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Abstract

PURPOSE: Conventional time-domain optical coherence tomography (OCT) has been shown to provide reproducible retinal nerve fiber layer (RNFL) measurements. Recently, high-speed, high-resolution Fourier-domain 3D-OCT has been introduced to improve OCT quality. It can provide 6-mm(2) high-density scans to provide RNFL thickness measurements. The purpose of this study was to test the reproducibility of 3D-OCT RNFL thickness measurements in healthy volunteers. METHODS: Thirty-eight eyes were included in the study. High-density 6-mm(2) 3D scans were registered by two independent operators. RNFL thickness was calculated for eight areas corresponding to the ETDRS areas and for two ring areas. The ETDRS grid was centered on the optic disc. Intraclass correlation coefficients (ICC) and coefficients of variation (COV) were calculated. Interobserver reproducibility was visualized by using Bland-Altman analysis. RESULTS: Intrasession reproducibility was good with a mean ICC of 0.90. The mean COV for operator 1 and 2 was 4.2% and 4%, respectively (range, 1.9%-6.7%). Highest reproducibility was found for the two ring areas and the superior and inferior quadrants. Mean differences in RNFL thickness measurements for ring 1 and 2 between operator 1 and 2 were 0.9 microm (limits of agreement, -11.4 to +9.6 microm) and 0.1 microm (limits of agreement -4.1 to +3.9 microm), respectively. CONCLUSIONS: 3D-OCT RNFL thickness measurements in healthy volunteers showed good intra- and interobserver reproducibility. 3D-OCT provides more RNFL thickness information compared to conventional time-domain OCT measurements and may be useful for the management of glaucoma and other optic neuropathies.
Reproducibility of Nerve Fiber Layer Thickness Measurements using 3D Fourier-Domain OCT (Topcon 3d-OCT1000)

Marcel N Menke MD¹, Pascal Knecht MD¹, Veit Sturm MD¹, Simeon Dabov², Jens Funk MD¹

¹) University of Zuerich, Department of Ophthalmology, Zuerich, Switzerland
²) University of Muenster, School of Medicine, Muenster, Germany

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Corresponding author and address for reprints:
Marcel N Menke MD
Dept. of Ophthalmology
University of Zuerich (USZ)
Frauenklinikstrasse 24
8091 Zuerich
Tel.: 0041762228845
Fax: 0041442554354
E-Mail: marcel.menke@gmail.com
Abstract:

Purpose:

Conventional time-domain optical coherence tomography (OCT) has been shown to provide reproducible retinal nerve fiber layer (RNFL) measurements. Recently, high-speed, high-resolution Fourier-domain 3d-OCT has been introduced to improve OCT quality. It can provide 6x6 mm high-density scans to provide RNFL thickness measurements. The purpose of this study was to test the reproducibility of 3d-OCT RNFL thickness measurements in healthy volunteers.

Methods:

38 eyes were included into the study. High-density 6x6 mm 3d-scans were registered by 2 independent operators. RNFL thickness was calculated for 8 areas corresponding to the ETDRS areas and for two ring areas. The ETDRS grid was centered on the optic disc. Intraclass correlation coefficients (ICC) and coefficients of variation (COV) were calculated. Inter-observer reproducibility was visualized by using Bland-Altman analysis.

Results:

Intrasession reproducibility was good with a mean ICC of 0.90. The mean COV for operator 1 and 2 was 4.2% and 4%, respectively (range 1.9% to 6.7%). Highest reproducibility was found for the two ring areas and the superior- and inferior quadrants. Mean differences in RNFL thickness measurements for ring 1
and 2 between operator 1 and 2 were 0.9 µm (limits of agreement -11.4 to 9.6 µm) and 0.1 µm (limits of agreement -4.1 to 3.9 µm), respectively.

