Article

Effect of Signal Intensity on Measurement of Ganglion Cell Complex and Retinal Nerve Fiber Layer Scans in Fourier-Domain Optical Coherence Tomography

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Purpose: We determined the effect of Fourier-domain optical coherence tomography (OCT) signal strength index (SSI) and cropping on retinal nerve fiber layer (RNFL) and macular ganglion cell complex (GCC) scan repeatability and measurement thickness.

Methods: Eyes were enrolled in the longitudinal Advanced Imaging for Glaucoma Study. At each visit, three repeat scans from the optic nerve head and macular protocols were obtained. Each measurement was associated with an SSI value from 0 to 100. Measurements with similar SSI scores were grouped to calculate repeatability defined as pooled standard deviation. Within-visit analysis was used to determine how measured thickness changed in relation to change in SSI level.

Results: The study included 1130 eyes of 569 patients. Cropped images yielded significantly worse repeatability and they were excluded from subsequent analyses. The within-visit repeatability for RNFL and GCC measurements were significantly better with higher signal strength, and optimal cutoffs were $SSI \geq 37$ and ≥ 44 , respectively. The coefficient of variation was $<1.8\%$ for RNFL scans with $SSI \geq 37$ and $< 2\%$ for GCC with $SSI \geq 44$. For scans above the cutoff SSI, higher SSI's were correlated with thicker RNFL among normal (slope = $0.056 \mu\text{m}/\text{SSI unit}$, $P < 0.001$) eyes and glaucoma suspect and perimetric glaucoma (GSPPG) eyes (slope = $0.060 \mu\text{m}/\text{SSI unit}$, $P < 0.001$), but not for perimetric glaucoma (PG) eyes. No significant correlation was found for GCC.

Conclusion: Repeatability of RNFL and GCC thickness measurements may be improved by excluding images with cropped anatomic features and weak signal strength below recommended SSI cutoffs.

Translational Relevance: Measurement precision and image quality of inner eye structure by advanced imaging modality are important for clinical diagnosis and tracking of glaucoma disease.

Introduction

Glaucoma is an optic neuropathy characterized by progressive degeneration of retinal ganglion cells (RGCs) and thinning of the retinal nerve fiber layer (RNFL).¹⁻⁴ These structural changes can be measured by optical coherence tomography (OCT), a noncontact imaging technique based on the principles of low-coherence interferometry, which was first introduced into ophthalmology practice in 1990.⁵ Early time-domain OCT (TD-OCT) systems provided clinicians

with an objective method to analyze ocular structures on the micron level, and has been shown to be highly sensitive and specific for diagnosing glaucoma.^{6,7} In recent years, Fourier-domain OCT (FD-OCT; also called spectral domain OCT) systems have been developed, which greatly improve scan time and depth resolution, resulting in increased data sampling, superior resolution, reduced motion artifact, and new segmentation capabilities, compared to TD-OCT.⁸ Among these segmentation capabilities is the ability to evaluate the macular ganglion cell layer (GCL), which has been shown to improve the diagnostic

Figure 1. Image quality of GCC and RNFL scans under different signal strength index scores. Figure 1 depicts ganglion cell complex and RNFL scans with low and high SSI scores. It can be seen that a low SSI scan has reduced resolution in the retinal layers compared to a high SSI scan.

accuracy of FD-OCT, when used in conjunction with peripapillary RNFL thickness measurements.^{9,10}

One of the fundamental requisites for any imaging modality is that accurate data output relies heavily on scan quality. In clinical practice, OCT scan quality can be highly variable and is dependent on a number of factors, including operator skill, eye movement, and media opacities. Some measurement variability has been mitigated by the reduced scan time in FD-OCT, however, test-retest variability still impacts measurement values.^{11,12} When interpreting a baseline scan, or comparing follow-up measurements to baseline values, it is important to examine carefully the quality of the acquired images. One indicator of scan quality inherent to the imaging device is the signal intensity score, which is calculated directly from the intensity of the image obtained by the device, as defined by the system manufacturer's proprietary guidelines. For instance, Stratus OCT (Carl Zeiss Meditec, Dublin, CA, USA) uses "signal strength" (SS) and recommends values ≥ 6 on a scale from 0 to 10, Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany) uses a "Q score" and recommends values ≥ 15 on a scale of 0 to 40, and RTVue (Optovue Inc., Fremont, CA, USA) uses "signal strength index" (SSI) and recommends values ≥ 45 for macular patterns and ≥ 35 for retinal scanning, on a scale from 0 to 100. Generally speaking, images with higher signal intensities yield better clarity and improved segmentation (See Fig. 1 for examples), in turn resulting in improved repeatability for the measurement.¹³

