Optical Coherence Tomography 3: Automatic Delineation of the Outer Neural Retinal Boundary and Its Influence on Retinal Thickness Measurements

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Purpose. To investigate the automatic delineation of the outer limits of the macular neural retina, by using the optical coherence tomography (OCT)-3 built-in software, and to determine its influence in assessing retinal thickness in the normal macula.

Methods. Retrospective analysis of the OCT3 data at a tertiary-care referral center was performed to study the automatic delineation of the outer neural retina boundary generated by the OCT built-in software. In parallel, a cross-sectional study was designed to compare retinal thickness measurements obtained at specific macular regions of nine normal eyes by the automatic measurement tool with those obtained using a manual-caliper-assisted technique.

Results. OCT data from 121 eyes were evaluated. Two parallel, linear highly reflective layers (HRL) were visible at the level of the outer retinal boundary in normal macular regions. Disappearance of the inner and maintenance of the outer HRL was noted in the presence of eye conditions affecting the external retinal layers. The automated software delineation for the outer retinal border was primarily guided by the presence of the inner HRL, whereas the correlation of the OCT findings with the expected clinical and angiographic features on eyes presenting specific macular conditions pointed toward a deeper retinal pigment epithelium–retina interface occurring at the level of the outer HRL. There was a statistically significant difference between the retinal thickness in specific normal macular regions obtained by the automatic measurement tool and the caliper-assisted technique in which the outer retinal border delineation was based on the outer HRL (P = 0.008, Wilcoxon signed rank test).

Conclusions. Incorrect delineation of the outer neural retina boundary is occurring with the automated retinal thickness measurement tool of the OCT3 software. At specific regions of the normal macula, retinal thicknesses were significantly underestimated due to such misalignment. (Invest Ophthalmol Vis Sci. 2004;45:2399–2406) DOI:10.1167/iows.04-0155

Axial measurements of ocular tissues have become important in both medical and surgical management of disease. With the advent of optical coherence tomography (OCT),1 a relatively new noncontact imaging technique, exceptional assessment of tissue thicknesses, such as those of the neural retina2 and the nerve fiber layer,3,4 has been demonstrated. Detailed accounts of the underlying principles of OCT image acquisition and its interpretation have been reported elsewhere.1,2 In summary, a scanning interferometer is used to obtain a cross section of the retina based on the reflectivity of different layers within the retina allowing detection of micro- metric changes in tissue thickness.1–5 OCT has been largely applied to assist the diagnostic of a large number of choroidal and retinal diseases.6–11 Presently, a growing requirement for the measurement of retinal thickness has become apparent, particularly in relation to the management of macular diseases.12–14 With the advent of modern therapeutic modalities that primarily modulate disease–lesion activity, such as feeder vessel treatment, photodynamic therapy, and intravitreal/ sub-Tenon/juxtascleral drug injections, angiography probably is not sufficient for accurate evaluation of the treatment response.

The release of the third generation of OCT offers the theoretical possibility of high-resolution measurements of the neural retina. Recent reports using such a device15,16 as well as an ultra–high-resolution OCT prototype17 have demonstrated two well-defined, parallel, red/white, highly reflective layers (HRLs) separated from each other by one thin layer of low to moderate reflectivity (green/yellow) at the outer aspect of the neural retina in presumably unaffected or normal macular regions. It has been suggested that the inner HRL may correspond to the junction between the inner and outer segments of the photoreceptors and that the outer HRL quite possibly corresponds to the retinal pigment epithelium (RPE)-choriocapillaris hyperreflective complex commonly seen in first-generation OCT scans.16,17

When automated measurements of retinal thickness are performed, the analysis protocol (retinal thickness [single eye]) of the OCT3 software automatically delineates the boundaries of the neural retina (Fig. 1). Apparently, the automatic software delineation has considered the inner HRL to be the outer boundary of the neural retina in some patients, which may lead to erroneous retinal thickness estimates. Therefore, the purpose of this study was twofold: to investigate the automatic delineation of the outer limits of the macular neural retina in normal and pathologic eyes as generated by the analysis protocol of the OCT3 built-in software and to compare the retinal thickness in specific normal macular regions obtained by the automatic software measurement tool with those obtained by manual-caliper-assisted technique, in which the outer HRL was considered the outer boundary of the neural retina.
METHODS

