

# The Humphrey optical coherence tomography scanner: quantitative analysis and reproducibility study of the normal human retinal nerve fibre layer

Adrian L Jones, Nick J L Sheen, Rachel V North, James E Morgan

## Abstract

**Background/aims**—To determine the reproducibility of the Humphrey optical coherence tomography scanner (OCT), software version 5.0, for measurement of retinal nerve fibre layer (RNFL) thickness in normal subjects and to compare OCT measurements with published histological thickness of the human RNFL.

**Methods**—Three independent measurements were obtained at each session for one eye from 15 normal subjects with a mean age of 30.8 (SD 10.9) years. Scans were taken in the peripapillary retina using the default setting (1.74 mm radius from centre of the optic disc) and were repeated 1 week later. Additional scans were obtained at the optic nerve head (ONH) margin overlying the scleral rim, for comparison with available histological data on the human RNFL.

**Results**—For the 1.74 mm circular scan, the mean coefficient of variation (COV) for the global RNFL thickness measurement was 5% (SD 3%). This increased to 8% (3%) for quadrant measurements and to 9% (3%) with further subdivision into 12 segments. Significant differences ( $p < 0.05$ ) between sessions were only found when the data were divided into segments. The mean RNFL thickness for the 1.74 mm scan was 127.87 (9.81)  $\mu\text{m}$ . The RNFL was maximal at the superior disc pole, 161.44  $\mu\text{m}$  (14.8), and minimal at the temporal pole, 83.1 (12.8)  $\mu\text{m}$ . Peak thickness values occurred superior temporal and inferior temporal to the vertical axis. RNFL thickness for every sector of the disc was greatest at the margin of the optic disc (mean 185.79  $\mu\text{m}$ ; SD 32.61). Although the variation in RNFL thickness around the disc follows published histology data, the OCT underestimates RNFL thickness by an average of 37% (SD 11; range 21–48%).

**Conclusion**—The OCT provides reproducible measurement of the retinal structures that are consistent with the properties of the RNFL. However, comparison with available studies of RNFL thickness in the human suggests that in its present form, the OCT underestimates RNFL thickness. Further refinement of this technology is required to improve the accuracy with which the OCT measures retinal nerve fibre layer thickness.

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Optical coherence tomography (OCT) is a new technique for the in vivo acquisition of cross sectional images of retinal structure, from which clinically relevant thickness estimates of the retinal layers can be made. The theoretical axial resolution of OCT is approximately 14  $\mu\text{m}$  in air and 10  $\mu\text{m}$  in optically clear biological tissues,<sup>1</sup> which is a significant improvement over the resolution of approximately 300  $\mu\text{m}$  for the scanning laser ophthalmoscope.<sup>2</sup>

The principles of image acquisition have been described in detail elsewhere.<sup>1-4</sup> In brief, the OCT uses low coherence interferometry to provide absolute measurements of retinal thickness, thereby eliminating the need for a reference plane.<sup>5</sup> Comparison of histological and OCT images from prototype devices have shown a good correlation between the OCT estimates and real measurements of retinal nerve fibre layer (RNFL) thickness.<sup>6-9</sup> OCT has been developed for use in the clinical setting and shows particular promise in the assessment of macular pathology, with OCT images showing a close correlation with the histological structure of macular holes,<sup>10,11</sup> cystoid macular oedema,<sup>12</sup> central serous retinopathy,<sup>13</sup> retinal detachment,<sup>14</sup> choroidal neovascularisation,<sup>15</sup> and retinal trauma.<sup>16</sup> Compared with prototype versions, parameters such as laser wavelength and intensity have been changed in the commercial version of the OCT that would be expected to alter the laser-tissue interaction and provide different estimates of retinal structure. While this may not be a critical issue when providing qualitative assessment of macular pathology it is important in the quantitative assessment of retinal structures such as nerve fibre layer thickness in the assessment of glaucoma.<sup>4,5,7,17-21</sup> In this study we have therefore evaluated the reproducibility of the Humphrey OCT and have compared estimates of RNFL thickness obtained using the latest software version with published histological measurements of the human RNFL.

