Reproducibility of Retinal Nerve Fiber Layer Thickness Measurements Using the Eye Tracker and the Retest Function of Spectralis SD-OCT in Glaucomatous and Healthy Control Eyes

Stefan J. Langenegger, Jens Funk, and Marc Töteberg-Harms

PURPOSE. To evaluate the impact of Spectralis self-acting eye-tracking (eye tracker) and retest software on the reproducibility of retinal nerve fiber layer (RNFL) thickness measurements in glaucomatous and healthy control eyes by SD-OCT.

METHODS. RNFL thickness was measured in 56 normal and 47 glaucomatous eyes by one operator within one session with a brief rest between measurements. Three measurements were taken with the eye tracker and the retest function engaged (method A), and three measurements were taken without the eye tracker and without the retest function (method B). Method A and B measurements were taken alternately.

RESULTS. Reliability, measured by intraclass correlation coefficient (ICC) for absolute agreement and coefficient of variation (COV), was calculated for the global mean RNFL thickness (G), for each sector and for the peripapillary bundle. The ICC (and lower 95% confidence interval [CI]) for the global mean RNFL thickness (G) for method A measurements in both normal and glaucomatous eyes was 0.99 (0.98 CI). In glaucomatous eyes, the COV for method B measurements was between 2.7% and 10.5%, and between 1.3% and 3.5% for method A measurements.

CONCLUSIONS. The reproducibility of RNFL measurements with Spectralis SD-OCT is excellent in both normal and glaucomatous eyes and can be significantly improved by using the eye tracker and retest software. The gain of reproducibility by using the software is significantly higher in glaucomatous eyes than in normal eyes. These findings suggest that software applications are capable of significantly improving the reproducibility of RNFL thickness measurements.

Reproduced from: Fellowship in Ophthalmology, University of Zurich, Switzerland. All subjects underwent a full ophthalmic examination including measurement of refraction, best corrected visual acuity, and intraocular pressure (Goldman applanation tonometry). The study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the local ethics committee. Written informed consent was obtained from each subject. Subjects were recruited from the ophthalmology outpatient service of the University Hospital Zurich, Switzerland. All subjects underwent a full ophthalmic examination including measurement of refraction, best corrected visual acuity, and intraocular pressure (Goldman applanation tonometry).
The inclusion criteria for both healthy and glaucomatous subjects were a visual acuity of more or equal to 0.5 Snellen, refractive error less than $\pm 5.00$ D spheric and $\pm 3.00$ D cylindrical, and no history of ocular trauma or of any other severe ocular disease (particularly diseases affecting the optic nerve) or surgery other than uncomplicated cataract surgery. Additional inclusion criteria for the glaucoma group were a diagnosis of POAG or PEX glaucoma. All glaucoma patients underwent visual field (VF) testing. Young patients in the control group with normal optic disc and no or nearly no cupping were included in the study without VF testing. If IOP or cupping was abnormal or even if there was a difference in sides, VF was tested in control patients. Regarding the VF perimetry (GI-program of the Octopus; Haag-Streit AG, Köniz, Switzerland), the mean defect (MD) for control patients had to be less than or equal to 2.0 dB and greater than 2.0 dB for glaucoma patients. Exclusion criteria for the control group were history of trauma or of any other severe ocular disease (particularly diseases affecting the optic nerve) or surgery other than uncomplicated cataract surgery.

In general, glaucoma was defined based on a clinical diagnosis of progressive optic nerve damage with abnormal optic disc cupping and MD $>2.0$ dB. We assessed the optic disc in accordance with the method published by Jonas et al.\textsuperscript{19} Primary open-angle glaucoma is characterized as an optic nerve neuropathy with atrophy of the optic nerve and loss of retinal ganglion cells and axons and in combination with characteristic VF abnormalities. The anterior chamber angle is open.\textsuperscript{20} PEX glaucoma is defined as elevated IOP causing progressive optic nerve damage. In PEX glaucoma, IOP elevation is caused by abnormal protein obstructing the trabecular meshwork.