A retrospective analysis of all patients who underwent OCT3 evaluation at the Unidade de Diagnóstico Avançado e Tratamento (UDAT), Hospital de Olhos de Araraquara, Araraquara-SP, between October 2002 and June 2003, was performed to study the automatic delineation of the outer neural retina boundary generated by the built-in software (versions 1.0/0002 and 2.0/0406) of the third-generation commercially available OCT system (Stratus model 3000; Carl Zeiss Meditec, Humphrey Division, Dublin, CA). In addition, a cross-sectional study was performed in parallel to assess the retinal thickness at specific regions of the central (3.00 mm) macular region of healthy subjects by means of using either the automated or manual-caliper-assisted measurement software tools (version 3.0/0052). The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local Institutional Review Board. All participants (healthy subjects) prospectively included in the cross-sectional study arm gave written informed consent before entering in the study.

The retrospective analysis included patients who had best corrected visual acuity equal to or better than 20/25, a refractive error limited to ≤1.00 D, intraocular pressures ≤21 mm Hg, no history of ocular surgery, and no clinical signs of indicative of eye disease (normal eyes). Patients who had evidence of well-established macular disease, defined as idiopathic macular holes, central serous chorioretinopathy without angiographic evidence of associated RPE detachment, drusen, serous RPE detachment, and diabetic or vascular occlusions related macular edema were also included (pathologic eyes). Patients were excluded if media opacities prevented acquisition of adequate OCT scans. Six linear scans, 6.00 mm in length (standard 512 A-scans per scan line acquisition protocol [radial lines]), through the central fovea were obtained. Each scan was performed using a standard protocol to ensure consistent positioning of the fovea. The scans were then processed using the built-in software for retinal thickness analysis.

The cross-sectional study utilized a horizontal, 3.00-mm long, OCT3 scan, after processing by built-in retinal thickness (single eye) analysis protocol. Top: automated retinal thickness measurement was obtained from corresponding A-scan at the fovea and in specific temporal and nasal regions (1250 ± 50 μm temporal and nasal to the foveal pit). Subsequently, accuracy of the manual-caliper-assisted tool by measurement of foveal height using the manual-caliper-assisted method was performed. Positioning the caliper crosses at the outer and inner boundaries used by the automated retinal thickness analysis tool. Middle and bottom: a manual-caliper-assisted measurement of retinal thickness at the same points previously evaluated was then performed, using the automated delineation of the inner retinal boundary by the software as one point (inner caliper cross) and positioning the outer caliper cross just above the outer HRL.
FIGURE 3. OCT3 evaluation of patients with specific macular diseases. Top left: Idiopathic macular hole: a 6.00-mm long linear OCT scan shows the hyper-reflective operculum (arrowhead) next to the minimally reflective membrane corresponding to the detached posterior hyaloid. The edges of the hole are thickened by cystic spaces. In the unaffected macular region, a similar outer retinal tomographic appearance as that in normal eyes is present. However, in the affected area (marked A and B), loss of optical reflectivity (A) and complete disappearance (B) of the inner HRL are shown. Top right: note that the automatic retinal thickness measurement tool uses the inner HRL at the level of external retina to delineate the outer neural retina border (outer white line). Manual-caliper-assisted measurement of the distance from the automatic outer generated line to the outer HRL was 66 μm in this case (two aligned crosses in blue). Middle left: Central serous chorioretinopathy and no clinical and angiographic evidence of retinal pigment epithelium detachment: 6.00-mm linear scan shows retinal elevation and a discrete increase in retinal thickness (asterisk) at the macula, due to subretinal fluid accumulation (dark region) and intraretinal edema. The inner HRL detaches from the outer HRL jointly with the rest of the retina at the edges of the neurosensory retinal detachment. Middle right: automatic delineation of the neural retina boundaries used the inner HRL to draw the outer limits in less-affected regions and the outer HRL in the area of the neurosensory retinal detachment (external white line). Note that the manual-caliper-assisted measurement (blue crosses) of the neural retina was 290 μm when the outer HRL was used to delineate the outer retinal boundary. In the same region, the automated retinal thickness measurement was to 248 μm. Bottom left: serous retinal pigment epithelial detachment (PED) as confirmed by clinical and angiographic evaluation: 6.00-mm linear scan shows both inner and outer HRL separate from other structures at the nasal edge of the serous PED. Subretinal fluid (above the outer HRL, #) as well as fluid accumulation beneath the outer HRL corresponding to the RPE (arrow) was seen in the temporal aspect of the OCT scan. Bottom right: Automatic delineation of the outer neural retina boundary was guided by the inner HRL in the nasal part of the scan (arrow) and by the outer HRL in the temporal aspect (arrowheads).

