

# The influence of axial length on retinal nerve fibre layer thickness and optic-disc size measurements by spectral-domain OCT

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## ABSTRACT

**Background** To evaluate the influence of axial length on measurements of the retinal nerve fibre layer (RNFL) thickness and optic nerve head (ONH) parameters in healthy subjects.

**Methods** Using Cirrus HD-OCT, RNFL thickness and ONH parameters (disc and rim area) were measured in 15 short (<22.5 mm), 15 medium (22.51–25.5 mm) and 15 long (>25.51 mm) eyes.

**Results** The mean axial length was 21.5±0.5 mm in short eyes, 24.1±0.8 mm in medium eyes and 26.6±1.0 mm in long eyes. The RNFL thickness decreased with longer axial lengths in the superior ( $r=-0.52$ ,  $r^2=0.27$ ,  $p=0.0003$ ), inferior ( $r=-0.72$ ,  $r^2=0.52$ ,  $p<0.0001$ ), nasal ( $r=-0.60$ ,  $r^2=0.37$ ,  $p<0.0001$ ) and temporal ( $r=-0.30$ ,  $r^2=0.09$ ,  $p=0.0485$ ) quadrants, as well as in the 360° mean measurement ( $r=-0.69$ ,  $r^2=0.48$ ,  $p<0.0001$ ). The optic-disc area ( $r=-0.74$ ,  $r^2=0.54$ ,  $p<0.0001$ ) and rim area ( $r=-0.41$ ,  $r^2=0.17$ ,  $p=0.0051$ ) decreased with longer axial lengths. Correcting for axial length-induced ocular magnification by means of the Littmann formula resolved the relationship between axial length and both RNFL thickness and ONH area.

**Discussion** Axial length influences measurements of RNFL thickness and ONH parameters in healthy subjects. Caution is recommended when comparing the measured values of myopic and hyperopic eyes with the normative database of the instrument.

## INTRODUCTION

Optical coherence tomography (OCT) is a non-invasive technology that has been extensively used to evaluate many diseases of the optic nerve. In most cases, scientists have focused their attention on the peripapillary retinal nerve fibre layer (RNFL) thickness.<sup>1–5</sup> However, OCT can also analyse and measure topographic parameters of the optic nerve head (ONH), including the disc area, neuroretinal rim area and cup-to-disc ratio.<sup>6–9</sup> Evaluation of these parameters is essential, since the ONH size affects the clinical course of several pathologies of the optic nerve, including glaucoma, non-arteritic anterior ischaemic optic neuropathy, optic disc drusen, Leber hereditary optic neuropathy and dominant optic atrophy.<sup>8–13</sup> Moreover, in the case of glaucoma, ONH parameters not only provide similar discriminating capabilities when compared with RNFL thickness,<sup>14</sup> but also can improve our diagnostic ability.<sup>6 15–17</sup> ONH analysis, which has been available with time-domain OCT (Stratus OCT, Carl Zeiss Meditec, Dublin, California) since 2002, is now available in last-generation devices

with spectral-domain technology, such as Cirrus HD-OCT (Carl Zeiss Meditec), and has been recently validated.<sup>18</sup>

Previous studies carried out with time-domain OCT revealed that axial length influences both RNFL thickness and ONH measurements.<sup>19–23</sup> If axial-length-related ocular magnification is not accounted for, the longer the eye, the thinner the RNFL, and the smaller the ONH size. These values may be misleading for a correct diagnosis of glaucoma when myopic eyes are compared with the normative database. Little is known about short hyperopic eyes, although it is logical to expect opposite results.

This study aimed to assess whether axial length exerts any effect on the measurements performed with spectral-domain OCT. To address this issue, we analysed a sample of healthy hyperopic, emmetropic and myopic subjects.

## METHODS

### Participants

Forty-five eyes of 45 healthy subjects, recruited among patients undergoing routine ophthalmological examination, were enrolled for this study between October 2009 and June 2010. Since ageing is known to reduce RNFL thickness,<sup>24 25</sup> we did not include subjects younger than 25 or older than 55 (mean age: 39.4±7.2 years).

Each subject underwent a comprehensive ophthalmological evaluation, including visual-acuity measurement, slit-lamp examination, intra-ocular-pressure measurement with Goldmann applanation tonometry, and dilated fundus examination. Visual field (VF) was examined with a SITA 24-2 standard test in all subjects using the Humphrey VF analyser (HFA II 750-4.1 2005, Carl Zeiss Meditec). Axial-length measurements were performed by Ocuscan (Alcon Laboratories, Fort Worth, Texas) ultrasound immersion biometry.

