.

Each participant underwent a routine ophthalmic examination, which included evaluating the patient's history, BCVA, refractive error without pupil dilation (autorefractor, Auto-Kerato-Refractometer KR-8900, Topcon, Tokyo, Japan), IOP (non-contact tonometer, Topcon CT-80, Topcon, Tokyo, Japan), axial length (MD-2300 A/B ultrasound, Meda, Tianjin, China), and central corneal thickness (ultrasonic pachymetry, PacScan 300P, Sonomed Escalon, Pennsylvania, USA). Examinations with slit-lamp biomicroscopy, gonioscopy, fundoscopy, and VF testing (Swedish Interactive Threshold Algorithm 30-2 algorithm, Humphrey visual field analyzer II 750, Carl Zeiss Meditec, Dublin, California, USA) were also performed.

Peripapillary retinal nerve fiber layer thickness measurements

All peripapillary RNFL thickness measurements were made by an experienced examiner using the Spectralis SD-OCT (Spectralis HRA + OCT; Heidelberg Engineering, Heidelberg, Germany) circular RNFL scan (Spectralis software version 6.9.0.0). The scan circle was 12 degrees in diameter (approximately 3.5 to 3.6 mm for a typical eye), with a total of 768 A-scans taken around the optic disc. Eye movements were compensated for by an eye-tracking system (TruTrack™ active eye tracking), and speckle noise was reduced by the Automatic Real-Time function. Measurement variability caused by differences in head positioning and/or tilting between OCT examinations was reduced by aligning the fovea-to-disc axis to previous scans (FoDi™ alignment technology). After selecting any prior scan as a reference scan, the OCT system automatically aligned the new fundus image to the selected reference fundus image, and the scan was then positioned to the original reference scan (AutoRescan™ automatic follow-up scan placement).

The Spectralis OCT uses the signal-to-noise ratio (SNR) to quantify image scan quality on a scale from 0 to 40 dB. As suggested by the manufacturer, acceptable scan quality was defined as an SNR >15 dB. However, a previous study also showed that additional criteria are important when determining scan quality [29] and the following criteria also had to be met for scan inclusion: clear fundus image with optic disc and scan circle visualization before and during image acquisition, visible RNFL with no interruptions, and a continuous scan pattern with no missing or blank areas. Scan quality was assessed during and immediately after image acquisition, and when necessary, scans were retaken until the scan quality met the criteria.

Study subjects first underwent a routine circular peripapillary scan with proper SE refractive error correction (baseline scan). The internal fixation target was used. The Spectralis OCT was put into "follow-up" mode and the first scan was defined as the subject's reference scan. The circular peripapillary scan was then repeated at the reference location when the subject refraction was corrected by SE correction –2.00D (SE–2D) and SE correction +2.00D (SE+2D). Two scans were performed in random order.

The Spectralis OCT software automatically performed RNFL segmentation. When segmentation errors were made, an experienced examiner manually controlled and corrected RNFL boundaries. Following segmentation, the Spectralis OCT software calculated average RNFL thickness (A, 360 degrees), quadrant RNFL thickness (superior [S], temporal [T], inferior [I], and nasal [N], 90 degrees], and additional sector RNFL thickness (superotemporal [TS, 45 to 90 degrees], superonasal [NS, 90 to 135 degrees], inferonasal [NI, 225 to 270 degrees], and inferotemporal [TI, 270 to 315 degrees]).

Statistical analyses

Statistical analyses were performed using SPSS Statistics (version 19.0, IBM Corp., Armonk, New York, USA). The Kolmogorov-Smirnov test was used to assess sample distribution. Differences in participant demographic and ocular characteristics were evaluated using chi-square tests for categorical variables and unpaired, two-tailed Student's t-tests for continuous variables. Analyses of variance was used to evaluate differences in RNFL thickness measurements and scan quality between the normal and glaucoma groups. The effects of refractive error on RNFL thickness measurements and scan quality were evaluated with two-tailed, paired Student's t-tests. Bland-Altman plots were used to assess agreement of average RNFL thickness measurements between SE correction and refractive correction error. The agreement was determined using calculations of mean bias and limits of agreement (LoA, defined as mean difference ±1.96× standard deviation of difference). Statistical significance was defined as $P<0.05$.

