

# Comparison of retinal nerve fibre layer measurements using optical coherence tomography versions 1 and 3 in eyes with band atrophy of the optic nerve and normal controls

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## Abstract

**Aims** To compare retinal nerve fibre layer (RNFL) measurements were carried out with two different versions of an optical coherence tomography device in patients with band atrophy (BA) of the optic nerve and in normal controls.

**Methods** The RNFL of 36 eyes (18 with BA and 18 normals) was measured using an earlier version of an optical coherence tomography device (OCT-1). The measurements were repeated using a later version of the same equipment (OCT-3), and the two sets of measurements were compared.

**Results** Using OCT-1, the peripapillary RNFL thickness (mean  $\pm$  SD, in  $\mu$ m) in eyes with BA measured  $80.42 \pm 6.94$ ,  $99.81 \pm 14.00$ ,  $61.69 \pm 13.02$ ,  $101.70 \pm 12.54$ , and  $57.36 \pm 16.52$  corresponding to the total RNFL average, superior, temporal, inferior, and nasal quadrants, respectively. Using OCT-3, the corresponding measurements were  $63.11 \pm 6.76$ ,  $81.22 \pm 13.34$ ,  $39.50 \pm 8.27$ ,  $86.72 \pm 15.16$ , and  $45.05 \pm 8.03$ . Each of these measurements was significantly smaller with OCT-3 than with OCT-1. In normal eyes, RNFL average and temporal quadrant OCT-3 values were significantly smaller than OCT-1 values, but there was no significant difference in measurements from the superior, inferior, and nasal quadrant.

**Conclusions** RNFL measurements were smaller with OCT-3 than with OCT-1 for almost all parameters in eyes with BA and in the global average and temporal quadrant measurements in normal eyes. Investigators should be aware of this fact when comparing old RNFL measurement with values obtained with later versions of the equipment.

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**Keywords:** optical coherence tomography; retinal nerve fibre layer; band atrophy

## Introduction

Optical coherence tomography (OCT) is a non-invasive technique for the acquisition of cross-sectional images of retinal structures from which estimates of the thickness of retinal layers can be made. The ability of OCT to provide quantitative and reproducible measurements of the retinal nerve fibre layer (RNFL) has been demonstrated in experimental and clinical studies.<sup>1–4</sup> Although most of the clinical studies using OCT have been performed in patients with glaucoma,<sup>5–9</sup> many other conditions have been studied, including band atrophy (BA) of the optic nerve from chiasmal compression.<sup>10,11</sup>

The first commercially available OCT equipment (OCT-1, Humphrey-Zeiss, Dublin, CA, USA) has been widely used and its ability

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to measure RNFL has been well documented.<sup>12-14</sup> The second commercial version of the equipment presented only slight modifications, but the recently released third version (OCT-3, or Stratus-OCT, Carl Zeiss Meditec Inc., Dublin, CA, USA) brought significant improvements over the original version.<sup>15</sup> As some patients examined with OCT-1 are likely to be examined with later versions of the equipment it is important to determine whether different versions produce comparable measurements in healthy and sick subjects. The purpose of this paper is to compare RNFL measurements obtained using two different versions of OCT, old (OCT-1) and new (OCT-3), when applied to patients with BA and to normal controls.

### Patients and methods

Between December 2002 and December 2003, 36 eyes were presented to OCT-1 analysis of the peripapillary RNFL. In all, 18 eyes were from 18 consecutive patients (10 males and eight females) with BA of the optic nerve owing to previous (already treated) chiasmal compression caused by suprasellar tumours. The remaining 18 eyes were from 18 normal age- and sex-matched controls. Mean  $\pm$ SD age of patients with BA and healthy individuals was  $45.70 \pm 15.83$  and  $45.58 \pm 14.06$ , respectively. Institutional Ethics Committee approval was obtained for the study and informed consent was obtained from all participants.