**Conclusion:**

3d-OCT RNFL thickness measurements in healthy volunteers showed good intra- and inter-observer reproducibility. 3d-OCT provides more RNFL thickness information compared to conventional time-domain OCT measurements and might be useful for the management of glaucoma and other optic neuropathies.
Introduction:

Evaluation of the retinal nerve fiber layer (RNFL) is fundamental for diagnosing and managing glaucoma and other optic neuropathies. In the past, RNFL could only be assessed subjectively by slit lamp examination. This method requires clinical experience and offers only qualitative data. In addition, comparisons over time are almost impossible. Successively, other techniques such as color photographs of the optic disc or red-free photographs of the RNFL became available, and facilitated comparisons over time. Scanning laser ophthalmoscopy and scanning laser polarimetry were the first instruments to allow objective and quantitative evaluation of the RNFL and the optic disc.\textsuperscript{1}

Optical coherence tomography (OCT) was first introduced in 1995 as an imaging technique for glaucoma diagnosis.\textsuperscript{2} Previous studies investigated the reproducibility of OCT retinal nerve fiber layer thickness measurements to assess the value of OCT as a clinical tool to distinguish between healthy and glaucomatous eyes.\textsuperscript{3-9}

However, all previous studies used conventional time-domain OCT for testing reproducibility of RNFL thickness measurements. Time-domain OCT uses a scanning interferometer and an 820-nm infrared light source which is split into two separate beams. One beam is scanning a tissue being analyzed, and the other one acts as a reference beam which is reflected by a reference mirror. The distance of the reference mirror can be adjusted and therefore the time it takes for the reference beam to reach the sensor can be changed. By comparing the
two light beams, time-domain OCT measures the optical backscattering of light to
generate a cross sectional image of the tested tissue.

Recently, improvements in OCT technology have been introduced.\textsuperscript{10-11} Fourier-Domain OCT (FD-OCT) provides increased resolution and scanning
speed by recording the interferometric information using a Fourier domain
spectrometric method instead of adjusting the position of a reference mirror.
Resolution is up to five times higher and imaging speed is 60 times faster than in
conventional time-domain OCT.\textsuperscript{12-13}

In addition to high image quality it is important to have reliable and
reproducible software programs to analyze the data acquired by FD-OCT.
Previous versions of OCT (Stratus OCT3) mostly used a 3.4mm diameter circle
scan centered on the optic disc to generate 512 A-scans. The RNFL thickness
profile showed a characteristic curve with two peaks, one in the superior and one
in the inferior quadrant. FD-OCT can perform a high-density raster-scan
(512x128 axial B-scans in a 6x6mm area). Recently, the peripapillary nerve fiber
layer thickness profile was determined with FD-OCT by using high density
scanning.\textsuperscript{14} These raster-scans provide considerably more data for RNFL
thickness analysis. The purpose of this study was to test the reproducibility of
RNFL thickness measurements in healthy subjects by using FD-OCT high-
density raster-scans (Topcon 3D-OCT1000).
Methods:

38 eyes of 19 healthy subjects (10 female) with a mean age of 26 ± 3 years were included into the study. Exclusion criteria were history of glaucoma, history of any other ocular disease, intraocular pressure greater than 21mmHg, or a refractive error of more than -5 or +5 dpt. FD-OCT high density scans were performed by using the Topcon 3d-OCT1000 system.

The Topcon 3d-OCT 1000 is a Fourier-domain OCT device providing OCT images up to 50 times faster than time domain OCTs using a sweep-scan technique. The device has a field angle of 45 degree with a color fundus camera included. The scanning range of the device is from 3x3mm up to 6x6mm. Horizontal resolution is ≤20µm and depth resolution is up to 5µm. As a light source the Topcon OCT uses superluminescent diodes with a wavelength of 840nm.