Results

A total of 68 participants (32 normal subjects, 36 glaucoma patients) were included in the study. In the glaucoma group, 7 subjects (19.44%) had primary open- angle glaucoma and 29 subjects (80.56%) had primary closed-angle glaucoma. Participant demographic and ocular characteristics are summarized in Table 1. Subject age, subject sex, refractive error, and the number of right eyes were not significantly different between the normal and glaucoma groups. However, VF mean deviation (MD, $P<0.001$) and pattern standard deviation ($P<0.001$) were significantly different between groups.

Average, quadrant, and sector peripapillary RNFL measurements of thickness were compared between the normal and glaucoma groups (Table 2). Average RNFL thickness was 105.88±10.47 µm in the normal group, which was significantly higher than that in the glaucoma group (67.67±17.27 µm, $P<0.001$).

Measurements of RNFL thickness were compared between baseline, SE+2.00D refractive correction, and SE–2.00D refractive correction scans (Table 3). For all examined eyes (n=68 eyes), RNFL thickness measurements were significantly higher for SE+2.00D scans than for baseline scans for average ($P<0.001$), quadrant (S: $P=0.005$; T: $P=0.003$; I: $P=0.001$; and N: $P=0.001$), and nearly all sector (TS: $P=0.041$; NS: $P=0.021$; NI: $P=0.001$; and TI: $P=0.609$) regions. However, there was no significant difference between baseline and SE–2.00D scans for any region examined. When normal and glaucomatous patients were examined separately, average ($P<0.001$) and inferior quadrant ($P=0.037$) RNFL thickness measurements were significantly different between baseline and SE+2.00D scans in normal eyes. In eyes with glaucoma, SE+2.00D RNFL thickness measurements were significantly higher than baseline measurements for average ($P<0.001$) and all four quadrant (S: $P=0.016$, T: $P=0.004$, I: $P=0.008$, and N: $P=0.003$) measurements. There were no significant differences between baseline and SE–2.00D scans for any region examined in normal and glaucomatous eyes except inferior quadrant in normal eyes ($P=0.048$).

Table 1. Patient demographic and ocular characteristics.

Parameter	Normal group	Glaucoma group	*P**
Number (eye, subjects)	32	36	–
Right eyes (% total)	53.1%	47.2%	0.808
Female (% total)	43.8%	55.6%	0.466
Age (years)	53.19±12.29	53.72±10.95	0.851
Spherical equivalent (D)	–0.29±1.41	–0.28±0.80	0.971
Axial length (mm)	23.00±1.05	22.96±0.90	0.867
VF mean deviation (dB)	–1.37±2.22	–13.65±8.45	<0.001
VF pattern standard deviation (dB)	2.19±1.09	5.66±2.91	<0.001

* Statistical comparisons made using chi-square tests for categorical variables and unpaired t-tests for continuous variables.
VF – visual field.

Table 2. Retinal nerve fiber layer thickness in normal and glaucomatous eyes.

	RNFL thickness (µm)		
	Normal group (n=32 eyes)	Glaucoma group (n=36 eyes)	*P*
Average	105.88±10.47	67.67±17.27	<0.001
Superior quadrant	134.31±18.57	82.24±22.93	<0.001
Temporal quadrant	76.53±12.92	57.58±14.56	<0.001
Inferior quadrant	134.00±16.02	79.99±28.26	<0.001
Nasal quadrant	78.66±18.24	50.83±14.84	<0.001
TS sector	146.50±16.90	83.53±22.36	<0.001
TI sector	146.81±24.69	86.56±35.47	<0.001
NS sector	122.13±28.05	80.94±27.64	<0.001
NI sector	121.19±22.71	73.41±26.86	<0.001

TS – superotemporal; TI – inferotemporal; NS – superonasal; NI – inferonasal.

Bland-Altman plots indicated that the mean difference in average RNFL thickness measured with proper SE correction and an induced refractive error of SE–2.00D was 0.35 µm (95% LoA interval: –3.08 to 3.79 µm), 0.56 µm (–2.81 to 3.94 µm), and 0.17 µm (–3.24 to 3.66 µm) in all eyes, normal eyes, and glaucomatous eyes, respectively. However, the mean difference in average RNFL thickness measured with proper SE correction and an induced refractive error of SE+2.00D was higher and was 1.10 µm (95% LoA interval: –1.95 to 4.15 µm), 0.86 µm (–1.39 to 3.14 µm), and 1.31 µm (–2.29 to 4.90 µm) in all eyes, normal eyes, and glaucomatous eyes, respectively. These results are illustrated in Figure 1.