Studies on how TD-OCT signal intensity impacts RNFL measurements have shown that higher scores

result in thicker measurement values,^{13–17} and these studies have concluded that TD-OCT signal intensity scores of at least 6 to 7 yield highly repeatable measurement.^{13,16,17} Similar results have been found for FD-OCT devices, namely the Cirrus OCT (Carl Zeiss Meditec), RTVue (Optovue), Spectralis (Heidelberg Engineering), and 3D OCT-1000 (Topcon Corporation, Tokyo, Japan);^{18,19} however, these study did not examine macular ganglion cell parameters. The concept of measurement repeatability and the direct impact of SSI on measurement values have important implications, since clinicians might misinterpret a relatively large change in measurement thickness over a short period of time as true change, when, in fact, it may be due to loss of signal intensity on a follow-up scan. This study was performed to determine the relationship between FD-OCT SSI scores and measurement thickness and repeatability, to determine an acceptable and optimal minimum SSI for RNFL and macular ganglion cell complex (GCC) scan protocols based on data collected in a prospective-longitudinal study, and to determine if measurement compensation may be necessary when SSI values fluctuate over time.

Methods

Subjects

The data used for the study were taken from participants enrolled in the Advanced Imaging for Glaucoma (AIG) Study (available in the public domain at www.AIGStudy.net), a multisite bioengineering partnership and longitudinal prospective clinical study sponsored by the National Eye Institute (ClinicalTrials.gov identifier: NCT01314326). Clinical data for the AIG study was collected from three clinical centers, including the Doheny Eye Institute at the University of Southern California (Los Angeles, CA, USA), the University of Pittsburgh Medical Center (Pittsburgh, PA, USA), and Bascom Palmer Eye Institute at the University of Miami (Miami, FL, USA). The study procedures adhere to the Declaration of Helsinki guiding studies involving human subjects. Written consent was obtained from all the participants and proper institutional review board approvals were obtained from all the participating institutions.

Eyes of all subjects were categorized into one of three study groups: normal volunteers (N), glaucoma suspect or preperimetric glaucoma (GSPPG), and perimetric glaucoma (PG). Normal subjects could

have no history of ocular disease except cataract, IOP ≤ 21 mm Hg, normal optic disc appearance based upon clinical stereoscopic examination and review of stereoscopic disc photographs, and normal visual field reading from Standard Automated Perimetry (SAP; Humphrey SITA 24-2 visual field): a mean deviation (MD) and corrected pattern standard deviation (CPSD) within 95% confidential limits of normal reference, and glaucoma hemifield test (GHT) within normal limits (95%). Glaucoma suspect (GS) was defined as ocular hypertension (IOP ≥ 22 mm Hg) with normal optic discs and normal SAP, or a diagnosis of glaucoma in the fellow eye. Preperimetric glaucoma (PPG) was defined as eyes with optic nerve head/nerve fiber layer defect on ophthalmoscopy with normal SAP. In the AIG study, GS eyes comprised 25% of the cases and PPG 75%; 29% had high ocular pressure and 13% were fellow eyes to glaucoma eyes. Perimetric glaucoma eyes had glaucomatous optic nerve damage and/or abnormal SAP defined as abnormal glaucoma hemifield test (GHT) and pattern standard deviation (PDS) outside 95% confidence interval. Glaucomatous optic nerve damage was defined as neuroretinal rim narrowing, notching, excavation, or nerve fiber layer defect. Patients with SAP abnormalities had at least one confirmatory visual field examination. Perimetric glaucoma and GSPPG participants were imaged every 6 months, and normal subjects were imaged annually.