The following individuals were excluded: those whose best-corrected visual acuity was less than 20/20; those with a history of severe ocular trauma, intraocular or refractive surgery or any ocular or neurological disease that could have affected the ONH or RNFL; those with glaucoma or an intra-ocular pressure higher than 21 mm Hg in either eye; those showing evidence of a reproducible visual-field defect (with significant SD at the <5% level or abnormal results in the glaucoma hemifield test) in either eye, as detected using the Humphrey VF analyser; those whose VF test results were unreliable (more than 15% false positives or false negatives, or more than 20% fixation losses).

All participants gave their informed consent according to the Declaration of Helsinki, and the study was approved by the internal review board of the Department of Neurological Sciences at the University of Bologna, Italy.

### Procedures

After pupil dilation, the eyes of the subjects who satisfied the study criteria were scanned using the Cirrus HD-OCT system with software version 5.0.

Data were acquired following the same procedure as previously described.<sup>26</sup> Briefly, each Optic Disc Scan captured a 6×6×2 mm 'cube' of data consisting of 200 A-scans from 200 linear B-scans (40 000 points) in 1.5 s (27 000 A-scans/s). Cirrus HD-OCT algorithms find the optic disc and automatically place a calculation circle of 3.46 mm diameter evenly around it. Layer-seeking algorithms find the RNFL inner (anterior) boundary and RNFL outer (posterior) boundary for the entire cube, excepting the optic disc. The system extracts from the data cube 256 A-scan samples along the path of the calculation circle. The resulting temporal, superior, nasal, inferior, temporal profile map is equivalent to the Stratus peripapillary RNFL scan.

The ONH parameters given by software version 5.0 result from a fully automatic algorithm that defines both the disc and cup margins within the 3-D data cube. The disc margin is defined as the termination of the Bruch membrane (also referred to as 'neural canal opening' or 'Bruch membrane opening').<sup>27 28</sup> The cup margin is defined using a proprietary algorithm that measures the neuroretinal rim as it exits the eye. From these landmarks, both the disc area and neuroretinal rim area were derived and analysed in this study.<sup>18</sup>

In order to correct axial length-related ocular magnification, we relied on the Littmann formula ( $t=p \cdot q \cdot s$ ), as modified by Bennet and later adopted by Leung *et al* and Kang *et al*.<sup>21 29–31</sup> This formula is based on the assumption that both the RNFL peripapillary scan circle and the optic-disc area ( $t$ ) are related to the camera magnification in the fundus imaging systems (factor  $p$ ) and the optical dimensions of the given eye (factor  $q$ ). Factor  $p$  is instrument-dependent and remains a constant in a telecentric imaging system: in both Stratus OCT and Cirrus HD-OCT, it is 3.382. The ocular magnification factor  $q$  of the eye can be determined with the formula  $q=0.01306 \cdot (\text{axial length}-1.82)$ .<sup>30</sup> Therefore, given a measurement,  $s$ , obtained with OCT, the actual size of the RNFL peripapillary scan circle can be calculated by means of the formula  $t=3.382 \cdot 0.01306 \cdot (\text{axial length}-1.82) \cdot s$ . For example, in the case of Cirrus, from this formula it follows that: actual radius of the scan circle on fundus= $3.3820 \cdot 0.01306 \cdot (\text{axial length}-1.82) \cdot 1.73$  mm.

As shown by Kang *et al*, if in the latter equation we use the RNFL thickness measured on the optic-disc cube scan as  $s$  (instead of the scan radius), we can determine the actual RNFL thickness in relation to axial length.<sup>31</sup>

Since the Littmann formula refers to linear rather than area magnification, when considering the ONH area we modified it according to the suggestion of Leung *et al* (ie,  $t^2=p^2 \cdot q^2 \cdot s^2$ ).<sup>23</sup>

### Statistics

Linear regression was performed to analyse the relationship between the axial length (independent variable) and each of the following dependent variables: RNFL thickness (mean 360° measurement, temporal, superior, nasal and inferior quadrants), ONH area and neuroretinal rim area. The same analysis was carried out with and without correcting for axial length-related ocular magnification. Analysis of variance (ANOVA) was performed to investigate differences in mean values among

short, medium and long eyes. A  $p$  value of  $<0.05$  was considered statistically significant. All statistical tests were performed using GraphPad InStat version 3a for Macintosh (GraphPad Software, San Diego, California; <http://www.graphpad.com>).