Image scan quality was significant lower than that of baseline scans for SE+2D scans ($P<0.001$), but not for SE–2D scans ($P=0.187$), when all eyes were examined together. This was also true for scans obtained from normal (+2.00D: $P<0.001$; –2.00D: $P=0.138$) and glaucomatous (+2.00D: $P<0.001$; –2.00D: $P=0.645$) eyes (Table 4). The quality of OCT images obtained from the glaucoma group was similar to that obtained for the normal group when eyes were properly corrected ($P=0.071$) and when the eye had a –2.00D defocus ($P=0.317$). However, image quality was significantly lower in the glaucoma group than in the normal group, with a refractive correction error of +2.00D ($P=0.002$).

Discussion

In this study, we investigated whether an incorrect refractive correction influenced RNFL thickness measurements obtained with the Spectralis SD-OCT in healthy and glaucomatous eyes. To the best of our knowledge, this type of study has not yet been published. Interestingly, we found that RNFL thickness measurements were significantly affected by a +2.00D refractive correction error, but not a –2.00D refractive correction error, in

Table 3. Retinal nerve fiber layer thickness measurements for different refractive correction.

RNFL thickness (µm)	SE	SE+2.00 D	*P**	SE–2.00 D	*P**
Normal eyes					
Average	105.88±10.47	**106.75±10.26**	**<0.001**	106.44±10.79	0.074
Superior	134.31±18.57	135.19±18.41	0.108	134.59±19.53	0.65
Temporal	76.53±12.92	77.09±12.68	0.22	76.66±12.11	0.801
Inferior	134.00±16.02	**134.72±15.97**	**0.037**	**134.72±16.23**	**0.048**
Nasal	78.66±18.24	79.94±17.78	0.061	79.44±18.01	0.424
TS sector	146.50±16.90	147.00±17.03	0.344	147.59±17.21	0.124
TI sector	146.81±24.69	146.69±24.03	0.878	147.59±24.15	0.331
NS sector	122.13±28.05	123.38±27.96	0.089	121.59±28.69	0.638
NI sector	121.19±22.71	**122.75±22.87**	**0.033**	121.84±22.53	0.439
Glaucomatous eyes					
Average	67.67±17.27	**68.97±16.93**	**<0.001**	67.83±17.51	0.567
Superior	82.24±22.93	**83.26±22.65**	**0.016**	82.29±23.27	0.864
Temporal	57.58±14.56	**59.41±14.24**	**0.004**	57.72±14.96	0.764
Inferior	79.99±28.26	**80.96±28.26**	**0.008**	79.82±28.86	0.776
Nasal	50.83±14.84	**52.31±14.55**	**0.003**	51.25±14.63	0.434
TS sector	83.53±22.36	84.69±22.27	0.062	83.86±22.45	0.499
TI sector	86.56±35.47	87.08±35.29	0.17	86.28±36.21	0.7
NS sector	80.94±27.64	81.83±27.53	0.115	80.72±28.14	0.689
NI sector	73.41±26.86	**74.83±27.19**	**0.017**	73.36±27.11	0.932

* Statistical comparison made to SE correction measurement using paired t-test. **Bolded** values were significantly different from SE correction. RNFL – retinal nerve fiber layer; SE – spherical equivalent; TS – superotemporal; TI – inferotemporal, NS – superonasal; NI – inferonasal.

both normal and glaucomatous eyes. Moreover, a larger number of RNFL thickness measurement areas were significantly affected by the +2.00D refractive correction error in glaucomatous eyes than in normal eyes (Table 3, Figure 1).

It is known that RNFL thickness varies with distance and direction from the optic nerve head (ONH) center [30–32] and that the size and position of the OCT scan circle heavily influence measurements of peripapillary RNFL thickness. In this study, the Spectralis OCT scan circle was fixed 12 degrees in diameter (Figure 2A), which changes with refractive error and axial length (typically 3.5 to 3.6 mm in an emmetropic eye). When the eye had a refractive overcorrection of +2.00D, it means there was a +2.00D convex lens in front of the eye. This additional lens power would focus the scan circle further in front of the retina, causing the scan circle to shrink in the retinal plane and be less than 12 degrees in diameter (Figure 2B). It has been well documented that ganglion cell fibers rapidly become thicker as they approach the optic disc [32]. Because a smaller scan circle results from this additional added power [33], an overestimation of peripapillary RNFL thickness likely resulted.