Inclusion criteria for the current analysis consisted of normal, GSPPG, and PG eyes (as described above) with at least 3 repeat scans from the RNFL and GCC protocols on ≥ 1 visit. Scans with blink artifact or that could not be processed by the segmentation algorithm were excluded. All final decisions on group assignment were done by the clinical site PI. Further details can be found from the website of AIG study (www.aigstudy.net).

OCT Imaging

An FD-OCT system (RTVue, software version 6.12) was used in this study to map GCC and RNFL thickness. The system works at 830 nm wavelength and has a scan speed of 26,000 A-scans/s and a depth resolution of 5 μm (full-width-half-maximum) in tissue. Taking advantage of the higher speed and resolution, a complex scan protocol (GCC protocol) was developed for the macular region that evenly samples the macula over a square area of 7 mm. The GCC scan pattern consists of one horizontal line and 15 evenly aligned vertical lines spaced 0.5 mm apart. A total of 14,928 A-scans was acquired over 0.6

Figure 2. Example of cropping artifacts. (A) OCT image with transverse cropping artifacts. Image is significantly decentered. (B) OCT image with axial cropping artifacts. Retina is out of view. (C) Recentered 3.45 mm NFL profile is out of scan area for transverse cropping artifact. (D) NFL map has significant error for axial cropping artifacts.

seconds and then an automated algorithm generated the thickness profile by segmenting the RNFL, GCL, and inner plexiform layer (IPL).¹⁰ The optic nerve head (ONH) scan protocol was used to measure RNFL thickness. The ONH pattern consists of six concentric circular scans with diameters ranging from 2.5 to 4 mm centered on the optic disc for RNFL measurement and twelve 3.4-mm radial scans centered on the disc for nerve head parameter measurement. The ONH protocol obtains a total of 9510 A-scans in 0.4 seconds.

Per the AIG study protocol (available in the public domain at www.AIGstudy.net), 3 repeat measurements were obtained from the GCC and ONH protocols on each visit. The operator was trained on OCT image acquisition before imaging subjects and was instructed to repeat scans, as necessary, until 3 quality scans were acquired and saved. Per the manufacturer's recommendations, an SSI ≥ 45 for macular scan patterns and ≥ 35 for all other scan patterns was considered acceptable. In the event the goal SSI could not be reached, lower SSI scans were saved. Eyes were imaged before pupillary dilation, unless dilation was needed to obtain quality scans. The SSI, on a scale from 0 to 100 (worst to best), and all measurement values from all saved scans were exported to the AIG study central database to forgo manual data entry.

The OCT image was processed using RTVue Software (v6.12). The RNFL and GCC thickness measurements were exported for later statistical analysis. Two quality factors were studied. The first was the SSI, which is the overall reflectance signal

strength, and is directly obtained using RTVue Software. The second factor was cropping, which evaluates transversal and axial cropping artifacts (Fig. 2). For ONH scans, cropping is tested by checking if the retina is out of view on the cross-section OCT images, or part of the 3.4-mm circle is out of scan area due to poor centration. For GCC scans, cropping was tested by checking if the retina is out of view or the reflectance signal is too weak in the parafoveal area.

An automated algorithm was developed and applied to OCT images and export thickness maps to test cropping. Axial cropping artifacts were detected by checking if the reflectance in the top and bottom 5 pixels of each a-scan is less than a threshold. If 1/10 of a-scans did not pass the check, the OCT image was classified as cropping. Transverse cropping was detected if recentered 6×6 GCC maps were out of a 7×7 mm scan area, or recentered RNFL profile was out of a 4.9 mm scan area. The recenteration estimation was based on the fovea and disc center calculated by RTVue software.