## Materials and methods

### SUBJECT CHARACTERISTICS

Subjects were recruited from staff and students of the Department of Optometry and Vision Sciences. Written informed consent was obtained in compliance with ethical standards of the Bro Taf Community Health Authority. Each subject undertook a complete ophthalmic examination, which included anterior segment and fundus biomicroscopy, refraction, keratometry, automated static perimetry, and

Retinal Imaging  
Laboratory,  
Department of  
Optometry and Vision  
Sciences, Cardiff  
University, Cardiff  
CF10 3YJ, UK  
A L Jones  
N J L Sheen  
R V North  
J E Morgan

Department of  
Ophthalmology,  
University of Wales  
College of Medicine,  
Heath Park, Cardiff  
CF14 4XW, UK  
J E Morgan

Correspondence to:  
Mr James E Morgan,  
Department of  
Ophthalmology, University  
of Wales College of  
Medicine, Heath Park,  
Cardiff CF14 4XW, UK  
MorganJE3@cardiff.ac.uk

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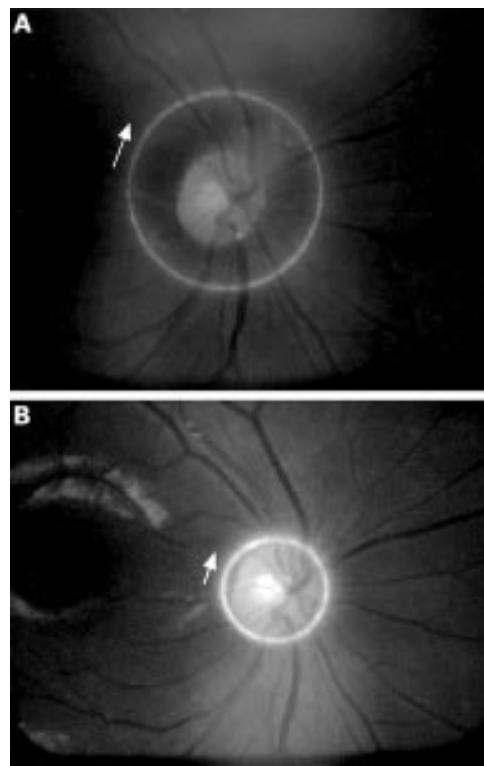


Figure 1 Operator monitor images showing the scan patterns with indication of scan direction (RE), (A) 1.74 mm circle scan, (B) ONH margin scan.

tonometry. Subjects were classified as having normal eyes if they had no history or evidence of ocular pathology or surgery (including refractive), absence of visual field defects (Humphrey 24–2 SITA-Standard), best corrected visual acuity of 6/9 or greater, and intraocular pressures of 21 mm Hg or less (Goldmann). The pupil of each subject's non-dominant eye was dilated using 1% tropicamide topical drops to give a minimum pupil diameter of 7 mm.

#### OCT IMAGE ACQUISITION

All images were acquired by a single experienced observer using the Humphrey OCT Model 2000, Humphrey Instruments, CA, USA (software V 5.0). The light source in the OCT is a superluminescent diode with a wavelength of 850 nm, the power was 750  $\mu$ W and the image acquisition time was approximately 1 second for each scan. OCT analysis was performed along a line consisting of 100 axial

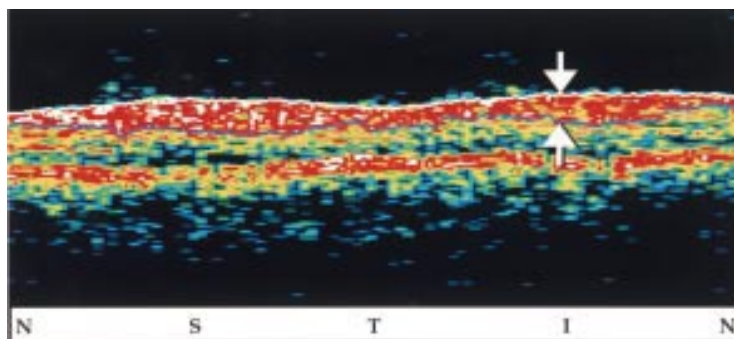


Figure 2 Raw data image as supplied by the commercial OCT software, the arrows indicate the RNFL. N = nasal, S = superior, T = temporal, I = inferior.

scans. Since the number of axial scans is fixed, the sample density varied as an inverse function of scan length. An internal fixation target offset nasally from the scan area has previously been shown to give the highest reproducibility<sup>22</sup> and was therefore used for all image acquisition. For each subject the image of the ONH was focused and aligned using the real time video monitor. Three<sup>23</sup> circular scans were taken around the optic nerve head at the default radius of 1.74 mm from the centre of the optic disc.<sup>22</sup> The coordinates of the retinal landmarks were recorded together with the scan and fixation coordinates. The procedure was repeated 1 week later.