### RESULTS

Cases were equally distributed among short ( $<22.5$  mm,  $n=15$ ), medium (22.51–25.5 mm,  $n=15$ ) and long eyes ( $>25.51$  mm,  $n=15$ ). The mean axial length and spherical equivalent were, respectively,  $21.5 \pm 0.5$  mm and  $+4.2 \pm 2.3$  dioptres (D) in short eyes,  $24.1 \pm 0.8$  mm and  $-2.2 \pm 3.0$  D in medium eyes,  $26.6 \pm 1.0$  mm and  $-7.6 \pm 1.9$  D in long eyes. As expected, axial length and refractive error were strongly correlated ( $r=0.95$ ,  $r^2=0.90$ ,  $p<0.0001$ ).

Linear regression revealed a statistically significant relationship between axial length and most OCT measurements (table 1). The longer the eyes, the smaller the optic-disc area and rim area. Similarly, all RNFL thickness measurements were lower with increasing axial length. The inferior quadrant showed the highest correlation between axial length and RNFL thickness ( $r=-0.72$ ,  $r^2=0.52$ ,  $p<0.0001$ ), while the temporal quadrant showed the weakest correlation ( $r=-0.30$ ,  $r^2=0.09$ ,  $p=0.0485$ ).

Correcting the measured values by means of the Littmann formula eliminated the relationship both between the axial length and optic-disc area and between the axial length and mean RNFL thickness (figure 1). Applying the same formula to the four quadrant measurements produced similar values, as the relationship disappeared in the superior ( $r^2=0.03$ ), inferior ( $r^2=0.01$ ) and nasal ( $r^2<0.01$ ) quadrants ( $p>0.5$  for all measurements); however, a slightly statistically significant relationship was evident in the temporal quadrant, where RNFL thickness was shown to increase with increasing axial length after the Littmann formula was applied ( $r=0.30$ ,  $r^2=0.09$ ,  $p=0.0415$ ).

According to ANOVA, there was a statistically significant difference among the mean values measured in short, medium and long eyes (table 2), the only exception being the RNFL thickness of the temporal quadrant. When the Littmann formula was applied, the statistically significant difference disappeared for RNFL thickness and became weaker for the optic-disc area.

Linear regression revealed a statistically significant relationship also between refractive error and rim area, optic-disc area and 360° mean RNFL thickness (table 3).

### DISCUSSION

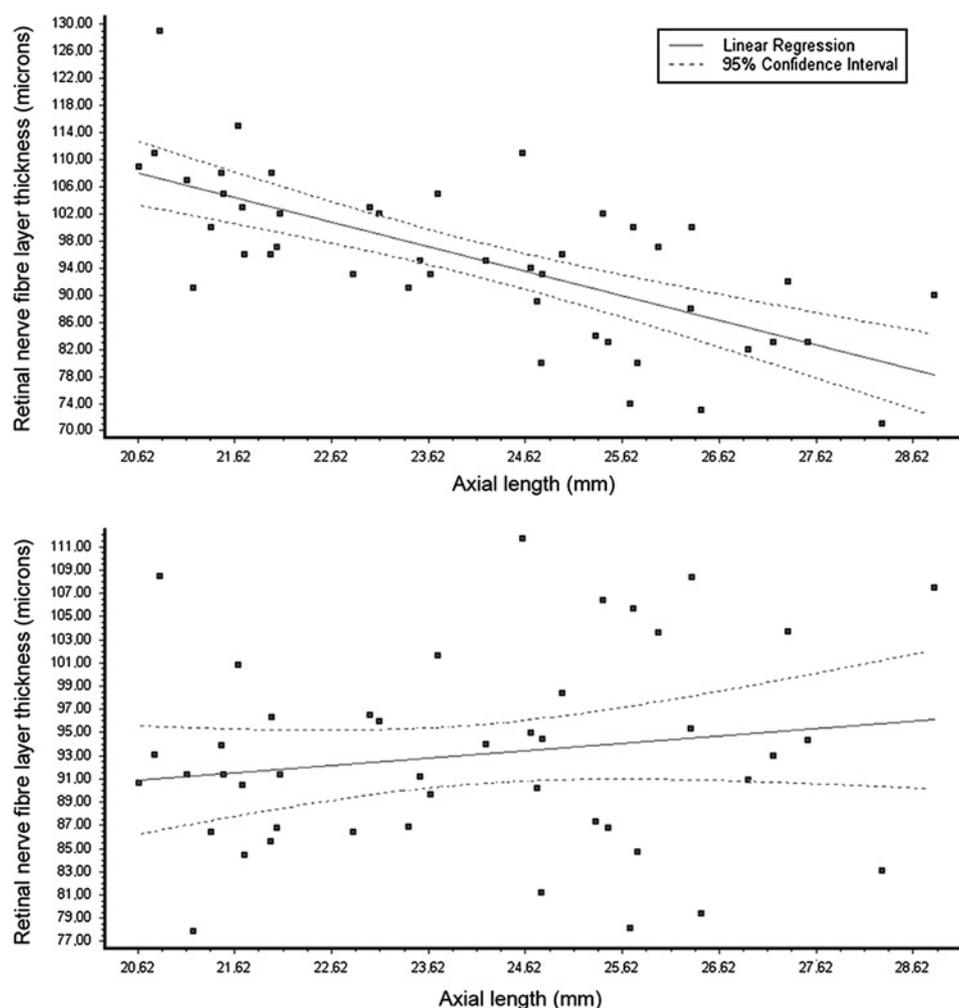
These data show that axial length influences spectral-domain OCT measurements of both RNFL thickness and ONH