**Image and Data Acquisition**

All RNFL circular scans were performed by one examiner using the Spectralis SD-OCT system (software version 5.1.2; Heidelberg Engineering GmbH). A pupil diameter of at least 4 mm was required for scanning. Within one session, three measurements were taken with the eye tracker and retest function engaged (method A) and three measurements were taken without the eye tracker or the retest function with manual positioning and repositioning of the scanning circle (method B). Method A measurements were alternated with method B measurements, to avoid systematic bias.

SD-OCT RNFL thickness measurements were performed by using circular scans with a scanning angle of 12°, which equates to a retinal diameter of 3.5 mm when assuming a standard corneal curvature of 7.7 mm. Equipped with a super luminescent diode with a center wavelength of 840 nm, the Spectralis SD-OCT obtains up to 40,000 A-scans/s with a depth resolution of 7 μm in tissue and a transverse optical resolution of 14 μm in an average human eye. The transverse digital resolution depends on the tightness of calculated A-scans (pixels) and can be adjusted. In high-resolution (HR) mode, the device provides a transverse digital resolution of 5 μm. High-speed (HS) mode doubles the distance between A-scans. As a result, transverse digital resolution decreases to 11 μm. A full RNFL circular scan contains 1536 A-scans in HR mode and 768 A-scans in HS mode along a peripapillary circle of 360°. In the present study, all scans were performed in HS mode, as HS mode is commonly used for RNFL examinations in the clinic in which the examinations were performed.

With the dual beam, a corresponding scanning laser ophthalmoscope (SLO) fundus image can be captured at the same time as the OCT measurement, enabling the system to link every OCT scan (Fig. 1A) to its corresponding position on the SLO fundus image (Fig. 1B). Processing of SLO data and identification of specific patterns in retinal structures such as blood vessels allows scans to be marked as a reference and baseline. In follow-up examinations, the system recognizes the former scanning area on the retina and automatically positions the retest scan on the same location. During the measurement, online eye tracking provides a real-time adjustment of the OCT scanner on the simultaneously gathered SLO image to decrease motion artifacts, and the high scanning speed reduces the time for distracting eye movements to occur. To increase image quality, the Spectralis SD-OCT includes an ART (automatic real-time) function. With ART activated, multiple frames (B-scans) are gathered during the scanning process, and images are averaged for noise reduction.\textsuperscript{21,22} The number of frames can be adjusted. In this study, the ART function was set to 16 frames per B-scan for all measurements performed.

The first A-scan is processed temporally (0°), after which the scanner moves through superiorly (90°), nasally (180°), inferiorly (270°) and back to temporally to complete a full circle. During the measurement, a quality bar visualizes the signal-to-noise ratio. The quality score range from 0 (poor) to 40 (excellent). For this study, scans with a quality of less than 25 were excluded and were repeated until good quality was achieved. Likewise, scans with blinks during the scanning process were excluded and repeated.

For the first RNFL measurement, the OCT scanning circle was manually positioned at the center of the optic disc while eye tracking.
was activated. The first acquired RNFL scan was set as a baseline for further retest scans (method A). Every retest was performed alternately with a traditional scan, with manual repositioning of the scanning circle on the optic disc (method B). Between each measurement, the subject was instructed to lean back before being repositioned on the headrest and the correction for spherical error was readjusted.

The Spectralis SD-OCT system provides an algorithm to determine the inner and outer boundary of the RNFL (Fig 1A). OCT data were analyzed by this algorithm, to detect RNFL thickness along the circular scan in micrometers. The median thickness was plotted in a pie chart diagram representing the six sectors of the optic disc (Fig 1D). For interpretation of the RNFL scan, the optic disc was segmented as follows: temporal (T, 315–45°), superior temporal (ST, 45–90°), superior nasal (NS, 90–135°), nasal (N, 135–225°), inferior nasal (NI, 225–270°), inferior temporal (IT, 270–315°), together with an averaged global classification (G) and the papillomacular bundle (PMB, 338–8°). The calculated thickness profile (Fig. 1C) refers to a set of normative RNFL thickness data collected from measurements in 170 healthy subjects. Green areas represent the 95% normal range found in healthy subjects of the same age, whereas values outside the 95% confidence interval (CI) of the normal distribution (0.01 < P < 0.05) are indicated in red. Yellow areas represent values outside the 95% CI but within the 95% CI of the normal distribution.