In a separate session, a single set of three scans was obtained from the margin of the optic disc as defined by the location of Elshnig's ring. Images were rejected if significant eye movement occurred during the scan.

The OCT software generates a colour coded image for the display of the interferometric signals. Estimates of RNFL thickness are made from these signals using proprietary algorithms within the OCT software.

For each scan, the global RNFL thickness was determined from 100 points around the disc. The mean RNFL thickness was also calculated for four 90 degree quadrants and twelve 30 degree segments by the OCT software. Statistical analysis was performed using SPSS V.8. (SPSS Inc, Chicago, IL, USA). Intersession variation was assessed using the Friedman test and intrasession variation using the Wilcoxon test. Coefficients of variation for segment, quadrant, and global values were calculated for each subject.

#### Results

OCT examinations were performed on 15 eyes of 15 subjects (five male, 10 female) with a mean age of 30.80 years (SD 10.86; range 20–53 years). The mean spherical refractive error was  $-1.18$  DS (1.88;  $+0.50$  to  $-5.50$  DS) and mean astigmatic error was 0.45 DC (0.37; 0 to 1.00 DC). Good quality images were acquired in every case. Videographic images of the region of interest are shown in Figure 1, indicating the scan pattern at the default setting (1.74 mm radius) and at the disc margin; the cross sectional image of peripapillary retina is shown in Figure 2.

The mean RNFL thickness for the 1.74 mm radius was 127.87  $\mu$ m (SD 9.81). The mean standard deviation was 6.33 (3.52)  $\mu$ m with a mean COV of 5% (3%). When RNFL thickness was considered in quadrants (superior, inferior, nasal and temporal) these values increased to 9.69 (2.05)  $\mu$ m and 8% (3%) respectively, increasing again to 11.33 (1.80)  $\mu$ m and 9% (3%) when RNFL thickness was further subdivided into 12 segments. Figure 3 shows the RNFL thickness plotted for both sessions and the COV calculated from each subject for every segment (range 5–20%). The nasal areas of the RNFL provided the least reliable measurements with mean COV value of 20%; however, this did not reach significance ( $p > 0.05$ ). When the RNFL thickness

measurements at this eccentricity were combined for the first and second imaging sessions the mean was found to be 127.79 (0.31)  $\mu\text{m}$ . Intersession significant differences were only found for the segment values ( $p < 0.05$ ). Those subjects with significant intersession differences tended to have lower mean RNFL thickness values.

For OCT scans taken at the margin of the optic disc, the mean RNFL thickness was 185.79 (32.61)  $\mu\text{m}$  with a mean standard

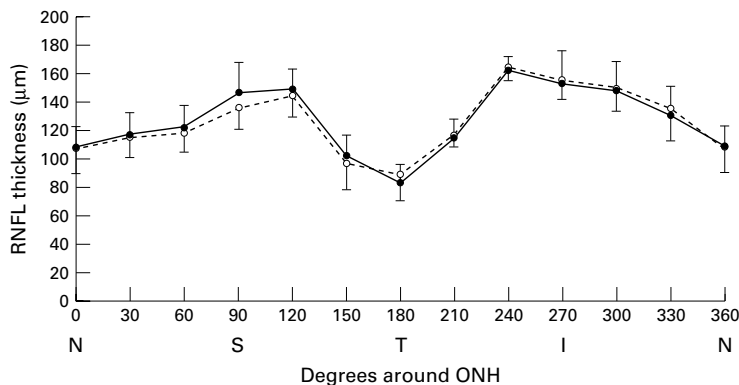


Figure 3 Plot of the mean RNFL thickness per segment for each session using the 1.74 mm circle scan, error bars indicate 1 SD from the mean. Open circles represent values from session 1; solid circles from session 2. N = nasal, S = superior, T = temporal, I = inferior.