Statistical Analysis

The key indicator to evaluate the effect of scan quality is the repeatability, which is defined as the pooled standard deviation obtained from the multiple measurements within a visit on each eye. In general, suppose a total of N eyes are involved in the calculation. Let x_{ijk} denote the k th thickness measurement (RNFL and GCC are treated the same in the calculation) on the j th visit for the i th individual eye ($i = 1, \dots, N$, $j = 1, \dots, N_i$, $k = 1, \dots, n_{ij}$); that is, the measurement is repeated n_{ij} times on the j th visit for the i th eye. The within-visit repeatability in term of pooled standard deviation can be calculated as:

$$\sqrt{\sum_{ijk} (x_{ijk} - \bar{x}_{ij})^2 / \sum_{ij} (n_{ij} - 1)},$$

where $\bar{x}_{ij} = \sum_k x_{ijk} / n_{ij}$ is the within-visit average. Under the assumption that the variance of the measurement is the same across eyes, the pooled variance is a χ^2 statistic divided by its degree of freedom, thus F -tests can be used to compare repeatability from different groups.

The RNFL and GCC scans were first grouped into groups with and without cropping. The repeatabilities from the two groups were compared to evaluate the effect of cropping.

To determine how SSI was related to the repeatability of the RNFL and GCC measurements, eyes SSI levels were stratified into bins by intervals of 5

(e.g., 45–50, 50–55). Scans of RNFL and GCC were grouped into these bins corresponding to their respective SSI values. To ensure there were enough measurements in each SSI bin (at least 10 per bin) for meaningful statistical analysis, we collapsed low and high ends of the SSI spectrum into two separate bins. The recommended SSI (per manufacturer) is slightly higher in GCC scans than in RNFL scans, so the SSI bins for GCC were set one step higher than RNFL. The repeatability of RNFL and GCC scans in each of the SSI bins then were calculated and compared.

After studying the effect of cropping and SSI on the repeatability of the measurement, the quality criteria for RNFL and GCC scans may be established regarding the existence of cropping and the SSI level. Scans on that meet the criteria were selected for further analysis to determine how the measured thickness would change proportionally in correspondence to the change in SSI level. We used a simple within-visit linear model to address this question. Among the three scans in each visit we chose the two measurements with the largest difference in SSI, and then we calculated the corresponding difference in the measured GCC/RNFL thickness. The SSI difference and GCC/RNFL thickness difference were fitted to a linear model without intercept. We used this parsimonious model to eliminate potential confounding effects from other factors, such as age, or different operators. The regression was performed and reported separately in three disease groups. In addition, we used mixed-effect model to study the effect of age on SSI. When applicable, Generalized Estimating Equation (GEE) method²⁰ also was used to account for intereye correlation. All the statistical analyses were performed with SAS software version 9.3 (SAS Institute, Inc., Cary, NC, USA).

Results

A total of 1130 eyes of 569 patients, consisting of 242 normal, 589 GSPPG, and 299 PG eyes, had an overall mean of 8.0 ± 4.3 visits over 52 ± 27 months of follow-up (Table 1); 353 (62%) participants were females, 60 (9.5%) were African Americans. Values for SSI ranged from 24 to 91, and the average SSI for GCC and RNFL scans among normal, GSPPG, and PG eyes are listed in Table 1. The overall mean SSI among GCC scans was significantly higher (60.6 ± 8.5) compared to RNFL scans (54.7 ± 9.4 , $P < 0.001$). Healthy eyes generally yielded higher SSI values in GCC and RNFL scans (Table 1).