had to be available for appraisal for all patients. Additional linear scans, 3.00 mm in length, at 0° (horizontal) and 90° (vertical) were occasionally available for evaluation. Each scan was processed using the standard analysis protocol (retinal thickness [single eye]) to verify the automatic delineation of the outer border of the macular neural retina. The borders as determined on OCT scans were further correlated with the expected clinical and angiographic features of the related macular diseases.

The cross-sectional study was design to assess the retinal thickness at specific regions of the central (3.00 mm) macular region of healthy subjects by using either the automated measurement software tool of the OCT3 unit or the manual-caliper-assisted technique. The inclusion criteria included patients aged between 20 to 40 years who had best corrected visual acuity $\geq 20/25$, a spherical equivalent refractive error limited to $\pm 1.00$ D and no clinical evidence of retinal or optic nerve disease. Each subject received a detailed ophthalmic examination including measurement of best corrected visual acuity according to a standardized refraction protocol using a retroilluminated Lighthouse for the Blind (New York, NY) distance visual acuity (VA) test chart (using modified ETDRS charts 1, 2, and R), slit lamp biomicroscopy, applanation tonometry, dilated biomicroscopic and indirect fundus examination, and automated perimeter using the Swedish Interactive Test Algorithm (SITA) standard test (Carl Zeiss Meditec). Participants were considered to have no evidence of retinal or optic nerve disease if they had no history of ocular disease or surgery, had a reliable SITA standard test result with no visual field defect, had intraocular pressures less than 21 mm Hg, and had no evidence of any optic nerve or retinal disease based on binocular biomicroscopic and indirect fundus examination performed by two different retinal specialists (RAC, JAC) and one glaucoma specialist (MS). Both eyes of each subject were included in the study, provided that no evidence of retinal or optic nerve disease was present in either eye.

All OCT acquisitions were performed under supervision of one of the authors (RAC). For OCT scan acquisition, a linear horizontal scan 3.00 mm in length was used to optimize tomographic imaging of the retinal structures within the central macular region. Scanning was performed through the macula of each eye by the same experienced examiner (DC). Three horizontal OCT scans were obtained at the center of the macula and based on one retinal specialist’s (RAC) judgment, one representative scan for each eye, characterized by strong signal quality and transecting the deepest portion of the foveal pit, was used in the data analysis. OCT data analysis was performed by a single retinal specialist familiar with the technique (RAC). In these scans, measurements in specific temporal and nasal regions ($1250 \pm 50$ μm temporal and nasal to the foveal pit), and measurements of foveal height were taken (Fig. 2, top). Initially, automated retinal thickness measurement was performed. Each scan was processed using the standard analysis protocol (retinal thickness [single eye]) and measures...
for the desired regions were obtained from corresponding A-scan results. Subsequently, manual measurement of the retinal thickness at the same points was performed using the manual caliper function of the OCT analysis software. A similar manual-caliper-assisted measurement technique was used by Costa et al., and the reproducibility of these manual measurements has also been demonstrated by Sanchez-Tocino et al. To verify the accuracy of the manual-caliper-assisted tool and avoid ascertainment bias, measurement of foveal height using the manual-caliper-assisted method was performed positioning the caliper crosses at the outer and inner boundaries used for the automated retinal thickness analysis (Fig. 2, top). The manually obtained measurements for studied eyes were within \( \pm 2 \mu m \) of the automated retinal thickness measurements, indicating good accuracy. Manual-caliper-assisted measurement of retinal thickness was then performed with the automated delineation for the inner retinal boundary by the software as one point (inner caliper cross) and positioning the outer caliper cross just above the outer HRL, which is thought to correspond to the RPE-choriocapillaris hyperreflective complex on first-generation OCT. Manual measurement of retinal thickness was performed at the same points previously evaluated by the automated retinal thickness measurement tool (nasal and temporal to the foveal pit, and for foveal height; Fig. 2, middle and bottom).