### Statistical Analyses

For statistical analyses, the RNFL thickness of all sectors, the PMB and the global mean RNFL thickness (G) were examined (PAWS/SPSS Statistics, ver. 18; IBM Corporation, New York, NY, and MedCalc, ver. 11.2; MedCalc Software, Mariakerke, Belgium).

A pilot study was conducted (five eyes of five healthy controls and five eyes of five patients with a glaucoma diagnosis), to calculate the sample size that would be needed to distinguish a relevant difference in COV of ±1% between methods A and B. Sample size calculation was based on the measurements of G in glaucomatosus eyes according to the same examination protocol, as described in the main study. The pilot study showed that when the sample size in the glaucoma group is 28, a single group t-test with a 0.05 two-sided significance level will have 95% power to detect the difference between a null hypothesis mean of 2.000 and an alternative mean of 1.000, assuming that the SD is 1.400.

Descriptive statistics for quantitative variables, such as means and standard deviations, as well as relative frequencies for qualitative variables such as sex, were conducted. A Student’s t-test was calculated for differences in age between healthy and glaucomatosus eyes.

One set of three RNFL thicknesses obtained from three measurements using the same measurement method (method A or B) in the same eye was used to calculate an intraclass correlation coefficient (ICC) and the coefficient of variation (COV). The COV was defined as the standard deviation divided by the arithmetic mean and is expressed as a percentage. For both the glaucoma group and the control group, this resulted in a set of COV values for measurements acquired by method A and another set acquired by method B. Thus, the population statistics for COV were calculated. The calculations were performed separately in all measurement areas (G, TS, T, TI, NI, N, NS, and PMB). Consequently, differences in the mean COV between the two measurement methods (method A minus method B) were computed.

Moreover, differences between the mean RNFL thickness in micrometers provided by methods A and B were computed for all measurement areas in both the glaucoma and healthy control groups.

A one-sample Student’s t-test was applied to the differences in means to determine whether method A provided a different measurement than method B provided. Moreover, agreement of methods A and B was investigated with a Bland-Altman plot, along with the corresponding 95% limits of agreement. A one-sample Student’s t-test was also applied to COV differences to determine whether method A was more reproducible than method B for healthy subjects and glaucoma patients (corresponding to a paired t-test). A two-sample t-test was used to explore possible differences in COV reduction (method A, method B) between the healthy controls and the glaucoma group. A two-sample t-test was used to determine whether significant differences existed in thickness (method A versus method B) between healthy and glaucomatosus eyes.

A linear regression analysis for reproducibility (COVs) and global mean thickness (G), with age and sex as predictors, was conducted to find associations between predictors and COVs for every sector, for the PMB, and for global mean RNFL thickness (G) for method A separately in healthy and glaucomatosus eyes.

A one-way ANOVA with a Scheffe post hoc test was conducted to investigate potential differences in the reproducibility of sectors for both eye tracker and traditional measurement.

Results of the statistical analyses with P < 0.05 were interpreted as statistically significant.

### Results

#### Demographic Parameters

One hundred seven subjects were examined. Four patients had to be excluded: three because of poor fixation and one because of poor measurement quality and failure of the RNFL thickness algorithm. Forty-two left and 61 right eyes were included. Table 1 shows the characteristics and demographics of the study sample.

A Student’s t-test showed a significant difference in age between the glaucoma patients and healthy controls (P = 0.007), whereas there was no significant difference in age between the men and women (P = 0.813) in either group.

#### Reliability of Measurements Performed with the Eye Tracker and Retest Function

The mean RNFL thickness is shown in Table 2 for each sector, the PMB, and G. As the table shows, RNFL thickness measured

### Table 1. Demographic Characteristics of Glaucoma Patients and Healthy Subjects

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Glaucoma</th>
<th>Total Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes, n (%)</td>
<td>56 (54.37)</td>
<td>47 (45.63)</td>
<td>103</td>
</tr>
<tr>
<td>Age, y</td>
<td>46.31 ± 19.56</td>
<td>68.96 ± 16.22</td>
<td>56.65 ± 21.30</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>28 (50)</td>
<td>23 (49)</td>
<td>51 (49.5)</td>
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</tbody>
</table>

* Mean ± SD.