Among all RNFL and GCC scans, 3% to 6% had

TABLE 1. Eye Characteristics

Variables	Normal, <i>N</i> = 242	GSPPG, <i>N</i> = 589	PG, <i>N</i> = 299	<i>P</i> *
Age	56.2 ± 9.7	60.5 ± 9.5	62.0 ± 9.4	< 0.001
Axial length, mm	23.8 ± 1.0	24.2 ± 1.3	24.3 ± 1.3	< .0001
Sphere refraction	−0.7 ± 2.0	−1.2 ± 2.3	−1.0 ± 2.5	0.069
Cylinder refraction	0.5 ± 0.7	0.6 ± 0.8	0.7 ± 0.7	0.16
Central corneal thickness, μm	562 ± 32	557 ± 39	545 ± 37	0.0002
IOP at baseline, mm Hg	14.8 ± 2.8	16.4 ± 4.0	14.9 ± 4.1	0.016
Mean deviation, dB	0.0 ± 0.9	−0.6 ± 1.5	−5.0 ± 4.5	< .0001
Pattern standard deviation, dB	1.4 ± 0.2	1.7 ± 0.4	5.8 ± 4.0	< .0001
Visual field index, %	99.5 ± 0.6	98.8 ± 1.8	87.9 ± 13.6	< .0001
Overall RNFL thickness, μm	99.6 ± 8.0	93.6 ± 10.1	81.3 ± 12.2	< .0001
RNFL SSI	57.0 ± 10.1	55.1 ± 9.3	52.6 ± 8.7	0.006
Overall GCC thickness, μm	97.3 ± 6.9	92.9 ± 8.1	83.5 ± 10.8	< .0001
GCC SSI	62.0 ± 9.1	60.9 ± 8.4	59.0 ± 8.1	0.01
Number of visits	5.5 ± 2.1	8.5 ± 4.6	8.9 ± 4.4	< 0.001

* Test to compare three groups.

cropping effect. The within-visit repeatabilities of the scans with or without cropping effect among all three disease groups and RNFL and GCC scans were calculated and compared, as shown in [Table 2](#). It appears that cropping effect significantly reduced repeatability across all groups and both types of scans by as much as 2 to 3 times ($P \leq 0.008$ in all six comparisons, *F*-tests). The reduced repeatability by cropping effect suggests that such scans should be considered “bad” quality scans and be excluded.

[Table 3](#) summarizes the repeatability of GCC and RNFL measurements for each SSI bin. There is no significant difference in age among SSI bins for either GCC or RNFL scans ($P = 0.4$).

Repeatability of GCC generally improved with higher SSI values, particularly when the SSI was >40,

with a plateau occurring beyond the 45 to 50 SSI bin. By pooling the measurements, the repeatability was 2.39 with $SSI < 45$ and 1.44 with $SSI \geq 45$ ($P < 0.001$, *F*-test). The optimal minimum SSI cut-point for GCC scans appears to fall in the 40 to 45 bin, whereby further improvement in SSI had little effect on measurement repeatability. Measurements of RNFL showed a similar improved repeatability with higher SSI values. The optimal minimum SSI cut-point for RNFL scans appears to fall in the 35 to 40 bin, with a less dramatic improvement in repeatability beyond these SSI values. To further refine the selection of the optimal SSI cutoffs, we plotted the repeatability of scans below certain SSI cutoffs and the proportion of qualified scans that passed the SSI cutoffs, as shown in [Figures 3a](#) and [3b](#) with SSI cutoffs ranging from 30

TABLE 2. Comparison of Within-Visit Repeatability Among Scans With or Without Cropping

	Scans With No Cropping			Scans With Cropping			P Value
	# Scans/# Eyes	Mean $\pm$ SD, Repeatability, μm	μm	# Scans/# Eyes	Mean $\pm$ SD, Repeatability, μm	μm	
GCC:							
Normal	2564/242	95.8 $\pm$ 7.5	1.3	94/9	91.2 $\pm$ 7.8	2.3	0.04
GSPPG	9551/589	90.4 $\pm$ 8.2	1.1	420/24	89.1 $\pm$ 9.9	3.3	0.003
PG	4410/299	81.2 $\pm$ 9.9	1.3	254/18	81.0 $\pm$ 14.3	4.1	0.004
RNFL:							
Normal	2559/242	99.8 $\pm$ 8.3	1.4	123/11	102.8 $\pm$ 11.9	4.3	0.006
GSPPG	9433/589	91.0 $\pm$ 10.8	1.3	480/28	93.8 $\pm$ 10.9	3.7	0.004
PG	4301/299	79.4 $\pm$ 11.6	1.4	255/19	83.7 $\pm$ 11.8	4.3	0.003