The retinal thicknesses obtained by the automated measurement software tool were compared to those obtained with the manual-caliper-assisted technique. The data were processed on computer (SPSS ver. 11.5; SPSS Sciences, Chicago, IL). Paired t-test and Wilcoxon signed rank test were used to compare neural retinal thickness measurements. Intraclass correlation was performed to verify the correlation of neural retinal thickness measurements between right and left eyes.

**Figure 4.** Macular retinal thickness at specific temporal regions obtained from OCT3 data evaluation in healthy subjects.

**Figure 5.** Macular retinal thickness at specific nasal regions obtained from OCT3 data evaluation in healthy subjects.
eyes for each technique. A significance level of 0.01 was adopted to adjust for multiple comparisons.

**RESULTS**

One hundred twenty-one eyes were included in the retrospective study, with 32 eyes (18 patients) having no clinical evidence of ocular disease (normal eyes) and 89 eyes (62 patients) with clinically evident pathologic conditions, including idiopathic macular holes (17 patients [23 eyes]; median [range] VA, 20/80 [20/25–20/400]); central serous chorioretinopathy (6 patients [7 eyes]; median [range] VA, 20/30 [20/25–20/80]); drusen (5 patients [9 eyes]; median [range] VA, 20/25 [20/20–20/40]); RPE detachment (4 patients [6 eyes]; median [range] VA, 20/60 [20/30–20/200]); and macular edema (30 patients [44 eyes]; median [range] VA 20/60 [20/25–20/400]). The median (range) age was 34 (17–45) years in patients with no clinical evidence of ocular disease (normal eyes) and 46 (31–66) years in the group of patients with macular diseases. Eleven (61%) in the normal patients group and 38 (61%) of the diseased cases were male.

Analysis of tomographic scans from the normal eyes revealed the presence of two parallel, highly reflective (red/white) layers separated from each other by one thin layer of moderate reflectivity (green/yellow) at the level of the posterior boundary of the retina in all cases (Fig. 1). Evaluation of the tomographic images obtained from the pathologic eyes revealed a similar appearance (two parallel HRL separated by one band of lower [green/yellow] reflectivity) in presumably non-affected areas of the macular region. However, tomographic peculiarities of the posterior boundary of the neural retina were encountered within the affected areas, depending on clinical disease: (1) idiopathic macular holes: the reflectivity of the inner HRL was diminished at the margins of the macular hole, resulting in its disappearance because of the similar reflectivity in the detached area (Fig. 3, middle); (2) RPE detachments and drusen: at the edge of the affected macular region, elevation from both HRLs was noted. As expected, no reflective signs were seen underneath the outer HRL in serous RPE detachments, whereas regions of moderate reflection (green/yellow) were seen in those eyes affected by drusen (Fig. 3, bottom); (3) macular edema: disappearance of the inner HRL was noted in the macular regions of increased retinal thickness because of intraretinal fluid accumulation. After processing each scan by using the OCT3 standard analysis protocol (retinal thickness [single eye]), we noted that delineation of the outer boundary occurred automatically at the level of the inner HRL whenever such a layer could be visualized (normal eyes and presumably unaffected macular regions of pathologic eyes). In the macular regions where peculiar changes in tomographic appearance resulted in fading of the inner HRL at the outer retinal aspect (as described earlier), delineation shifted automatically to the level of the outer HRL in those regions (Fig. 3, right column).