Visual field (VF) testing was performed using the Goldmann perimeter. The V-4-e, I-4-e, I-3-e, I-2-e, and I-1-e stimuli were used to draw the isopters. Patients were also submitted to standard automated perimetry (Humphrey, 24-2 Full-threshold test).

The inclusion criteria for the patients were: best corrected visual acuity of 20/30 or better in the study eye; complete temporal hemianopia on Humphrey perimetry and complete or almost complete temporal hemianopia on Goldmann perimetry; nasal hemifield within normal limits; spherical refraction within  $\pm 3$  D; cylinder correction within  $\pm 3$  D; intraocular pressure less than 22 mmHg; and reliable VF. A reliable Humphrey VF test was defined as one with fewer than 25% fixation losses, false-positive or false-negative responses. All patients had VF defects stable for at least 1 year. Patients with clinical signs of glaucoma were excluded. In 14 patients only one eye met the inclusion criteria. In four patients, one eye was chosen at random for the study. The control group consisted of normal healthy volunteers recruited from hospital staff members, with a normal ophthalmic examination including Humphrey perimetry.

VF and OCT-1 examinations were performed on the same day or within a maximum period of 2 weeks. OCT-1

examination was performed after pupillary dilatation to a minimum diameter of 5 mm. Circular 360-degree OCT scans were obtained with a diameter of 3.4 mm centered on the optic disc. The scan was initiated at the 9 O' clock position and proceeded clockwise. Each scan consisted of 100 evenly distributed transverse A-scans along the circumference with an acquisition time of 1 s.

Three good-quality circular scans were taken around the centre of the optic disc in each eye. OCT software version A5 was used for analysis. The RNFL was differentiated from other retinal structures using the automated edge detection algorithm provided by the OCT software. The average RNFL thickness values were calculated globally and separately for the superior, inferior, temporal, and nasal quadrants (90 degrees each), and for the twelve 30-degree segments using OCT software. The average RNFL thickness value was calculated automatically by the OCT software. We examined both left and right eyes; left eyes were considered to be mirror images of right eyes. Therefore, when indicating the 30-degree segments as clock hours, we considered the hours from 1200 to 0600 as nasal hours, and those from 0600 to 1200 as temporal hours.

Within a maximum interval of 6 months, the same group of individuals was presented to a second OCT examination using a recent version of the same equipment (OCT-3). There was no significant change on a repeat VF test. We used the fast RNFL algorithm to obtain RNFL thickness measurements. Three images were acquired from each subject, each one consisting of 256 A-scans along a 3.4 mm-diameter circular ring around the optic disc. The parameters of RNFL thickness calculated automatically by the OCT-3 software (version 3.1) were average thickness, temporal, superior, nasal, and inferior quadrant thickness and thickness for each of the 12 30-degree segment of the disc. The parameters were the same for measurements obtained with OCT-1.

Good-quality scans had focused images, an adequate signal-to-noise ratio ( $> 40$  dB), a ring around and concentric with the optic disc, and absence of overt misalignment of the surface detection algorithm. From the three scans judged to be of acceptable quality obtained with each equipment, mean values were calculated for each studied parameter.

RNFL values with OCT-1 were compared to those from OCT-3 using paired *t*-test. Analysis of histograms and the Shapiro-Wilk test confirmed that the distributions satisfied the normality assumption. *P*-values of less than 0.05 were considered significant. Results of statistical significance were provided after Bonferroni's correction based on the number of comparisons within each analysis. Bland and Altman plots were constructed to assess agreement between OCT-1 and OCT-3 RNFL thickness measurements.<sup>16,17</sup> Both absolute and the

percentage difference between OCT-1 and OCT-3 were evaluated. The number of disagreement measurements within 10 and 20  $\mu\text{m}$  and within a certain percentage (10 and 20%) was compared between the two groups using the  $\chi^2$ -test. Corresponding RNFL values of patients with BA measured both with OCT-1 and OCT-3 were also compared with those of normal controls using unpaired Student's *t*-test.