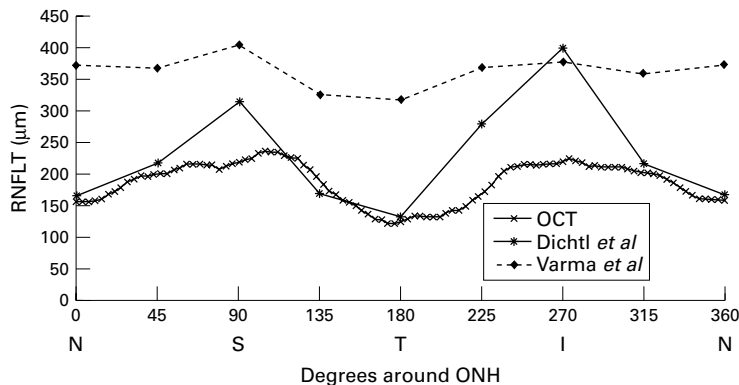


Figure 4 Plot of RNFL thickness according to angular location around the optic disc circumference. N = nasal, S = superior, T = temporal, I = inferior. Plots marked "OCT" represent values obtained by the OCT scan at the ONH margin. Those marked "Dichtl et al" and "Varma et al" represent RNFL thickness at the ONH margin as published by Dichtl et al,<sup>36</sup> and Varma et al,<sup>5</sup> respectively. Note the difference in modulation of the RNFL as measured by histology.

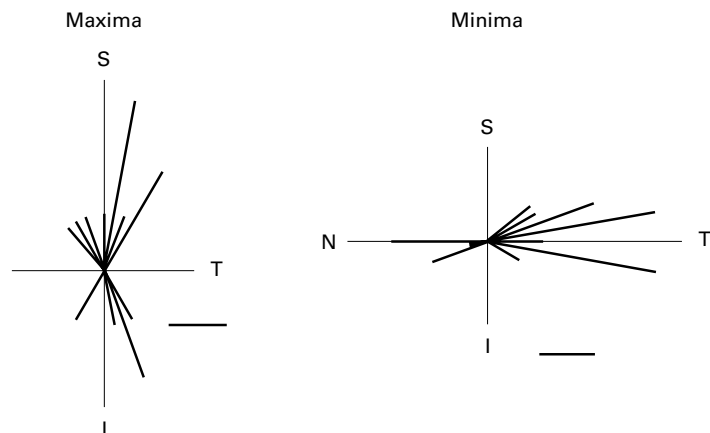


Figure 5 Polar frequency histogram showing the distribution of the maxima and minima for RNFL thickness at the ONH margin as measured by the OCT. N = nasal, S = superior, T = temporal, I = inferior. Scale bar shows representation for one subject.

deviation of 44.61 (7.48)  $\mu\text{m}$ . The variation in mean RNFL thickness as estimated by the OCT is shown in Figure 4. Published histological measurements of RNFL thickness from two studies have been overlaid for comparison, which are greater at every point around the ONH. In Figure 5 the location of the peak RNFL thickness has been represented as a polar histogram, showing that peak RNFL thickness occurred at the superior and inferior poles with a skew to the temporal aspect of the disc.

## Discussion

Our results are generally in agreement with previous studies using prototype OCT devices that report mean peripapillary RNFL thickness in the range of 80–150  $\mu\text{m}$ ,<sup>4 17 22 24–30</sup> with mean standard deviations of 3–20  $\mu\text{m}$ <sup>27 28</sup> when using the 1.74 mm radius scan. Our mean COVs are also similar to those obtained earlier studies of 7.5% to 20.2%,<sup>30 31</sup> although the mean standard deviations are larger than those recorded previously of (6.33 v 2.8  $\mu\text{m}$ ).<sup>27 28</sup> As with these studies, we have shown that COV increases with increasing subdivision of peripapillary RNFL thickness measurements and that it varies around the optic disc, being highest in the nasal retina and lowest at the superior and inferior poles. Studies with other imaging devices such as scanning laser polarimeters<sup>32 33</sup> and confocal scanning laser tomographer<sup>34</sup> give similar COV values, suggesting that a change of at least 20  $\mu\text{m}$  in the global RNFL thickness would be required to produce a clinically significant change in the OCT measurements.