For the cross-sectional study, 18 eyes of nine participants prospectively included were examined. The mean age was 29.7 ± 4.8 years and the group included three men and six women. Bilateral, consecutive OCT scans acquisition was well tolerated by all patients. Pupillary dilation with eye drops was

<table>
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<th>Eye</th>
<th>Region</th>
<th>Caliper-Assisted (μm)</th>
<th>Automatic (μm)</th>
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<tbody>
<tr>
<td>Right</td>
<td>Temporal</td>
<td>315 (286–325)</td>
<td>277 (255–292)</td>
</tr>
<tr>
<td></td>
<td>Foveal</td>
<td>199 (190–215)</td>
<td>156 (141–175)</td>
</tr>
<tr>
<td></td>
<td>Nasal</td>
<td>540 (315–356)</td>
<td>304 (271–324)</td>
</tr>
<tr>
<td>Left</td>
<td>Temporal</td>
<td>298 (290–331)</td>
<td>271 (253–294)</td>
</tr>
<tr>
<td></td>
<td>Foveal</td>
<td>199 (190–215)</td>
<td>144 (138–169)</td>
</tr>
<tr>
<td></td>
<td>Nasal</td>
<td>331 (307–356)</td>
<td>298 (265–322)</td>
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Data are shown for temporal-, foveal-, and nasal-specific regions of the macula for right and left eyes as measured by manual-caliper-assisted method and automatic software measurement tools. Data are the median with the range in parentheses. \( P = 0.008 \) by the Wilcoxon signed rank test.
not performed, and approximately 2.5 to 3 minutes were necessary to obtain the three optimal horizontal scans in each eye.

Analysis of tomographic 3.00-mm macular scans in all studied eyes revealed tomographic appearance of the outer neural retina boundary similar to that described earlier. The inner HRL, which probably corresponds to the junction between the inner and outer segments of the photoreceptors\(^{16,17}\) assumed a forward bowl-shaped configuration in the center of the macula consistent with the well-known increase in length of the outer segments of the cones in such a region. The outer HRL, which appeared approximately two times thicker than the inner HRL, probably corresponds to the RPE-choriocapillaris reflective complex. The outer HRL was used for manual-caliper-assisted measurements, to reflect the outer boundary of the neural retina (Figs. 1, 2).

Using the automatic software measurement tool that analyzes retinal thickness from A-scans at the designated regions,

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**Figure 7.** Box-plot showing the retinal thickness at specific temporal and nasal regions as well as at the fovea obtained from right eyes of healthy subjects by using either automatic OCT3 software or manual-caliper-assisted measurement tools.

**Figure 8.** As in Figure 7, but for the left eye.
TABLE 2. Correlations of Neural Retina Thickness of Temporal, Foveal, and Nasal Specific Regions of the Macula between Right and Left Eyes as Measured by Manual-Caliper-Assisted Technique and Automatic Software Measurement Tools

<table>
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<tr>
<th>Region</th>
<th>Caliper-Assisted</th>
<th>Automatic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Temporal</td>
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<td>Foveal</td>
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<td>0.023</td>
</tr>
<tr>
<td>Nasal</td>
<td>0.926</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

r = intraclass correlation coefficient.