**Results**

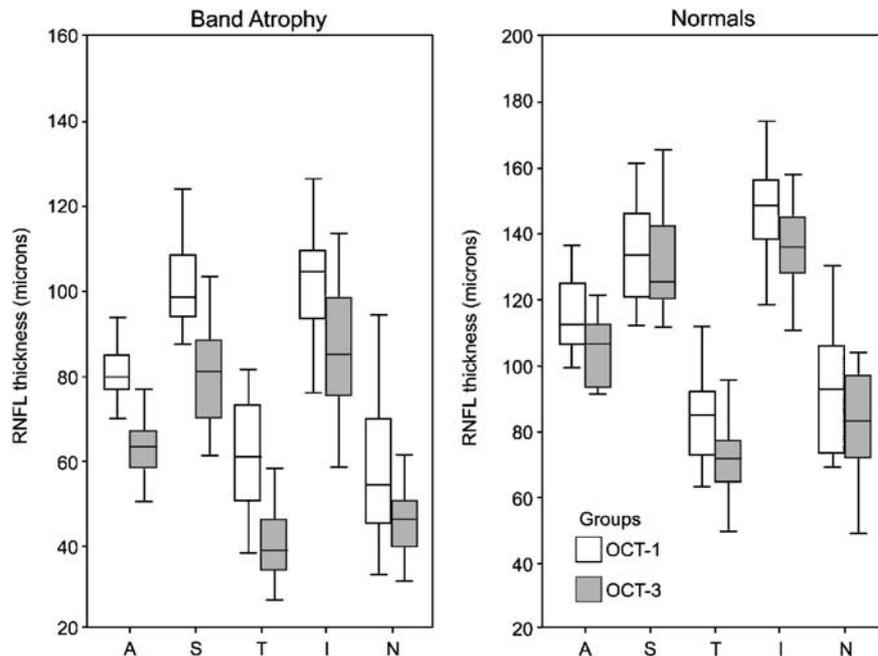
Figure 1 depicts the distribution of average RNFL thickness as well as the parameters measured by OCT-1 and OCT-3 in each quadrant of BA and normal eyes. In eyes with BA, the peripapillary RNFL (mean  $\pm$  SD, in  $\mu\text{m}$ ) measured with OCT-1 was  $99.81 \pm 14.00$ ,  $61.69 \pm 13.02$ ,  $101.70 \pm 12.54$ , and  $57.36 \pm 16.52$  in the superior, temporal, inferior, and nasal quadrants, respectively. The mean global RNFL was  $80.42 \pm 6.94$ . The corresponding figures for OCT-3 were  $81.22 \pm 13.34$ ,  $39.50 \pm 8.27$ ,  $86.72 \pm 15.16$ , and  $45.05 \pm 8.03$ . The mean global RNFL was  $63.11 \pm 6.76$ . Comparisons were also made in each of 12 clock hours measurements. After Bonferroni's correction ( $\alpha = 0.003$ ; 17 comparisons), average measurements as well as measurements in each quadrant with OCT-3 were significantly smaller ( $P < 0.003$ ) than values obtained with OCT-1. Measurements in the 30-degree segments of the optic disc were significantly smaller for OCT-3 at 10, 9, 8, 2, and 1 O' clock (Table 1).

In normal eyes, the peripapillary RNFL (mean  $\pm$  SD, in  $\mu\text{m}$ ) measured by OCT-1 was  $134.26 \pm 16.35$ ,  $83.50 \pm 14.57$ ,  $145.37 \pm 14.68$ , and  $91.89 \pm 18.80$  in the superior, temporal, inferior, and nasal quadrants, respectively. The mean

**Table 1** Mean values ( $\pm$ SD) of retinal nerve fiber layer thickness (in  $\mu\text{m}$ ) in each segment for the 18 eyes with band atrophy using OCT-1 and OCT-3