**Table 1** Correlation coefficients between axial length and optic disc measurements performed using Cirrus HD-OCT

Parameter	Correlation coefficient (r)	Coefficient of determination ( $r^2$ )	p Value
Optic-disc area	-0.74	0.54	<0.0001
Optic-disc area (LF)	0.24	0.06	NS
Rim area	-0.41	0.17	0.0051
RNFL mean	-0.70	0.48	<0.0001
RNFL mean (LF)	0.17	0.03	NS
RNFL temporal	-0.30	0.09	0.0485
RNFL superior	-0.52	0.27	0.0003
RNFL nasal	-0.60	0.37	<0.0001
RNFL inferior	-0.72	0.52	<0.0001

LF, measurement corrected by means of the Littmann formula; RNFL, retinal nerve fibre layer.

**Figure 1** Top: linear regression showing that the mean peripapillary retinal nerve fibre layer thickness becomes thinner as the axial length increases ( $r=-0.70$ ,  $r^2=0.48$ ,  $p<0.0001$ ). Bottom: after correction for ocular magnification. This relationship disappears ( $r=0.17$ ,  $r^2=0.03$ ,  $p>0.05$ ).



parameters: as previously reported by other authors for both time-domain and spectral-domain OCT, the longer the eye, the thinner the RNFL, and the smaller the optic-disc area and neuroretinal rim area.<sup>19–23 31–33</sup> For the first time, we also reveal that the opposite is true for short hyperopic eyes, whose RNFL thickness and ONH size may result, respectively, in higher and larger measurements compared with average eyes. These findings are ascribable to axial length-induced ocular magnification. If we consider, for example, the scan circle and apply the the Littmann formula to the standard radius (1.73 mm), the radius will decrease to 1.39 mm in a 20 mm long eye and increase to 2.00 mm in a 28 mm long eye (table 4).

Given that both histological and OCT studies have shown that RNFL thickness is inversely related to the distance from the ONH centre in healthy subjects,<sup>34 35</sup> and that the larger

diameter of the scan circle in myopic eyes results in a larger distance from the ONH centre, it is not surprising to observe that RNFL thickness measurements are lower in longer eyes.

However, if the axial-length induced ocular magnification is accounted for by correcting the measured values,<sup>29 30</sup> the statistical relationship disappears for RNFL and ONH. Hence, from this point of view, Cirrus HD-OCT behaves similarly to Stratus OCT.<sup>19–23</sup> Since neither device performs any adjustment to correct ocular magnification, clinicians must be aware that comparing the RNFL thickness measurements with the normative database of the internal software is likely to be misleading for short and long eyes. Correcting the measured values with the above-mentioned mathematical formula is quite easy if the axial length is known and may help to decide if the eye is within normal limits. An alternative solution would be to

**Table 2** Mean measured values in short, medium and long eyes as measured by Cirrus HD-OCT

Parameter	Short eyes	Medium eyes	Long eyes	ANOVA
Optic-disc area (mm <sup>2</sup> )	2.00±0.28	1.83±0.25	1.35±0.20	<0.0001
Optic-disc area (LF) (mm <sup>2</sup> )	1.51±0.22	1.78±0.24	1.61±0.23	0.0115
Rim area (mm <sup>2</sup> )	1.42±0.25	1.46±0.23	1.19±0.17	0.01
RNFL mean (µm)	105.13±9.29	94.93±7.93	86.53±10.13	<0.0001
RNFL mean (LF) (µm)	91.24±7.20	93.33±7.34	94.71±10.66	NS
RNFL temporal (µm)	72.13±11.35	66.00±7.06	64.93±14.09	NS
RNFL superior (µm)	127.80±13.96	122.60±14.76	108.60±17.15	0.0079
RNFL nasal (µm)	82.87±10.03	67.67±5.66	65.33±11.22	<0.0001
RNFL inferior (µm)	137.53±16.29	116.60±10.22	107.67±15.22	<0.0001

LF, measurement corrected by means of the Littmann formula; RNFL, retinal nerve fibre layer.