An important finding of the present study is that even with the latest RNFL algorithm (software V 5.0), the Humphrey OCT underestimates the RNFL thickness when compared with histological data.<sup>35 36</sup> It can be seen from Figure 4 that the OCT on average gives only 63% (range 52%–79%) of the histological RNFL thickness. Assuming 10–15% shrinkage for histological preparation,<sup>35</sup> it is possible the OCT could be underestimating RNFL thickness by as much as 47%. The discrepancy may increase further when account is taken of the age between the subjects in this study. Significant age related thinning of the human RNFL has been reported in both histological<sup>35 36</sup> studies and with other imaging devices.<sup>24</sup>

Entire retinal thickness measurements (rather than RNFL thickness) have been shown to provide greater reproducibility<sup>37</sup> and better agreement with human histology.<sup>6 9 38</sup> This may be due to a greater change in refractive index, and hence reflectance, at the retinal pigment epithelial/photoreceptor interface than between that of the retinal ganglion cell/retinal nerve fibre layer, thereby providing a higher definition of tissue boundary. This study has not addressed the method by which the OCT software calculates RNFL thickness from the digital interference signal recorded by the Michelson interferometer within the OCT hardware. These values are displayed on the monitor using a proprietary colour palette to represent the digital values. Correlation of the

OCT interference signals with retinal histology has to date been mostly empirical and is currently under investigation in our laboratory.

Comparison of the variation in RNFL thickness around the ONH is also instructive. One of the problems in drawing comparisons with histological measurements of RNFL thickness in the human is that existing reports do not provide a consistent picture (Fig 4). It can be seen that the OCT measurements show better agreement with data from Varma *et al*<sup>35</sup> in that it displays a more gradual modulation in RNFL thickness. Within the literature, the profile of the peripapillary RNFL thickness has been suggested to follow a “double hump” configuration. However, the degree to which this is shown by the various imaging devices is inconsistent. For example, a recent study with confocal scanning laser ophthalmoscopy (Heidelberg retina tomograph; Heidelberg Engineering GmbH, Heidelberg, Germany)<sup>39</sup> suggested a much lower modulation of the RNFL profile that was more consistent with Varma *et al*.<sup>35</sup> Other studies using computer topographic analysis of simultaneous stereoscopic videographic images (Rodentstock Analyser; Rodentstock Instruments, Munich, Germany)<sup>39</sup> and scanning laser polarimetry (Nerve Fibre Layer Analyser; Laser Diagnostic Technologies, San Diego, CA, USA)<sup>40</sup> reported modulation similar to that found by Dicht *et al*.<sup>36</sup> In the present study, the peak RNFL thickness was most frequently located along the vertical meridian with slight skew to the temporal aspect of the ONH. Minimum RNFL thickness was also located most frequently at temporal pole of the ONH, providing a greater relative change in RNFL thickness between the vertical and the temporal meridians of the ONH than between the vertical and nasal meridians.

A further artefact to consider is the shape of the scan used in comparison with the shape of the human ONH. In this study we utilised a circle scan, but since the ONH is elliptical in shape the information gathered from the temporal and nasal areas would be further from the ONH margin than the information from the poles. This would give lower RNFL thickness values for the temporal and nasal quadrants, thus artificially increasing the overall modulation in RNFL thickness. This suggests less modulation in the RNFL thickness at the nasal pole, and implies that the RNFL thickness profile of the human retina is more similar to that provided by Varma *et al*.<sup>35</sup> Further work is needed to verify the modulation of RNFL thickness in the human retina.

Although the OCT data are consistent with the reduction in RNFL thickness with increasing eccentricity, this does not appear to be symmetrical when comparing the superior and inferior poles of the ONH. In several studies<sup>41</sup> this can be expressed as the ratio of RNFL thickness superior to the ONH to that of the RNFL inferior to the ONH at the same eccentricity (S/I ratio). At the ONH margin, the S/I ratio was approximately 1.0; by contrast, in the peripapillary retina (1.74 mm) this had increased to 1.26. This implies a tendency for a greater relative RNFL thickness at the superior

pole; however, this difference did not reach significance. This is in contrast with results obtained using other topographic devices at similar eccentricities from the ONH margin<sup>42</sup> and the clinical evaluation of the neuroretinal rim<sup>43</sup> where the inferior aspect often provides a thicker RNFL. It is possible that this results from subtle differences in axon trajectory away from the ONH and emphasises the importance of eccentricity when comparing RNFL thickness around the ONH.

The OCT provides reproducible cross sectional images of the human retina from which estimates of RNFL and total retinal thickness can be made. These measurements may be of clinical value in the management of diseases such as glaucoma. Our study suggests that the current version of the OCT consistently underestimates RNFL thickness. In view of the considerable clinical potential of this technology, further work is required to improve the accuracy with which it measures retinal structure.

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