we found the median (range) retinal thickness for temporal and nasal regions to be 277 (255–292) and 304 (271–324) μm, respectively, in right eyes and 271 (253–294) and 298 (265–322) μm, respectively, in left eyes. In contrast, the median (range) retinal thickness for temporal and nasal regions obtained by manual-caliper-assisted measurements were 315 (286–323) and 340 (315–356) μm, respectively, in right eyes and 298 (290–331) and 331 (307–356) μm, respectively, in left eyes (Figs. 4, 5). The median (range) foveal height for right and left eyes was 156 (141–175) and 144 (138–169) μm, respectively, when using the automated analysis tool, and 199 (190–215) and 199 (190–215) μm, respectively, when using the manual-caliper-assisted technique (Fig. 6). The difference in retinal thickness obtained by the two techniques was statistically significant in each of the measured regions in both right and left eyes (P = 0.008; Table 1; Figs. 7, 8). The correlations of neural retinal thickness for specific temporal and nasal regions of the macula between right and left eyes as measured by the automated and manual-caliper-assisted techniques were statistically significant (P < 0.01); for foveal height, the correlation between right and left eyes was also statistically significant for automated measurements (P = 0.009) and marginally significant for manual-caliper-assisted measurements (P = 0.023; Table 2).

DISCUSSION

Objective, quantifiable, and reproducible measurements of the retinal layers have been possible in the clinical scenario because of the recent advances in diagnostic technology. OCT evaluates the reflectance of posterior segment structures and incorporates a mathematical algorithm capable of determining the anterior and posterior boundaries of the neural retina or retinal nerve fiber layer. Our study suggests that the automated retinal thickness measurements generated by the current available OCT3 software versions should be viewed with caution. Our observations in subjects with no clinically evident macular conditions, as well as in patients with well-established macular diseases, demonstrate that the outer boundary of the neural retina has been inaccurately delineated with the automated measurement tool, because it considers the inner HRL, whenever present, as the outer boundary of the neural retina. It is quite likely that the inner HRL at the level of the outer retina belongs to the neural retina, thus excluding a significant part of the structure for ultimate retinal thickness measurements at specific regions of the normal macula, as demonstrated herein. In accordance with recent reports, we believe that the inner HRL corresponds to the junction between the internal and external photoreceptor segments, as the outer one may correspond to the RPE–choriocapillaris hyporeflective band seen on the first generation of OCT. The increase in the distance between the inner and outer HRLs on the fovea of healthy subjects in this study, which is consistent with the well-known increase in length of the external segments of the cones in such region, provides additional support for our beliefs.

Inconsistencies in the alignment of the outer retinal boundaries of the macular region using the automated software measurement tool were more easily perceptible in patients with macular holes and central serous chorioretinopathy. Delineation of the outer HRL as the outer boundary occurred automatically whenever loss of the inner HRL in affected outer retinal areas occurred. In a clinical scenario, the mistaken measurement of the retinal thickness may be particularly relevant, because affected areas tend to have correct alignment of the outer neural retina boundary generated by the OCT3 software (versions 1.0, 2.0, and 3.0), whereas underestimation of these thicknesses are occurring in nonaffected areas. Moreover, the automated measurements of retinal thickness may be underestimated, particularly in the fovea, by the scanner software, because the inner HRL has a forward bowl-shaped configuration in this region. This forward bend is at its maximum in the deepest portion of the fovea, and underestimating of foveal heights may be greatly affected (>30%) by identifying the inner highly reflective layer as the outer retinal boundary, as demonstrated. Therefore, analysis of tomographic retinal measurement data generated by the actual available software versions should be viewed with extreme caution.

In conclusion, we have demonstrated that two HRLs were visible at the level of the outer retina in the macular region of healthy subjects. A similar appearance was also evidenced in nonaffected macular regions of patients with selected eye diseases, whereas affected regions generally demonstrated a single-layer, highly reflective appearance with disappearance of the inner HRL. The use of the OCT3 automated retinal thickness measurement tool (software versions 1.0, 2.0, and 3.0) is generating erroneous values because of incorrect interpretation of the inner HRL as the outer neural retina boundary. Measurements using the automated tool of the current OCT3 software (version 3.0) in comparison to a manual-caliper-assisted technique, in which the outer HRL was interpreted as the outer boundary, demonstrated that a significant difference existed in the generated measurements of retinal thickness at specific macular regions in healthy subjects, caused by the misalignment.

References