Pupil diameter had to be at least 4mm for scanning. High density raster scans (512x128 B-scans in a 6x6 mm area) were centered on the optic disc by moving the patient’s fixation target on the OCT observer screen. Scans were performed 6 times in one session by 2 operators (3 scans each in changing order). All subjects gave informed consent to participate in the study which adhered to the tenets of the Declaration of Helsinki. The FD-OCT software provides a quality factor (Q-factor) comparable to the scan strength number given in Stratus OCT3 for each examination. Scans with a Q-factor less than 45 were excluded and measurements were repeated until 6 scans of good quality were acquired. In addition, scans with blinks during the scanning process were
excluded and repeated. Eighteen scans had to be repeated because of low Q-
factors or blinks (7.9%). The Topcon 3d-OCT1000 system contains a high-
resolution camera for color fundus pictures. Pictures are automatically taken after
each examination. Before data analysis, stored infrared fundus images were
registered with the corresponding color fundus image. Scans were automatically
aligned to compensate for eye movement artifacts during the scanning process.
The FD-OCT system provides a software algorithm for RNFL thickness
measurements. Each high-density raster scan was separately analyzed by using
the RNFL algorithm to generate RNFL thickness values in µm. Mean RNFL
thickness values can be plotted as an area of 6x6 mm containing 36 squares of
mean RNFL values, or alternatively as 9 areas corresponding to the 9 ETDRS
areas also known from Stratus OCT3. The 3.4 mm circle scan for RNFL
measurements known from the Stratus OCT was not available in the software
version of the Topcon OCT. To obtain good centration on the optic disc it is
beneficial to use a circle shaped target-area which can easily be centered on the
optic disc. Therefore, for testing RNFL thickness reproducibility the ETDRS plot
(see Fig. 1) was chosen because one can easily center the inner ring of the plot
on top of the optic disc. The inner circle of the ETDRS plot has a diameter of
500µm. The middle circle represents a diameter of 3mm and the outer circle
represents a diameter of 6mm. Both left and right eyes were analyzed. Therefore,
data was adjusted such that all quadrants could be appropriately assessed. Left
eyes were treated as mirror images of right eyes. In all tables area 3 and 7
correspond to temporal quadrants and areas 5 and 9 correspond to nasal
quadrants. Figure 1 shows an example of a RNFL thickness measurement showing mean values of RNFL thickness for each of the 9 EDTRS areas. The most inner ring (area1) of the EDTRS plot was excluded from analysis as measuring RNFL thickness is not possible directly on the optic disc cup. In previous measurements we observed that the Topcon OCT actually performed RNFL thickness measurements on the optic disc rim. Therefore, we decided to include areas 2 to 5 (inner ring) to test reproducibility of such measurements. Areas 2-5 are between inner ring and middle ring and were included into the analysis even if parts of the optic disc rim crossed the inner ring. Areas 5-9 were unaffected by the optic disc and included into the analysis (Figure 1).

For statistical analysis areas 2-9 were analyzed. In addition, mean RNFL thickness for the inner ring (ring 1, consisting of areas 2-5) and outer ring (ring 2, consisting of areas 6-9) was calculated. Square root of variance components and 95% confidence intervals (95%CI) were determined for subjects, eyes, operators and scans using a linear mixed effect model. In addition, the bias between operators was tested. The software STATA\textsuperscript{tm} (Version 9.2, StataCorp, Texas, USA) was used for analysis. Three different kinds of Intraclass correlation coefficients (ICCs) were determined: ICC\textsuperscript{1} (for measurements within the same subject, eye and operator), ICC\textsuperscript{2} (inter-operator) and ICC\textsuperscript{3} (intra-operator).

Inter-observer reproducibility was visualized by providing limits of agreement in Bland-Altman plots to compare every first measurement of operator 1 and 2 of the inner and outer ring and areas 2 to 9, based on the assumption of equal imprecision between operators. Additional limits of agreement were provided to
take into account that eyes were nested within subjects. Bland-Altman plots were
created using the program MedCalc (MedCalc Software 9.3.9.0, Mariakerke,
Belgium). Coefficients of variation (COV) were determined for each area and
both rings for operator 1 and 2.

Results:

RNFL thickness measurements were done 3 times by each operator. Mean
RNFL thickness values were calculated out of the 3 measurements for areas 2 to
9 and ring 1 and 2 separately for operator 1 and 2 (Table 1).