	OCT-1 (n=18)	OCT-3 (n=18)	P <sup>a</sup>
Total average	$80.42 \pm 6.94$	$63.11 \pm 6.76$	<i>&lt;0.001</i>
<i>RNFL quadrants</i>			
Superior	$99.81 \pm 14.00$	$81.22 \pm 13.34$	<i>&lt;0.001</i>
Temporal	$61.69 \pm 13.02$	$39.50 \pm 8.27$	<i>&lt;0.001</i>
Inferior	$101.70 \pm 12.54$	$86.72 \pm 15.16$	<i>&lt;0.001</i>
Nasal	$57.36 \pm 16.54$	$45.05 \pm 8.03$	<i>0.003</i>
<i>Clock hours</i>			
12	$107.76 \pm 23.54$	$93.16 \pm 24.40$	0.016
11	$107.91 \pm 21.79$	$93.66 \pm 26.11$	0.007
10	$69.32 \pm 14.77$	$46.38 \pm 17.41$	<i>&lt;0.001</i>
9 (temporal)	$52.78 \pm 19.42$	$32.38 \pm 7.76$	<i>&lt;0.001</i>
8	$62.08 \pm 20.41$	$39.94 \pm 10.30$	<i>&lt;0.001</i>
7	$106.30 \pm 29.93$	$93.33 \pm 29.51$	0.152
6	$112.00 \pm 19.17$	$103.05 \pm 20.97$	0.059
5	$84.05 \pm 31.89$	$63.33 \pm 17.87$	0.005
4	$55.70 \pm 21.29$	$44.00 \pm 7.09$	0.026
3 (nasal)	$48.28 \pm 19.38$	$40.05 \pm 10.66$	0.133
2	$70.22 \pm 18.58$	$51.33 \pm 13.68$	<i>&lt;0.001</i>
1	$82.11 \pm 17.89$	$57.50 \pm 17.46$	<i>&lt;0.001</i>

<sup>a</sup>Paired *t*-test. Significant values are in italic.



**Figure 1** Box plot chart showing the distribution of retinal nerve fibre layer (RNFL) thickness measurements with OCT-1 and OCT-3 in 18 eyes with band atrophy (left) and 18 eyes from normal controls (right). A = average, S = superior, T = temporal, I = inferior, N = nasal.

global RNFL was  $114.35 \pm 11.02$ . The corresponding figures for OCT-3 were  $130.16 \pm 15.54$ ,  $70.83 \pm 11.29$ ,  $135.25 \pm 16.00$ , and  $80.88 \pm 16.47$ . The mean global RNFL was  $104.76 \pm 9.81$  (Table 2). Measurements with OCT-3 were significantly smaller ( $P < 0.003$ ) than those obtained with OCT-1 in the global measurement as well as in the temporal quadrant. Comparisons of measurements for each of the 12 30-degree segments showed significantly smaller values for OCT-3 than for OCT-1 only at 9, 8, and 4 O' clock (Table 2).

Bland–Altman<sup>17</sup> plots were made depicting the agreement between OCT-1 and OCT-3 RNFL thickness measurements in eyes with BA and in normal controls. The difference (OCT-1 RNFL thickness minus OCT-3 thickness) was plotted against the mean of the two measurements for the average value as well as for measurements of each quadrant around the disc (Figure 2). There was considerable disagreement between the 90 measurements made with OCT-1 and OCT-3. In most cases, measurements were greater with OCT-1 than with OCT-3. The discrepancies between OCT-1 and OCT-3 RNFL thickness measurements were more evident in eyes with BA compared with normal eyes. The difference between the OCT-1 and OCT-3 measurements was within  $20 \mu\text{m}$  in 53 (58.8%) measurements in eyes with BA and in 73 (81.1%) measurements in normal eyes. The  $\chi^2$ -test showed a significant difference between the two groups ( $P = 0.001$ ). The difference was within  $10 \mu\text{m}$  in 30

(33.3%) measurements in eyes with BA and in 38 (42.2%) measurements in normal eyes. No significant difference was observed ( $P = 0.219$ ) in this comparison.