Systematic, device-dependent errors may result when refractive error is positive. Balasubramanian et al. [34] found that Spectralis SD-OCT retinal thickness measurements increased as image quality decreased in all eyes examined. This indicates that image quality metrics should be considered when evaluating SD-OCT thickness measurements [35]. Therefore, we compared image quality when patients were corrected for different refractive errors. We demonstrated that image quality was significantly lower when eyes had a +2.00D refractive correction error than when they were properly corrected. This was true when all eyes were examined together and when normal and glaucomatous eyes were examined separately.

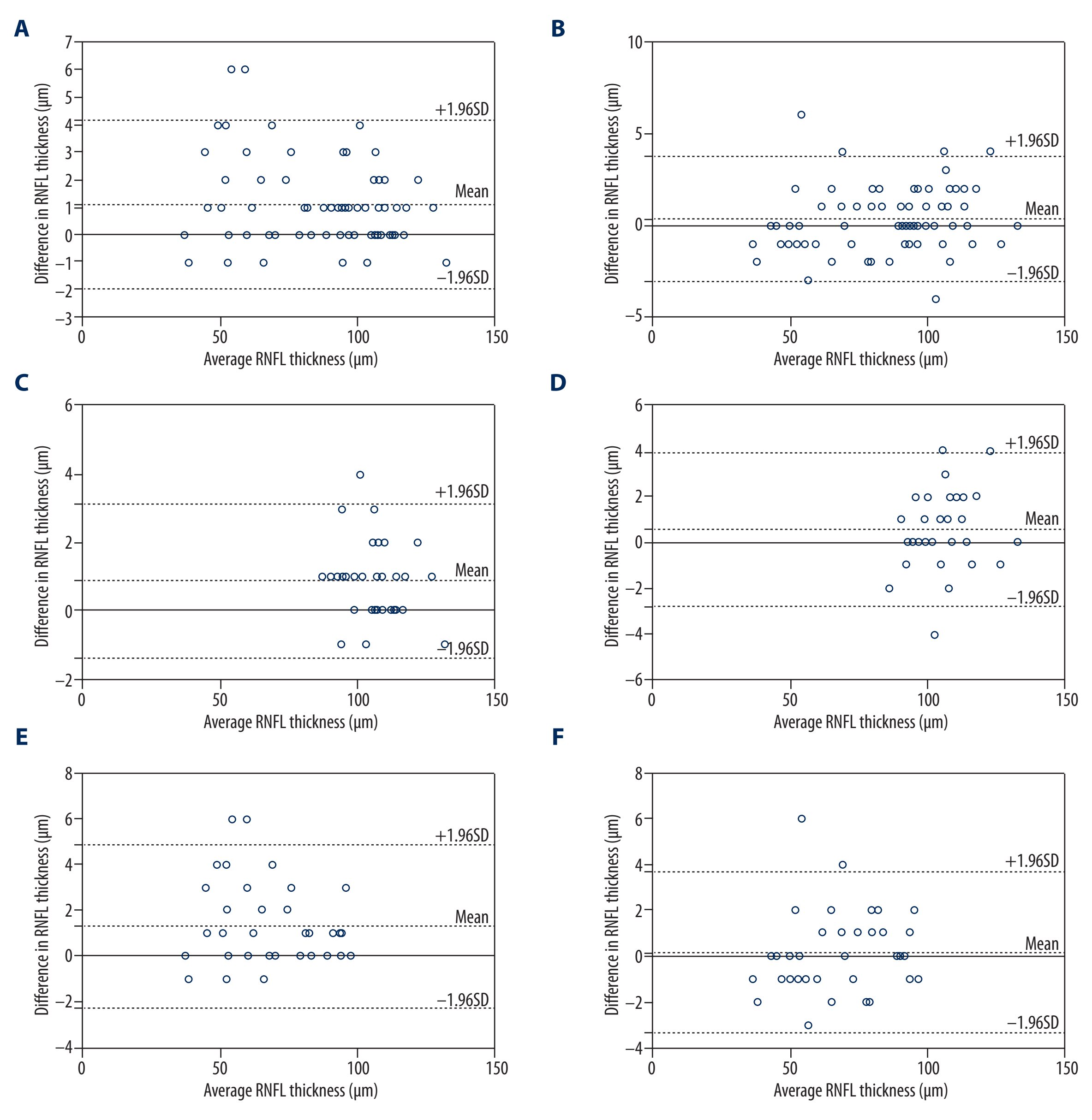

Figure 1. Bland-Altman plots showing differences in average RNFL thickness measured with the proper and improper refractive error correction. Refractive error correction of the spherical equivalent (SE) versus the following: SE+2.00D in all study eyes (**A**), SE–2.00D in all study eyes (**B**), SE+2.00D in normal eyes (**C**), SE–2.00D in normal eyes (**D**), SE+2.00D in glaucomatous eyes (**E**), and SE–2.00D in glaucomatous eyes (**F**). RNFL, retinal nerve fiber layer.