TABLE 3. Repeatability by SSI Bins

SSI Bins	# Scans/# Eyes	Mean $\pm$ SD, μm	Repeatability, μm	CV, %
GCC				
<35	34/17	87.6 $\pm$ 14.7	3.5	4.0
35–40	176/96	87.6 $\pm$ 11.1	2.2	2.6
40–45	506/346	87.1 $\pm$ 10.4	1.7	2.0*
45–50	1623/536	86.8 $\pm$ 10.5	1.4	1.6
50–55	2371/692	87.7 $\pm$ 10.3	1.2	1.4
55–60	2964/799	88.8 $\pm$ 10.1	1.0	1.1
60–65	3002/769	88.6 $\pm$ 10.2	1.0	1.1
65–70	2610/674	89.0 $\pm$ 9.0	1.0	1.1
≥ 70	2939/516	90.9 $\pm$ 9.0	1.1	1.2
RNFL				
<35	299/168	89.7 $\pm$ 9.7	2.7	3.0
35–40	699/377	88.3 $\pm$ 11.5	1.6	1.8*
40–45	1917/629	87.1 $\pm$ 12.0	1.4	1.6
45–50	2507/767	87.7 $\pm$ 11.4	1.3	1.5
50–55	2877/795	87.2 $\pm$ 12.7	1.2	1.4
55–60	2908/799	89.5 $\pm$ 12.8	1.1	1.3
60–65	2344/702	90.1 $\pm$ 13.1	1.2	1.3
65–70	1577/523	91.1 $\pm$ 12.3	1.1	1.2
≥ 70	1165/331	94.5 $\pm$ 12.5	1.3	1.3

CV, coefficient of variable.

* A recommended cut-point by considering repeatability alone should be in 40 to 45 bin for GCC and 35 to 40 bin for RNFL.

to 80, for GCC and RNFL, respectively. Both Figures demonstrate that if the minimum acceptable SSI score is set to 30, zero scans would be excluded, but the repeatability would be unacceptable among the low SSI scans. The optimal minimum SSI for GCC scans was determined to be 44, a level where 95% of the saved scans would be included, yet still produce an acceptable level of repeatability (Fig. 3a). For RNFL scans, the optimal minimum SSI was determined to be 37, again, a level where 95% of the saved scans would be included, yet still produce an acceptable level of repeatability (Fig. 3b).

From the mixed effect models, we found that that age was not a statistically significant factor for SSI in the ONH and macular scans.

In the within-visit linear regression models among normal eyes, an increase in SSI was significantly correlated with the increase in RNFL thickness measurement, but not GCC (Table 4). The regression analysis yielded similar results among GSPPG eyes. However, we did not find any association between SSI change and thickness change among PG eyes ($P = 0.65$ for RNFL and $P = 0.14$ for GCC; Table 4).

Discussion

Signal intensity scores are a proxy for OCT scan quality and are used commonly in the clinical setting to determine image reliability. Adequate structural illumination, the basis of the signal intensity score, is important for accurate and repeatable OCT measurements, as shown by several studies using the previous generation TD-OCT system.^{14–17} It has been reported that higher signal strength results in thicker measurement values^{16,17} and this phenomenon also has been shown for RNFL thickness using FD-OCT systems.¹⁸ Figure 1 shows example FD-OCT scans with SSI values above and below the manufacturer's recommendations, and we performed this study to determine the effect of SSI on measurements and their within-visit repeatability. To our knowledge, this study is the first to analyze the effect of SSI on GCC and RNFL measurements using FD-OCT data collected in a prospective longitudinal study.