RNFL thickness values were higher in the superior and inferior quadrant (areas
2, 4, 6 and 8) compared to the temporal and nasal quadrants. Regardless of the
observer, mean RNFL thickness values were very similar. COVs of
measurements for each area are shown in Table 2. Square root of variance
components with 95% confidence intervals for patients, eyes, operator, and
residuals and the corresponding ICCs are shown in Table 3.

Reproducibility was good with a mean ICC\(^1\), ICC\(^2\), and ICC\(^3\) of 0.9, 0.88,
and 0.9, respectively. Mean COV for operator 1 and 2 was 4.2% and 4%,
respectively. COVs ranged from 1.9% to 6.7% (Table 2). Best reproducibility was
found for ring 1 and 2 with highest ICCs (ring 1: 0.95; ring 2: 0.96), and lowest
COVs (operator 1 for ring 1: 2.9%; ring 2: 1.9%; operator 2 for ring 1: 2.3%; ring
2: 2.0%) Lowest reproducibility was found for area 5, 9 (nasal quadrants) and
area 7 (outer temporal area) with lowest ICCs (area 5: 0.82; area 7: 0.8; area 9:
Mean differences in RNFL thickness measurements for ring 1 and 2 between operator 1 and 2 were 0.9 µm (range -14.0 to 9.8 µm) and 0.13 µm (range -4 to 4.8 µm), respectively. To assess inter-observer reproducibility, limits of agreement were provided as 2-times SD with upper and lower limits of the differences between measurements of operator 1 and operator 2 for RNFL thickness measurements of ring 1 and 2. For ring 1 bias was -0.9 µm with limits of agreement from -11.4 µm to 9.6 µm. For ring 2 bias was -0.1 µm with limits of agreement from -4.1 µm to 3.9 µm. Figure 2 shows Bland-Altman plots for RNFL measurements of ring 1 and 2 to demonstrate inter-observer differences. Bland-Altman plots for all other areas tested looked similar. In addition, adjusted limits of agreement were provided to take into account that eyes were nested within subjects. No significant bias was found between operators (p> 0.09).

Discussion:

FD-OCT represents the latest commercially available generation of OCT. With higher axial resolution and higher scan acquisition speed FD-OCT enables high-density scanning of larger retinal areas compared to conventional time-domain OCT. Before FD-OCT, only circle scans could be registered to calculate RNFL thickness at certain points around the disc. RNFL thickness maps were available with Stratus OCT3, but data was interpolated out of only 3 circle scans with increasing diameters, centered on the disc. This can cause considerable
errors. In addition, localized RNFL defects can be missed. With higher scan
density of FD-OCT, RNFL thickness information of a 6x6 mm area around the
disc becomes available. This additional information might be helpful for
diagnosing and following glaucoma or other diseases that might affect the RNFL.
Reproducibility of any diagnostic test is important for diagnostic accuracy.
Especially in glaucoma, reproducibility of RNFL measurements is critical if the
device is used to monitor progression of the disease. Consequently, the goal of
this study was to determine the reproducibility of high-density FD-OCT RNFL
thickness measurements. Data on the reproducibility and reliability of first-, second-, and third-generation OCT (time-domain OCT) RNFL thickness
measurements have been reported before in normal and glaucomatous eyes. To
our knowledge, this study reports the first data on reproducibility of high density
FD-OCT RNFL thickness measurements in healthy subjects.

Schuman et al. used first-generation OCT, performing a 3.4 mm circle
scan on 11 normal and 10 glaucoma subjects on 5 separate occasions. He
reported ICCs of 0.56 and 0.52, respectively. The authors concluded that
measurements were reproducible, particularly when stable fixation could be
maintained during measurements.\textsuperscript{15} Carpineto et al. tested reproducibility by
comparing 24 glaucomatous patients with 24 age-matched controls using first-
generation OCT.\textsuperscript{7} He reported an ICC of 0.50 for mean RNFL thickness, but poor
ICCs for temporal and nasal quadrants (0.36 and 0.31, respectively).
Blumenthal et al. tested reproducibility of RNFL measurements of a second-
generation OCT in 10 normal and 10 glaucomatous eyes and reported COVs for
mean RNFL thickness of 7% in normal eyes and 13% in glaucomatous eyes. Measurements in the nasal quadrant were more variable (COV of 28%). In addition, Jones et al. studied reproducibility of the second-generation OCT finding a COV for mean RNFL thickness of 5% in normal subjects. Measurements in the nasal quadrant were more variable with a COV of 20%. One would expect an improvement in reproducibility of RNFL measurements with further developments in OCT technology. Especially increasing scan resolution and improvements in OCT software regarding the RNFL thickness algorithm should improve reproducibility. 