**TABLE 1.** Demographic Characteristics of Glaucoma Patients and Healthy Subjects

**TABLE 2.** Mean RNFL Thicknesses for Both Examination Methods in Glaucoma Patients and Controls

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th></th>
<th>Glaucoma</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Method B</td>
<td>Method A</td>
<td>Method B</td>
</tr>
<tr>
<td>G</td>
<td>95 ± 11.6</td>
<td>96 ± 12.6</td>
<td>71 ± 16.5</td>
</tr>
<tr>
<td>TS</td>
<td>130 ± 17.8</td>
<td>128 ± 18.2</td>
<td>96 ± 29.4</td>
</tr>
<tr>
<td>T</td>
<td>71 ± 10.8</td>
<td>70 ± 11.2</td>
<td>61 ± 14.6</td>
</tr>
<tr>
<td>TI</td>
<td>138 ± 19.2</td>
<td>138 ± 20.2</td>
<td>91 ± 36.7</td>
</tr>
<tr>
<td>NI</td>
<td>103 ± 23.7</td>
<td>106 ± 26.2</td>
<td>75 ± 20.1</td>
</tr>
<tr>
<td>N</td>
<td>71 ± 15.8</td>
<td>73 ± 18.2</td>
<td>56 ± 17.0</td>
</tr>
<tr>
<td>NS</td>
<td>108 ± 25.8</td>
<td>108 ± 28.4</td>
<td>70 ± 26.4</td>
</tr>
<tr>
<td>PMB</td>
<td>55 ± 9.9</td>
<td>54 ± 11.3</td>
<td>51 ± 11.2</td>
</tr>
</tbody>
</table>

Data are expressed in mean micrometers ± SD. G, 0–360°; T, 315–45°; TS, 45–90°; NS, 90–135°; N, 135–225°; NI, 225–270°; TI, 270–315°; PMB, 338–8°. Method B, measurements without eye tracker and without retest software, with manual positioning of the scanning circle; method A, measurements with eye tracker and retest software engaged.
Reproducibility of RNFL Measurements

Table 3 shows the COVs calculated from the three measurements. In healthy subjects, COVs for RNFL thickness measurement of the six sectors of the optic disc (T, TS, TI, N, NS, and NI) measured with method B ranged from 3.5% (TI, SD 0.023) to 7.4% (N, SD 0.040). In glaucoma patients, COVs for measurements with method A ranged from 1.6% (T, SD 0.012) to 2.7%, respectively 1.0% in healthy subjects; 2.7%, respectively 1.3% in glaucoma patients).

Effect of Eye Tracker and Retest Function on Reproducibility and RNFL Thickness Measurements

Differences in mean RNFL thickness and COVs between the two measurement methods are presented in Table 4. Significant differences in mean RNFL thickness between the two measurement methods were found in the control group for G (method A + 0.565 μm; P = 0.043) and sectors TS (–2.042 μm; P = 0.018), NI (+3.464 μm; P = 0.002), and N (+2.304 μm; P = 0.009). There was no significant difference in mean RNFL thickness between the two measurement methods within the glaucoma group. Differences in COVs for the two measurement methods (method A minus method B) were all negative, providing strong evidence that method A is more reproducible than method B. Figure 3 displays the reduction of COVs by method A, together with its corresponding 95% CI.

Linear regression analysis did not unveil any significant effect of sex nor age on reproducibility (COV) of measurements with method A or B.

For method A, ANOVAs revealed that there are no differences in COV between sectors. For method B there are differences (P = 0.001) in COVs between sectors in both the healthy subjects and glaucomatous patients. The TI sector measurements were the most reproducible.