The difference in measurements of eyes with relatively thinner RNFL became more evident when the percentage difference between the two methods was plotted against the mean of the two measurements (Figure 3). The difference was within 20% of the mean in 75 (83.3%) measurements in normal eyes but only in 37 (41.1%) measurements from eyes with BA ( $P < 0.0001$ ,  $\chi^2$ -test). The percentage difference was within 10% of the mean in 38 (42.2%) of measurements in normal eyes and in only 20 (22.2%) of measurements in eyes with BA ( $P = 0.004$ ).

OCT-1 and OCT-3 values were both significantly smaller ( $P < 0.001$ ) in eyes with BA than in normal eyes, in the average measurements as well as in all four quadrants studied.

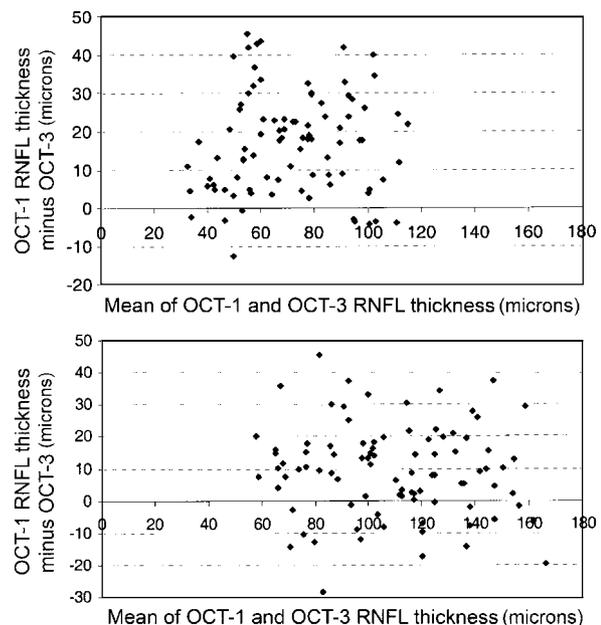
### Discussion

Several studies have demonstrated improvement in examinations performed with OCT-3 when compared to previous versions of the same equipment.<sup>15,18,19</sup> One of the most significant improvements of the OCT-3 version is its shorter acquisition time, especially in the FAST version used in the present study. In the OCT-1 version,

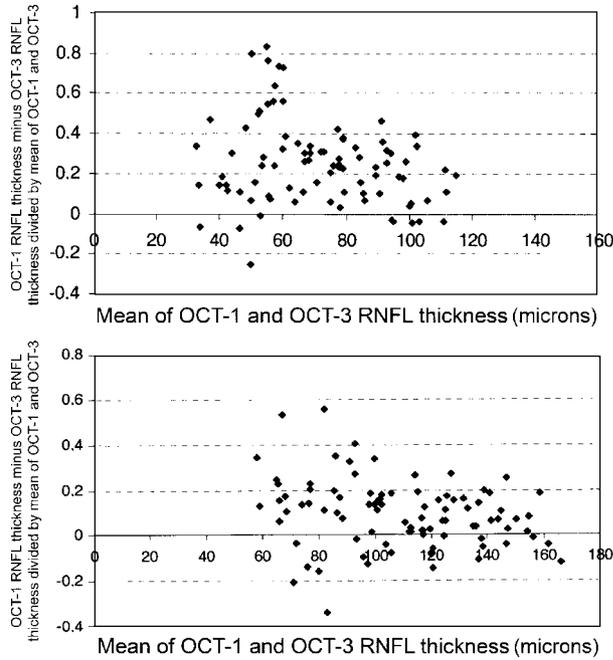
**Table 2** Mean values ( $\pm$ SD) of retinal nerve fibre layer thickness (in  $\mu\text{m}$ ) in each segment for the 18 normal eyes using OCT-1 and OCT-3