Paunescu et al. reported on reproducibility of RNFL thickness measurements of the third-generation Stratus OCT in 10 normal subjects. Subjects were scanned 6 times per day on 3 different days over a 5-months period. ICC for mean RNFL thickness was 0.79 by using the fast scan algorithm. ICCs for the superior, inferior, nasal, and temporal quadrant were 0.75, 0.71, 0.75, and 0.84, respectively. When using the standard circle scan with higher resolution compared to the fast scan, ICCs for mean, superior, inferior, nasal, and temporal measurements were 0.79, 0.73, 0.65, 0.68, and 0.79, respectively. In addition, Budenz et al. tested reproducibility of RNFL thickness measurements with Stratus OCT3. Intrasession variability of measurements between 88 normal and 59 glaucoma subjects was tested. ICCs ranged from 0.84 to 0.97 in normal eyes with a range of COVs from 1.7% (mean RNFL) to 8.2% (nasal quadrant). In glaucomatous eyes the ICC ranged from 0.79 to 0.98 with a range of COVs of 3.7% (mean RNFL) to 11.9% (nasal quadrant). In a second study in 59
glaucoma subjects ICCs, COVs and test-retest variabilities were virtually identical
despite using a different operator and different subjects. The study tested
variations of measurements between different days. Our results indicate that RNFL measurements with the Topcon 3D-
OCT1000 showed good reproducibility with a mean ICC, ICC, and ICC of 0.9,
0.88, and 0.9, respectively. Mean COV for operator 1 was 4.7% (range 2.4% to
6.7%) for areas 2 to 9. Mean COV for ring 1 and ring 2 for operator 1 was 2.9%
and 1.9% respectively. Mean COV for operator 2 was 4.4% (range 2.9% to
6.4%) for areas 2 to 9. Mean COV for ring 1 and ring 2 for operator 2 was 2.3%
and 2.0% respectively. As shown in table 3 some confidence intervals for
operator variance components were fairly wide. This might mainly be due to the
relatively small sample size and due to general difficulty in estimating variance
components.

All data was acquired by using the automated RNFL thickness algorithm
provided by the Topcon 3d-OCT1000 software. Although changes can be made
manually to the algorithm if borders of the RNFL are not correctly recognized, in
this study no corrections have been made. Therefore the reproducibility data of
this study can only be applied to automated RNFL measurements. Additional
studies are needed to test if the manually corrected algorithm shows even more
reliable and reproducible results for RNFL measurements compared to the fully
automated measurement.