Therefore, image quality differences may have contributed to the increase in RNFL thickness observed with a SE+2.00D refractive correction. Image quality was also compared between normal and glaucomatous eyes and showed that image quality was similar in the normal and glaucoma groups when eyes were correctly refracted. However, when eyes had a +2.00D refractive correction error, imaging quality was significantly lower in the glaucoma group than in the normal group (Table 4). Previous studies [23,36] also found that image quality was lower in the glaucoma group than in the normal group. The reason for this is not clear and may be because glaucoma patients have decreased fixation stability [37,38] and their eye movements could affect image quality [23]. Moreover, when eyes had a +2.00D overcorrection, the internal fixation target became blurred and the fixation stability decreased further. This observation may explain why RNFL thickness was more significantly affected by the +2.00D refractive correction error in glaucomatous eyes than in normal eyes.

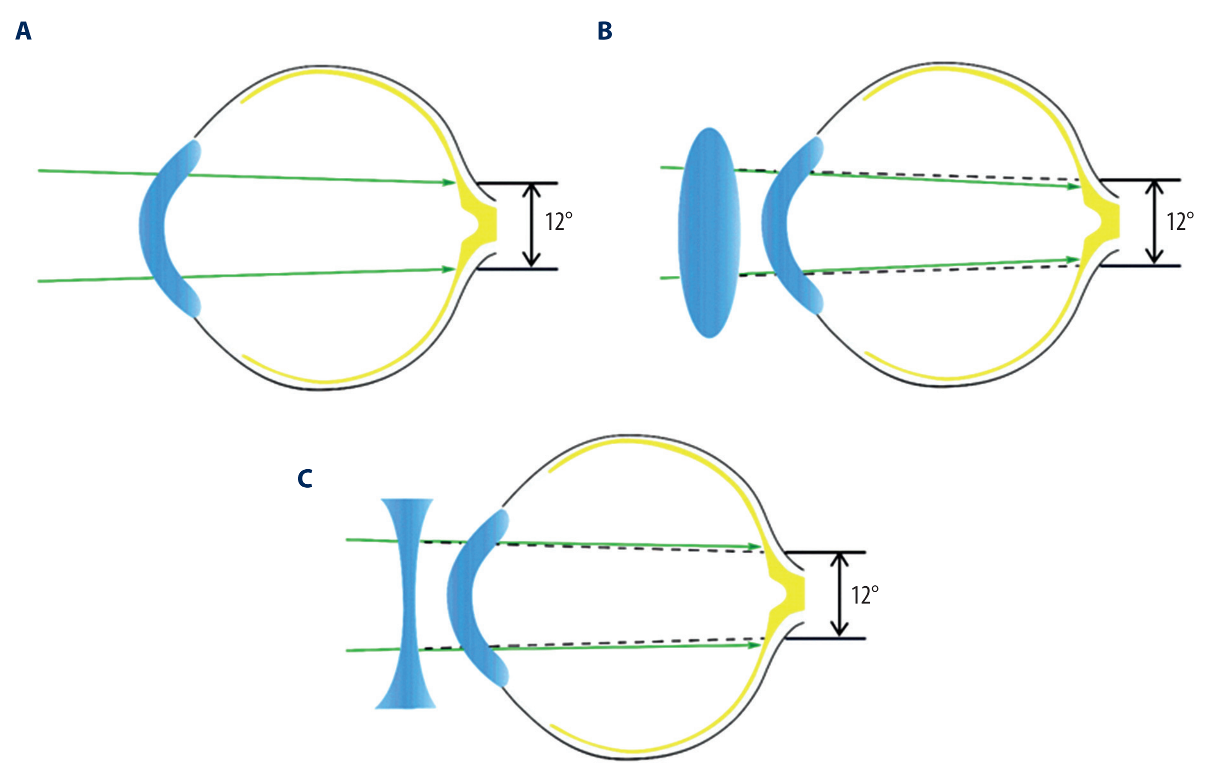

Figure 2. Diagram comparing the effect of positive and negative overcorrection on the scanning circle. (**A**) When proper spherical equivalent correction (SE) is applied, the scan circle diameter is 12 degrees. (**B**) When a SE+2.00D correction is applied, light is focused further in front of the retina, causing scan circle diameter to be <12 degrees. (**C**) When an SE–2.00D correction is applied, light is focused further behind the retina, causing scan circle diameter to be >12 degrees.