DISCUSSION

Since its introduction in 1991, OCT technology has experienced a dramatic evolution and has quickly become a popular resource in ophthalmic imaging and diagnostics. Despite its popularity and role in glaucoma research, its role in diagnostics is still developing. The correlation between glaucoma progression and RNFL thinning is well-documented.8,11 Evidence that the thinning of RNFL precedes the loss of VF function indicated that OCT could help detect the onset of glaucomatous changes more sensitively than by VF testing.12 An earlier diagnosis of glaucomatous changes gives clinicians more time to establish an effective therapy and may help maintain patients’ VF function.

![Bland-Altman plot for differences in mean global RNFL thickness measured with method A (meanGA) minus method B (meanGB) in normal and glaucomatous eyes. Method A, measurements with eye tracker and retest software engaged; method B, measurements without eye tracker and without retest software, with manual positioning of the scanning circle.](image-url)
RNFL thickness measurements with SD-OCT, the uniqueness of these measurements has been well documented in the literature. Although other methods for achieving more reliable and more highly reproducible RNFL thickness measurements when using the eye tracker and retest function are reliable and comparable to those obtained with method B. P value and 95% CI were obtained with one-sample Student’s t-tests on differences. Methods A and B and the optic disc sectors are described in Table 2.

Previous studies have kept record of the increasing reproducibility of RNFL thickness measurements performed with first, second, and third generation TD-OCT technology, as well as for the different SD-OCT devices that are currently available. The purpose of this study was to investigate the contribution of two specific software applications to the eye tracker and retest software (method A) of Spectralis measurements more reproducible. The present study demonstrates that reproducibility can be further improved by the use of specific software for retest recognition and compensation for involuntary eye movement (eye tracker).

The Spectralis software algorithm automatically detects the RNFL. In some cases, the software has problems detecting the correct boundary of the RNFL. In these cases, it is possible to manually correct the boundary in the Spectralis software. To avoid bias by a glaucoma specialist who uses manual correction of the RNFL boundary, we did not use manual correction in the present study. If it was obvious that the automatic detection had failed, those study eyes were excluded.

Our results show good reliability for measurements obtained with the eye tracker and retest function (method A). The Bland-Altman plot with 95% limits of agreement (Fig. 2) shows that RNFL thicknesses for G were comparable between the two methods. The only significant difference in RNFL thickness was found in control patients where method A resulted in higher values for the NI and N sectors and for G and in lower values in the TS sector. As the differences are as low as 0.2 to 3.0 μm, we consider them to be clinically irrelevant. Measurements with the new eye tracker and retest function (method A) are reliable and comparable to the thickness values measured without the eye tracker and retest function (method B).

The results of this study show excellent reproducibility and significant improvement of reproducibility of RNFL measurements when using the eye tracker and retest software. Using the eye tracker and retest software (method A) of Spectralis SD-OCT enhanced reproducibility significantly (COV 2.7%–1.3% for G in glaucomatous eyes; P = 0.000). In glaucoma patients, the improvement in reproducibility was significantly higher than in control eyes. To our knowledge the present measurements more reproducible. The present study demonstrates that reproducibility can be further improved by the use of specific software for retest recognition and compensation for involuntary eye movement (eye tracker).

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### Table 4. Differences in COV and mean RNFL Thicknesses in Glaucoma Patients and Healthy Controls

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls</th>
<th></th>
<th>Glaucoma Patients</th>
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<tbody>
<tr>
<td></td>
<td>Δ</td>
<td>Mean Difference</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>G</td>
<td>&lt;0.001</td>
<td>-0.005</td>
<td>-0.007</td>
<td>-0.003</td>
</tr>
<tr>
<td>TS</td>
<td>&lt;0.001</td>
<td>-0.030</td>
<td>-0.038</td>
<td>-0.023</td>
</tr>
<tr>
<td>T</td>
<td>&lt;0.001</td>
<td>-0.041</td>
<td>-0.05</td>
<td>-0.033</td>
</tr>
<tr>
<td>TI</td>
<td>&lt;0.001</td>
<td>-0.021</td>
<td>-0.029</td>
<td>-0.013</td>
</tr>
<tr>
<td>NI</td>
<td>&lt;0.001</td>
<td>-0.042</td>
<td>-0.052</td>
<td>-0.032</td>
</tr>
<tr>
<td>N</td>
<td>&lt;0.001</td>
<td>-0.049</td>
<td>-0.065</td>
<td>-0.035</td>
</tr>
<tr>
<td>NS</td>
<td>&lt;0.001</td>
<td>-0.040</td>
<td>-0.049</td>
<td>-0.051</td>
</tr>
<tr>
<td>PMB</td>
<td>&lt;0.001</td>
<td>-0.036</td>
<td>-0.049</td>
<td>-0.022</td>
</tr>
</tbody>
</table>