Mean RNFL thickness values measured with Topcon OCT seemed to be
slightly higher compared to values acquired with Stratus OCT. For ring 1 mean
RNFL thickness was 107 µm for both operators. When measured with Stratus OCT 3.4 mm circle scans RNFL thickness values are expected to range around 100 µm depending on age of the study subject. Direct comparison of RNFL values between different OCT machines might be difficult due to different technical specifications, imaging protocols and different thickness measurement algorithms. For example, retinal (macular) thickness measurements with the Topcon OCT are performed differently compared to Stratus OCT. Both instruments outline the inner limiting membrane as the inner retinal border. The outer retinal border is defined in Stratus OCT on top of a signal believed to correspond to the junction between inner- and outer segments of the photoreceptors. However, the Topcon OCT software defines the outer retinal border as to be right above the RPE but underneath the photoreceptor signal which results in larger retinal thickness measurements. Algorithm definitions of the outer border for RNFL thickness measurements are not so obvious, but such differences might account for larger RNFL thickness measurements as well. In addition, measurements closer than 3.4mm diameter around the disc were included in the analysis of Topcon RNFL thickness measurements. It is well known that RNFL thickness increases with increasing proximity to the optic disc. That might also explain higher RNFL thickness values measured with Topcon 3dOCT 1000. To this point no specific scan protocol has been implemented in the Topcon 3d-OCT1000 software to measure the RNFL thickness in a specific area around the disc. In this study the ETDRS grid (normally used for macular
thickness measurements) was used to divide RNFL thickness in 4 quadrants around the disc. However, the central ring (area 1) of the ETDRS grid has a diameter of only 500 µm. The average optic disc has a larger diameter than area 1. RNFL thickness measurements cannot be performed directly on the optic disc cup. Therefore, area 1 had to be excluded from analysis. Frequently, the optic disc rim reached into areas 2 to 5. The Topcon RNFL software algorithm was able to identify RNFL boundaries on the optic disc rim as seen in the corresponding OCT B-scans. To our knowledge, RNFL measurements on the optic disc rim have not been tried previously with OCT since such measurements where not possible with the standard 3.4mm circle scans provided by previous OCT versions. Despite the problem that reliability of RNFL thickness measurements on the optic disc rim is not known yet, areas 2 to 5 and ring 1 were included in the analysis. Surprisingly, reproducibility was good for areas 2, 4 and ring 1 and acceptable for areas 3 and 5.

As found in previous studies, reproducibility was higher in the superior and inferior quadrants and lowest in the nasal quadrants. The reason for that is not clear. Knighton et al. suggested that the angle of incidence of the illuminating beam makes the RNFL image on the nasal side dimmer and therefore harder to be identified by the measurement algorithm. In addition, ICCs might be reduced mathematically because of a smaller population variance nasally. Our data showed better reproducibility in the larger sample areas (Ring 1 and 2) compared to the smaller areas 2 to 9. These findings are consistent with previous studies using time-domain OCT. Larger sampled areas contain more individual
measurements that add into the mean of that area. This signal averaging leads to
more reliable measurements. Gurses-Ozden et al. actually showed that
increasing the sampling density or the number of A-scans can increase the
reproducibility of OCT measurements.\textsuperscript{18}

In addition to intra-observer reproducibility our study tested inter-observer
reproducibility. Differences between the two operators were small. Mean
difference between RNFL thickness measurements of operator 1 and operator 2
for ring 1 and ring 2 were only 0.9\(\mu\)m and 0.1\(\mu\)m, respectively. Limits of
agreement were calculated as 2-times the SD of the mean difference between
multiple measurements of the two operator for ring 1 (2xSD= 9.6\(\mu\)m) and ring 2
(2xSD=4.4\(\mu\)m). Our data suggest that reproducibility of RNFL thickness with FD-
OCT is good, regardless of the operator. However, an analysis
based on Bland-Altman plots depends on the assumption of equal
imprecision. If this assumption would be unreasonable, the conclusion from the
Bland-Altman plot could be incorrect.

Scan quality is important to facilitate the recognition of the RNFL by the
measurement algorithm. Eighteen scans (7.9\%) with poor quality factors (Q-
Factor) or because of blinks during scanning process had to be excluded. In
clinical routine, there might be a greater difference in RNFL measurements
between experienced operators and un-experienced operators which are
expected to produce scans with lower Q-factors. In our study, the experience
level of both operators was the same. Therefore we assume that the imprecision
for both operators is about the same allowing the use the linear mixed effect
model for analysis and ICC calculation.