There are several reasons SSI values may impact measurement values. Optical coherence tomography

Figure 3. (a) Effect of SSI cutoff on GCC repeatability and yield. Repeatability of GCC measurements for scans with SSI scores ranging from 30 to 80; as SSI increases, measurement repeatability improves; however, the percent of qualifying scans decreases. (b) Effect of signal strength index cutoff on RNFL repeatability and yield. Repeatability of RNFL measurements for scans with SSI scores ranging from 30 to 80; as SSI increases, measurement repeatability improves; however, the percent of qualifying scans decreases.

software algorithms rely on reflectance properties specific to each retinal layer to delineate the inner and outer boundaries of the structure being measured. Software engineers design scan protocols that segment layers with sufficiently different reflectance from their bordering layers, such that the measured layer(s) can be identified by an automated algorithm. When an acquired image has a low overall illumination, which can be caused by media opacities, floaters, cropping, blink artifacts, eye movement, or operator error, the clarity of the image decreases, resulting in less accurate segmentation and increased measurement variability. The directional reflectance of RNFL

TABLE 4. Within-Visit Regression Analysis of Retinal Sublayer Thickness Versus Signal Strength

Retinal Layer	Regression Slope ($\mu\text{m}/10$ SSI Unit)
Normal eyes	
GCC	0.19 ($P = 0.20$, $R = 0.05$)
RNFL	0.56 ($P < 0.0001$, $R = 0.2$)
GSPPG eyes	
GCC	-0.14 ($P = 0.12$, $R = 0.03$)
RNFL	0.60 ($P < 0.0001$, $R = 0.18$)
PG eyes	
GCC	-0.23 ($P = 0.14$, $R = 0.045$)
RNFL	0.048 ($P = 0.65$, $R = 0.014$)

is another important factor that affects SSI and measured thickness. Along the plane parallel to its length, RNFL has mirror-like directionality,²¹ so that the reflected OCT signal is much stronger near perpendicular incidence, and weakens with more oblique incidence angles. Therefore, oblique incidence would reduce SSI through reduced reflectance. It also could artifactually reduce measured RNFL thickness by decreasing the contrast between the usually brighter RNFL and subjacent GCL.

The arrow of causality also could run the other way – a thinner RNFL could lower the SSI of an OCT image because the RNFL usually is one of the more brightly reflecting layer in the peripapillary OCT scan.

Our study found that SSI significantly affects GCC and RNFL measurement repeatability, particularly in scans with SSI values that are on the extreme low end of the spectrum (i.e., $\text{SSI} < 35$). The repeatability of GCC measurements deteriorated more when the SSI scores are very low. It is intuitive that higher quality scans will result in improved measurement repeatability; however, it is important to determine the minimum SSI that generates an acceptable level of repeatability, since, in real world practice, the operator often must accept less than ideal scans. We determined the optimal minimum SSI should be 44 for GCC scans and 37 for RNFL scans. These values result in measurements that are sufficiently repeatable, should be easily obtainable by OCT operators, and further increases in SSI produces only minor improvements in measurement repeatability. In the current study, the best repeatability among RNFL parameters came from scans in SSI bins between 50 and 70; however, only accepting images with these SSI

values would result in the exclusion of >30% to 90% of saved scans, and, thus, is an unrealistic requirement for use in clinical practice. Similarly, the best repeatability for GCC scans came from SSI bins between 55 and 70; however, this also represents too strict an SSI range for use in clinical practice.

We also found cropping was a significant source of variability. When the retina is out of view or scan is not centered at designed location, every effort should be made to retake the scan to eliminate these artifacts.

To determine whether SSI affects RNFL thickness, we performed within-visit correlation between variation in SSI and RNFL thickness. This correlation does not measure the effect of RNFL thickness on SSI, since the true RNFL thickness should be constant for the same eye within a visit. Therefore, any correlation would be due to the effect of SSI on RNFL thickness measurement, not the other way around. Using this method, we found a significant correlation between SSI value RNFL measurement thickness among normal and GSPPG eyes (Table 4). For instance there was a statistically significant ($P < 0.001$) 0.056 μm increase in RNFL thickness for each point increase in SSI (SSI scale ranges from 0–100) among normal eyes. This value is less than those found in studies using a TD-OCT device, although it is difficult to make exact comparisons due to different signal intensity scales used by TD-OCT versus RTVue FD-OCT.^{13,15,22} Samarawickrama et al.¹⁶ found a small but significant difference in inner macular thickness between scans with moderate and good signal strength scores. These findings suggest that in FD-OCT and TD-OCT devices, weaker SSI is associated with thinner RNFL thickness. The weaker association in later FD-OCT devices could be due to the effort of programmers to reduce this artifactual bias in later generations of segmentation software.