The difference in COV was determined by subtracting the resulting COVs obtained with method A from those obtained with method B. P value and 95% CI were obtained with one-sample Student’s t-tests on differences. Methods A and B and the optic disc sectors are described in Table 2.

![Figure 3](image-url)
study is the first to report on the reproducibility of RNFL measurements using the Spectralis SD-OCT device.

Budenz et al.29 studied reproducibility of RNFL measurements with TD-OCT Stratus OCT3 (Carl Zeiss Meditec, Dublin CA) in 88 normal and 59 glaucomatous eyes and found COVs ranging from 3.7% (G) to 11.9% (N) in glaucomatous eyes and from 1.7% (G) to 8.25% (N) in normal eyes, respectively. Similar to the present study, N measurements were the least, whereas T measurements were the most reproducible. Also in that study, glaucomatous eyes showed less reproducibility than normal eyes, which was also reported by Blumenthal et al.27 Although reproducibility found in the present study is still higher in normal eyes than in glaucomatous eyes, our results show that the eye tracker and retest software (method A) had a higher impact on reproducibility in glaucomatous eyes than in normal eyes. This finding indicates that such software may help to reduce the gap in reproducibility previously found between measurements of glaucomatous and normal eyes. Mwanza et al.35 studied intra- and intersession reproducibility of RNFL thickness and optic nerve head parameters measured with the SD-OCT Cirrus HD-OCT (Carl Zeiss Meditec, Inc.) in 55 glaucomatous eyes. Similar to the present study, the mean RNFL thickness showed the best intravisit reproducibility, with a COV of 1.9%. Menke et al.34 reported on the reproducibility of the 3D Fourier domain OCT (3D OCT1000; Topcon, Tokyo, Japan) in 38 normal subjects by having two operators perform three RNFL thickness measurements. The mean COV was 4.1%. Highest reproducibility was found for the inner ring area (ETDRS-scheme) with a COV of 1.9%, which compares with results of the present study for measurement method B in normal eyes (1.6%). Lee et al.52 used the test–retest function of Spectral OCT/SLO (Ophthamlic Technologies Inc., Toronto, ONT, Canada) to investigate the reproducibility of RNFL thickness measurements in 98 normal and 79 glaucomatous eyes performing three measurements within one session. RNFL measurements showed good reproducibility for that device. As in the present study, best reproducibility was found for the global mean RNFL thickness, with a COV of 1.9% in normal and 2.0% in glaucomatous eyes. As all scans were performed with one method (retest) only, no conclusion regarding the specific effect of the retest function to enhance reproducibility could be drawn.

Reproducibilities found in different studies are not directly comparable, as different eyes and different study protocols were used. The results of the present study showed excellent reproducibility for measurements using the eye tracker and retest protocol (method A) of Spectralis SD-OCT. With lowest COVs of 1.0% in normal eyes and 1.3% in glaucomatous eyes, the results show one of the best reproducibilities ever reported for RNFL thickness measurements for any OCT device available today. The HS mode used in the present study used only half the transverse resolution the device is capable of. If one were to use the full resolution of the HR mode, even higher reproducibility may be achieved. Whereas other currently available OCT devices provide a scanning resolution comparable to the device used in the present study and also include test–retest software, the Spectralis SD-OCT is the first device to integrate real-time eye tracking. The significant improvement of reproducibility attained by using this software in the present study indicates that improvement in reproducibility cannot entirely be accounted for by higher resolutions and faster scanning speeds of the latest SD-OCT devices, but also has to be understood as a result of more sophisticated software applications. These findings may have implications for the design and development of the next generation of OCT devices.

References