**Table 3** Correlation coefficients between refractive error and optic-disc measurements performed using Cirrus HD-OCT

Parameter	Correlation coefficient (r)	Coefficient of determination (r <sup>2</sup> )	p Value
Optic-disc area	-0.72	0.52	<0.0001
Rim area	0.35	0.13	0.0189
Retinal nerve fibre layer mean	0.72	0.51	<0.0001

LF, measurement corrected by means of the Littmann formula.

improve the Cirrus database by stratifying patients according to the axial length.

A strong correlation was also observed between patients' spherical equivalent and optic-disc measurements; however, we believe that the direct influence of refractive error (as opposed to axial length) is negligible for two reasons: (1) the refractive error is clearly dependent on the axial length, as shown in table 3, and (2) previous studies have shown that RNFL thickness values do not change before and after excimer laser surgery to correct myopia.<sup>36,37</sup> Although these studies were performed using time-domain OCT, it is likely that their results can be applied to spectral-domain OCT as well.

Our findings agree with those previously reported by other studies mostly or exclusively focused on myopic eyes: Kang *et al* and Wang *et al* showed that the RNFL thickness of the superior and inferior quadrants and the mean peripapillary RNFL thickness, as measured by Cirrus HD-OCT, are inversely correlated to the axial length.<sup>31,32</sup> However, their results revealed some differences for the temporal and nasal quadrants, as they detected thicker RNFL values in the temporal quadrant of high myopes (as opposed to thinner RNFL values as in our own study) and no relationship between axial length and RNFL thickness in the nasal quadrant (whereas we found an inverse correlation). Both discrepancies can be explained by the different composition of the study populations: although our sample was considerably smaller, it included a higher percentage of hyperopic eyes, as one-third of the eyes were shorter than 22.25 mm, whereas no patients in the above-mentioned studies had an axial length shorter than this value. Based on the data shown in tables 1 and 2, it can be argued that the inverse correlation between axial length and RNFL thickness in the temporal and nasal quadrants is mainly related to the presence of short eyes, whose mean RNFL thickness values are considerably higher than those of medium and long eyes, whereas the difference between medium and long eyes is much lower. Accordingly, our results

**Table 4** Radius size of the peripapillary scan circle of Cirrus HD-OCT as calculated according to the Littmann formula in eyes with different axial lengths

Axial length (mm)	Scan circle radius (mm)
20	1.39
21	1.47
22	1.54
23	1.62
24	1.69
24.46	1.73
25	1.77
26	1.85
27	1.92
28	2.00
29	2.08
30	2.15

When the axial length is 24.46 mm, there is no magnification.

would have been closer to those of Kang *et al* and Wang *et al* if we had excluded hyperopic eyes, as in this case the negative correlation between axial length and RNFL thickness would have disappeared in both nasal and temporal quadrants ( $p>0.05$ ).

Another interesting finding of our study was the positive correlation between axial length and temporal RNFL thickness after correcting for ocular magnification. Such a relationship may be explained by the findings of Hong *et al*, who recently reported that patients with a higher axial length are more likely to show a temporally deviated RNFL thickness profile.<sup>38</sup>

We also observed an inverse correlation between axial length and ONH parameters (disc and rim areas). Using Stratus OCT, Leung *et al* had already demonstrated that axial length influences ONH size measurements, as longer eyes had smaller discs. In their study, this relationship disappeared once the measured values were corrected by means of the Littmann formula.<sup>23</sup> Our results with spectral-domain OCT are similar and suggest that ONH size must be appropriately corrected to account for axial length.

This study has some limitations, and further investigation is warranted. First, the sample size was large enough to detect the influence of axial length on the measured parameters but would be insufficient to develop a clinically useful normative database. Second, we did not include eyes with peripapillary atrophy, which may influence ONH edge detection. Third, we did not have the possibility of investigating this relationship by means of other spectral-domain OCT instruments, so our results can be applied only to the values provided by Cirrus HD-OCT.

In conclusion, we found that axial length influences the measurements of both RNFL thickness and ONH parameters. To ensure better accuracy, clinicians should consider measuring the axial length in each patient undergoing optic disc analysis by Cirrus HD-OCT and correcting for axial length-induced magnification by using the Littmann formula.

**Competing interests** GS: speaker honorarium (Carl Zeiss Meditec).

**Patient consent** Obtained.

**Ethics approval** Ethics approval was provided by the Department of Neurological Sciences, University of Bologna, Bologna, Italy.

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