	OCT-1 (n = 18)	OCT-3 (n = 18)	P <sup>a</sup>
Total average	$114.35 \pm 11.02$	$104.76 \pm 9.81$	<i>&lt; 0.001</i>
<i>RNFL quadrants</i>			
Superior	$134.26 \pm 16.35$	$130.16 \pm 15.54$	0.169
Temporal	$83.50 \pm 14.57$	$70.83 \pm 11.29$	<i>&lt; 0.001</i>
Inferior	$145.37 \pm 14.68$	$135.25 \pm 16.00$	0.014
Nasal	$91.89 \pm 18.80$	$80.88 \pm 16.47$	0.029
<i>Clock hours</i>			
12	$135.63 \pm 26.14$	$134.88 \pm 19.84$	0.899
11	$141.07 \pm 17.84$	$138.61 \pm 25.76$	0.646
10	$98.74 \pm 13.47$	$87.66 \pm 17.82$	0.013
9 (temporal)	$74.92 \pm 16.23$	$56.66 \pm 10.68$	<i>&lt; 0.001</i>
8	$84.79 \pm 17.35$	$68.72 \pm 12.86$	<i>0.001</i>
7	$131.53 \pm 22.77$	$132.94 \pm 20.19$	0.818
6	$160.35 \pm 20.32$	$154.88 \pm 20.31$	0.333
5	$136.87 \pm 20.40$	$123.11 \pm 21.56$	0.005
4	$93.68 \pm 22.45$	$77.05 \pm 20.71$	<i>0.002</i>
3 (nasal)	$77.28 \pm 23.10$	$64.22 \pm 16.46$	0.017
2	$105.28 \pm 22.49$	$92.83 \pm 19.55$	0.061
1	$123.33 \pm 22.29$	$119.16 \pm 22.85$	0.441

<sup>a</sup>Paired *t*-test. Significant values are in italic.



**Figure 2** Bland–Altman plots of the agreement in RNFL thickness between OCT-1 and OCT-3. The difference (OCT-1 RNFL thickness minus OCT-3 RNFL thickness) was plotted against the average of the two measurements for the global measurements and for measurements in each quadrant (90 measurements). *Above*: eyes with band atrophy. *Below*: normal control eyes.



**Figure 3** Bland–Altman plots of the agreement in RNFL thickness between OCT-1 and OCT-3. The percentage difference ((OCT-1 RNFL thickness minus OCT-3 RNFL thickness)/mean of OCT-1 and OCT-3) was plotted against the average of the two measurements for the global measurements and for measurements in each quadrant. *Above:* eyes with band atrophy. *Below:* normal control eyes.

images are produced from 100 A-scans performed during 1 s.<sup>6,20</sup> On the other hand, when using the FAST protocol with OCT-3, images are produced from 256 A-scans performed during a four-times shorter acquisition time.<sup>15</sup> Thus, three images are captured from which the software automatically calculates the mean. As a result inter- and intra-examiner variability is smaller and reproducibility higher in OCT-3 than in OCT-1.

The higher variability observed for OCT-1 should in theory lead us to expect values both greater and smaller than those provided by later versions; however, we observed consistently smaller values with OCT-3 than with OCT-1. As the same patients were presented by the same examiner to both OCT-1 and OCT-3 within a short period of time, we may conclude that the observed difference may be accounted for by differences in the specifications of the two devices. Our study also shows that such difference may be observed with both normal individuals and patients with optic atrophy. However, differences were much more evident in eyes with BA than in normal eyes. This finding is important since interpretation of data from eyes with RNFL loss from glaucoma or other condition causing loss of RNFL fibres may become strongly biased in sequential

examinations if results from different devices are treated as equivalent.