In contrast to RNFL, we did not find a statistically significant correlation between SSI and GCC thickness (Table 4). This is unusual because GCC and RNFL measure ganglion cells,²³ though in different regions. It would appear that the effect of varying SSI values on measurement thickness does not apply equally to both regions. The reflectance measured by an optical instrument depends not only on the proportion of incident light that is reflected, but also on whether the direction of the reflected light reached the viewing aperture. The directional reflectance of RNFL means that the OCT beam position within the pupil aperture could affect the brightness of RNFL signal on the OCT image. A dim RNFL would reduce the SSI score and reduce the contrast between the

RNFL and the GCL, leading to artifactually thinner RNFL measurement. Knighton and Qian²¹ have reported the reflectance of the nasal RNFL is particularly sensitive to aperture location and this may account for an increased dependence on adequate signal intensity when scanning a peripapillary pattern. If we hypothesize that the correlation between RNFL thickness measurement and SSI is primarily mediated through variation in OCT beam incidence angle, this could explain why GCC is not affected by SSI as much. The primary segmentation for GCC thickness measurement is the boundary between the IPL and the inner nuclear layer. These layers do not have strong directional reflectance like RNFL, and the signal contrast between them would not be strongly influenced by beam incidence angle.

Another discrepancy was that the within-visit correlation between variations in SSI and RNFL was not found in the PG group, unlike the strong correlation among normal and GSPPG eyes (Table 4). The reason may be that among PG eyes, the larger loss of nerve fibers or decrease in microtubule content makes reflectance less directional. So change occurs in SSI mainly due to scatter defocus or beam blocking, which does not alter the contrast between RNFL and GCL; therefore, segmentation is not affected. The lack of SSI-RNFL correlation in PG group suggests that direct compensation of SSI for glaucoma progression analysis is not recommended. Although compensating for SSI might reduce variation in RNFL thickness in the normal group, it also would make RNFL appear thicker in the PG group; hence, the contrast between PG and N groups would not be improved by SSI-based compensation. Furthermore, monitoring of RNFL thinning over time as a way of measuring glaucoma progression is useful primarily in the PG group. And SSI-based compensation is not necessary in the PG group.

Several confounding factors, such as age, race, and other variables, are known to affect RNFL and GCC thickness.^{17,22,24–28} Older age also had been linked to lower SSI.²⁶ In the current study, we did not find a significant correlation between age and SSI.

This study has several potential limitations. First of all, the SSI cut-points were determined solely based on repeatability of the measurement under different SSI levels, and the choice of the cut-point is arbitrary, hence there may exist a more optimized cut-point when other factors are considered; also the test comparing repeatability requires same variance across eyes, which may not be met in reality. Secondly, the study used data across multiple tests over multiple

visits on both eyes of the participants to compensate for the rather small sample size; correlation and between-visit variation might have a negative effect on the analysis. In an ideal situation, it is preferable to have a large number of same-day repeat sessions on a single eye to evaluate the SSI effect. Additionally, factors other than signal intensity, such as cataract, vitreous floaters, or disc margin delineation algorithm failure,²⁹ may impact measurement values and lead to increased variability independent of SSI. It also is unknown to what extent measurement variability caused by extreme high and low SSI values affects OCT diagnostic classifications (i.e., green/yellow/red color code), which may complicate interpretation. Finally, although higher SSI values yield improved repeatability, it remains to be determined whether an SSI correction model would improve our ability to track glaucoma progression. Having said that, any advancement that increases our ability to detect true structural changes in GCC and RNFL thickness over time is welcome, and future studies are warranted to determine the role of a SSI thickness-response model in the clinical setting.

In conclusion, higher SSI results in improved repeatability. For the RTVue FD-OCT instrument, we recommend SSI values ≥ 44 for GCC measurements and ≥ 37 for RNFL measurements.

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