Table 4. Optical coherence tomography scan quality for images measured with the proper and improper refractive error correction.

Image Scan Quality (dB)	SE	SE+2.00 D	P*	SE–2.00D	P*
All eyes	22.32±3.44	19.10±3.34	<0.001	21.65±3.20	0.187
Normal group	23.13±3.45	20.41±3.25	0.001	22.06±3.12	0.138
Glaucoma group	21.61±3.31	17.94±3.01	<0.001	21.28±3.30	0.645
P**	0.071	0.002	----	0.317	----

* Statistical comparison to SE correction measurement made using paired t-tests. ** Statistical comparison between normal and glaucoma groups made using unpaired t-tests. SE – spherical equivalent.

Measurements of RNFL thickness were negligibly influenced by a –2.00D refractive correction error. This result was somewhat surprising because the effect of this refractive error was supposed to influence RNFL thickness, as opposed to the +2.00D refractive correction error. A –2.00D lens is concave, which causes light divergence. Therefore, a negative refractive defocus error results in a scan circle that is larger than 12 degrees (Figure 2C). As already stated, peripapillary RNFL thickness rapidly thins [32], and a larger scan circle would lead to an underestimation of RNFL thickness. It may be accommodation allows people to compensate for negative refractive correction errors, which would minimize the effects of a –2.00D refractive correction error on both scan circle and image quality.

In our study, the difference in average RNFL thickness between SE correction and +2.00D optical defocus was only 1.10±1.56 μm; however, that does not imply that the difference can be ignored. As shown in Figure 1A, the average RNFL thickness measured by +2.00D overcorrection was thicker (1–6 μm) than SE correction in 60.29% of eyes, and the average RNFL thickness was thinner in only 8.82% of eyes. The difference was not only significant (*P*<0.001) but also prevalent. Moreover, in glaucomatous eyes, the difference in average RNFL thickness between SE correction and +2.00D optical defocus was increased to 1.31±1.83 µm. Bendschneider et al. [39] reported a mean annual loss in RNFL thickness of only 0.19 µm using SD-OCT, which is similar to the annual decrease reported by Feuer et al. [40] and Parikh et al. [41]. Wessel et al. [42] investigated the longitudinal loss of RNFL thickness measured using Spectralis SD-OCT and revealed an estimated annual loss of RNFL thickness of 2.12 µm in glaucomatous eyes with progressive optic disc changes, whereas glaucomatous eyes without progression lost 1.18 µm of RNFL thickness per year. Therefore, for every individual with glaucoma, a change of even 1 micron in RNFL thickness is important for the detection glaucoma progression and for deciding whether intensifying treatment is necessary.

Recent studies have reported that average and inferior quadrant RNFL thicknesses are best at discriminating between normal and glaucomatous eyes [43,44]. We showed that these two measurement parameters were significantly different when a positive refractive error was induced, even in normal eyes. In the glaucoma group, measured RNFL thickness in all of four quadrants and over the entire retina was significantly increased when a positive refractive defocus error was induced (Table 3). Therefore, it is important to image normal and glaucomatous eyes with the proper refractive correction to avoid errors in RNFL thickness measurements.

Our study had several potential limitations. First, only a single SD-OCT device was examined, and other systems may have different error characteristics. Therefore, caution should be used when extending our results to other OCT systems. Second, we did not measure subject fixation stability, and studies that monitor gaze tracking should be performed. Third, a relatively low number of subjects were included in our study, but our sample size is comparable to that in similar reports [16,17,20,45]. A study with a larger sample size is needed to better understand the effects of refractive error on RNFL thickness measurements. Finally, cycloplegic examination would be better to avoid the influence of accommodation. In clinical practice, the best and simplest way to avoid SD-OCT RNFL thickness measurement errors is to compensate for each patient's refractive error (with SE correction) when baseline scans are obtained. Any refractive shifts that occur during patient follow-up should also be compensated for.

Conclusions

To the best of our knowledge, this is the first study to investigate the effect of refractive error on RNFL thickness measurements made with the Spectralis SD-OCT. Our study showed that a positive, but not a negative, error in refractive correction leads to an overestimation of RNFL thickness on the Spectralis SD-OCT. Careful correction of refractive error is important for both initial and follow-up OCT measurements in patients with and without glaucoma.

Statement

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