Bourne *et al*<sup>21</sup> recently evaluated the comparability of RNFL thickness measurements obtained by two versions of OCT (Stratus OCT and OCT 2000). These authors analysed the results obtained by both devices obtained on the same day in 104 eyes of 104 individuals (28 with open-angle glaucoma, 40 suspected of having glaucoma, and 36 healthy subjects) and reported thinner RNFL measurements with Stratus OCT compared to OCT 2000. Application of a correction factor to OCT 2000 measurements predicted Stratus OCT RNFL thickness within 10  $\mu\text{m}$  of the observed measurement in 75% of the eyes, although they observed considerable variability that exceeds the limits of resolution afforded by the instruments. We therefore suggested that comparisons between instruments using these approximations should be interpreted with caution.<sup>21</sup> Our results agree with those from Bourne *et al* in that RNFL thickness measurements obtained with the Stratus OCT were significantly thinner than those measured by older versions of this technology. However, contrary to Bourne *et al*, we found that the discrepancies between the two devices tended to become worse in patients with thinner RNFL. It should be noted that our study included eyes with more severe optic atrophy and thinner RNFL measurements than those studied by Bourne *et al*,<sup>21</sup> which may have contributed to reveal more discrepancies between the two OCT devices in the presence of severe RNFL loss. We are unaware of previous reports comparing measurements between different versions of OCT in eyes with BA of the optic nerve. Such eyes represent an important model when investigating RNFL measuring equipments, as the pattern of RNFL loss is markedly different from the one occurring in glaucoma.

We could not find an exact explanation for the consistent difference observed between the measurements from the two versions of the technology. This difference could be related to modifications in the hardware as well as in the software of the instrument. Differences in the axial and longitudinal resolution as well as in the algorithm for detection of the RNFL borders between the two versions of the instrument may explain in part our findings. High-density scan measurements of the RNFL tend to result in lower values of thickness than standard-density scans, perhaps owing to the higher accuracy of detection of the retinal posterior border of the RNFL with the former.<sup>19</sup> Our data analyses show that the differences between OCT-1 and OCT-3 were more evident in areas with lower values of RNFL thickness. In normal eyes, differences were significant for average values as well as for thickness in the temporal quadrants and in some of the 30-degree segments located

temporally (9 and 8 O' clock) and nasally (4 O' clock). The RNFL is known to be thinner in these areas than in the superior and inferior areas of the optic disc. On the other hand, in eyes with BA OCT-1 and OCT-3 measurements differed in every quadrant and in most 30-degree segments. Bland and Altman<sup>17</sup> plot analyses of BA and normal controls examinations lend support to this conclusion because measurement discrepancies between the two OCT devices were more evident for the smaller measurements (Figures 2 and 3).

As there is no external validation for measurements obtained with either version of the equipment it is not possible to be certain about which one of the devices is more accurate. However, if we assume that the newest generation of the equipment (OCT-3) is the more accurate one, one possible interpretation would be that the OCT-1 overestimates the thickness in areas with thin RNFL. In these areas, the OCT-3 would be able to provide better estimates of thickness because of its superior performance in correctly identifying the borders of the RNFL. These findings are in agreement with those of Ishikawa *et al*<sup>22</sup> who observed significantly lower values of RNFL thickness using a refined and more accurate algorithm for detection of the inner and outer borders of the RNFL, compared with the commercially available OCT-1 software (version A5). These authors also observed that the commercially available algorithm, which is similar to the one used in the present study, lacks the flexibility required to adapt to various conditions of reflectivity. This is particularly important in glaucomatous eyes owing to their thin and uneven RNFL reflectivity.<sup>22</sup> Thus, it would seem that the more recently developed software (version 3.1) used by the OCT-3 equipment improves detection of inner and outer borders of the RNFL, yielding smaller values particularly for the thinner areas in both normal and BA-affected eyes, but mainly in the latter.

In conclusion, our study shows RNFL measurements obtained with OCT-3 to be significantly smaller than those obtained with OCT-1 in almost all study parameters in eyes with BA and in the nasal and temporal disc areas of normal eyes. Although both OCT devices were able to differentiate eyes with BA from normal eyes, the values of RNFL thickness obtained by the two instruments were significantly different. Researchers and clinicians should be aware of this fact when comparing older RNFL measurements with values obtained with later versions of the same equipment.

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