Multiple prior studies with previous generations of OCT tested
reproducibility. However, ICCs cannot directly be compared since measurements
were done in different groups of subjects and with different OCT models. The
ICC is calculated as the ratio of variability due to differences between subjects to
variability from all sources such as noise and/or fluctuations within subjects. The
best way to compare study results would probably be a comparison of the
residual error variance components as an absolute measure of imprecision of
measurements. Residual error variance components of this study are given in
table 3. In our case, ICCs were calculated from measurements of young healthy
volunteers. One would expect only little between subject variance in such group.
Caution should be used when applying these data on older subjects or patients
with glaucoma which are expected to have a greater between subject variance.
Additional studies testing the reproducibility of RNFL thickness measurements in
glaucoma patients are needed before the Topcon 3D-OCT1000 can be safely
used as a tool for diagnosing and monitoring glaucoma and other optic
neuropathies.
Figure legends:

Figure 1:

A: ETDRS-scheme applied for retinal nerve fiber layer (RNFL) thickness measurements. The central black area (area 1) was excluded for analysis. Mean RNFL thickness was calculated for ring 1 (white) and ring 2 (grey).

B: Fundusphotograph taken during an RNFL measurement. The ETDRS grid was centered on the disc. The white arrow indicates the scan location and direction of the B-scan shown in C.

C: One out of 128 B-scans taken during a high-density 3d-OCT scan. The white lines represent the RNFL thickness algorithm. One can see that measurements directly on the optic disc cup cannot be performed correctly. Therefore area 1 was excluded from analysis.

D: 6x6mm RNFL thickness map. RNFL thickness is color-coded from blue (0-60 µm) over green (80-140 µm), red (160-180 µm), to white (over 200 µm).

Figure 2:
Bland-Altman plots of differences in retinal nerve fiber layer (RNFL) thickness in measurement 1 by operator 1 and 2 for ring 1 and 2 versus the means of the two operator's measurements in a set of 38 eyes. Units for both axes are microns.

adj: adjusted limits of agreement taking into account that eyes were nested within subjects

References:


Figure 1:
Figure 2:
Table 1

Mean RNFL thickness values for areas 2 to 9, ring 1 and ring 2. (n=38)

<table>
<thead>
<tr>
<th>Operator 1</th>
<th>Area 2</th>
<th>Area 3</th>
<th>Area 4</th>
<th>Area 5</th>
<th>Area 6</th>
<th>Area 7</th>
<th>Area 8</th>
<th>Area 9</th>
<th>Ring 1</th>
<th>Ring 2</th>
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<tbody>
<tr>
<td>Mean RNFL thickness (µm):</td>
<td>132</td>
<td>82</td>
<td>140</td>
<td>106</td>
<td>92</td>
<td>55</td>
<td>91</td>
<td>56</td>
<td>107</td>
<td>74</td>
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<tr>
<td>Operator 2</td>
<td>Area 2</td>
<td>Area 3</td>
<td>Area 4</td>
<td>Area 5</td>
<td>Area 6</td>
<td>Area 7</td>
<td>Area 8</td>
<td>Area 9</td>
<td>Ring 1</td>
<td>Ring 2</td>
</tr>
<tr>
<td>Mean RNFL thickness (µm):</td>
<td>133</td>
<td>82</td>
<td>142</td>
<td>107</td>
<td>94</td>
<td>55</td>
<td>91</td>
<td>57</td>
<td>107</td>
<td>74</td>
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</tbody>
</table>

RNFL= retinal nerve fiber layer;
Table 2

COVs for all areas measured by operator 1 and 2 are shown. Both operators performed 3 measurements in each eye (n=38).

<table>
<thead>
<tr>
<th></th>
<th>Area 2</th>
<th>Area 3</th>
<th>Area 4</th>
<th>Area 5</th>
<th>Area 6</th>
<th>Area 7</th>
<th>Area 8</th>
<th>Area 9</th>
<th>Ring 1</th>
<th>Ring 2</th>
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<tbody>
<tr>
<td>Operator 1 COV (%)</td>
<td>5.1</td>
<td>5.8</td>
<td>3.5</td>
<td>6.7</td>
<td>2.4</td>
<td>4.1</td>
<td>3.1</td>
<td>6.6</td>
<td>2.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Operator 2 COV (%)</td>
<td>3.5</td>
<td>4.6</td>
<td>4.2</td>
<td>6.2</td>
<td>2.9</td>
<td>4.5</td>
<td>2.9</td>
<td>6.4</td>
<td>2.3</td>
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</table>

COV = Coefficient of variation (%)
Table 3:
Square root of variance components with 95% confidence intervals for patients, eyes, operators, and residuals are shown for each measured area. The linear mixed model was used to calculate ICCs:

<table>
<thead>
<tr>
<th>Areas tested</th>
<th>SqRVar. Patient 95% CI</th>
<th>SqRVar. Eye 95% CI</th>
<th>SqRVar. Rater 95% CI</th>
<th>SqRVar. Residual 95% CI</th>
<th>ICC 1</th>
<th>ICC 2</th>
<th>ICC 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ring 1</td>
<td>14.1 (10.1, 19.7)</td>
<td>4.1 (2.8, 6.0)</td>
<td>1.4 (0.6, 2.8)</td>
<td>3.5 (3.2, 3.9)</td>
<td>0.95</td>
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<td>0.95</td>
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<td>Ring 2</td>
<td>8.3 (6.0, 11.6)</td>
<td>2.3 (1.4, 3.2)</td>
<td>1.0 (0.6, 1.5)</td>
<td>1.7 (1.4, 2.0)</td>
<td>0.96</td>
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<td>0.96</td>
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<tr>
<td>Area 2</td>
<td>18.7 (13.2, 26.4)</td>
<td>7.2 (5.1, 10.4)</td>
<td>1.0 (0.05, 20.3)</td>
<td>6.3 (5.7, 7.1)</td>
<td>0.91</td>
<td>0.91</td>
<td>0.91</td>
</tr>
<tr>
<td>Area 3</td>
<td>9.8 (5.7, 17.1)</td>
<td>10.7 (7.7, 15.1)</td>
<td>1.0 (0.14, 9.5)</td>
<td>5.4 (4.8, 6.1)</td>
<td>0.88</td>
<td>0.88</td>
<td>0.88</td>
</tr>
<tr>
<td>Area 4</td>
<td>14.8 (10.2, 21.7)</td>
<td>8.6 (6.1, 12.4)</td>
<td>1.4 (0.17, 11.1)</td>
<td>6.3 (5.9, 7.4)</td>
<td>0.87</td>
<td>0.86</td>
<td>0.87</td>
</tr>
<tr>
<td>Area 5</td>
<td>15.5 (10.3, 23.5)</td>
<td>10.7 (7.5, 15.4)</td>
<td>2.3 (0.4, 9.9)</td>
<td>8.5 (7.6, 9.5)</td>
<td>0.83</td>
<td>0.82</td>
<td>0.83</td>
</tr>
<tr>
<td>Area 6</td>
<td>10.3 (6.1, 15.8)</td>
<td>7.6 (5.5, 10.8)</td>
<td>2.4 (1.7, 3.5)</td>
<td>2.8 (2.4, 3.2)</td>
<td>0.96</td>
<td>0.92</td>
<td>0.95</td>
</tr>
<tr>
<td>Area 7</td>
<td>4.6 (2.8, 7.1)</td>
<td>3.7 (2.8, 5.3)</td>
<td>0.8 (0.2, 2.8)</td>
<td>2.8 (2.4, 3.2)</td>
<td>0.82</td>
<td>0.8</td>
<td>0.81</td>
</tr>
<tr>
<td>Area 8</td>
<td>12.3 (8.7, 17.4)</td>
<td>5.0 (3.5, 7.2)</td>
<td>1.7 (1.0)</td>
<td>3.3 (2.8, 3.6)</td>
<td>0.94</td>
<td>0.93</td>
<td>0.96</td>
</tr>
<tr>
<td>Area 9</td>
<td>10 (6.9, 14.6)</td>
<td>4.4 (2.8, 6.5)</td>
<td>1.7 (1.4, 2.1)</td>
<td>5.0 (4.5, 5.6)</td>
<td>0.83</td>
<td>0.81</td>
<td>0.83</td>
</tr>
</tbody>
</table>

All units are µm. SqRVar: Square root of variance; CI: 95% confidence interval; ICC: Intraclass correlation coefficient; P-values for operator bias were always > 0.09.