RPE-Normalized RNFL Attenuation Coefficient Maps Derived from Volumetric OCT Imaging for Glaucoma Assessment

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PURPOSE. We present spatial retinal nerve fiber layer (RNFL) attenuation coefficient maps for healthy and glaucomatous eyes based on optical coherence tomography (OCT) measurements. Quantitative analyses of differences between healthy and glaucomatous eyes were performed.

METHODS. Peripapillary volumetric images of 10 healthy and 8 glaucomatous eyes were acquired by a Spectralis OCT system. Per A-line, the attenuation coefficient of the RNFL was determined based on a method that uses the retinal pigment epithelium as a reference layer. The attenuation coefficient describes the attenuation of light in tissue due to scattering and absorption. En-face maps were constructed and visually inspected. Differences between healthy and glaucomatous eyes were analyzed (Mann-Whitney U test), both globally (average values) and spatially (concentric and per segment).

RESULTS. RNFL attenuation coefficient maps of healthy eyes showed relatively high and uniform values. For glaucomatous eyes, the attenuation coefficients were much lower and showed local defects. Normal and glaucomatous average RNFL attenuation coefficients were highly significantly different (P < 0.0001) and fully separable. The RNFL attenuation coefficient decreased with increasing optic nerve head distance for both groups, with highly significant differences for all distances (P < 0.001). The angular dependency showed high superio- and inferiortemporal and low nasal values, with most significant differences superio- and inferiortemporally.

CONCLUSIONS. Maps of RNFL attenuation coefficients provide a new way of assessing the health of the RNFL and are relatively insensitive to imaging artifacts affecting signal intensity. The highly significant difference between normal and glaucomatous eyes suggests using RNFL attenuation coefficient maps as a new clinical tool for diagnosing and monitoring glaucoma.

Since its inception, optical coherence tomography (OCT) in ophthalmology has predominantly been used to assess morphological features. These features represent architectural properties, such as thickness, and have been used to assess, for example, total retinal thickness, mainly for retinal applications, or the thickness of the retinal nerve fiber layer (RNFL), aimed at glaucoma diagnosis and management. Although the application of ophthalmic OCT has been broadened considerably since the introduction of Fourier-domain or spectral-domain OCT,1,2 a recent review showed that its diagnostic accuracy in glaucoma detection has not improved significantly.3

Although the aforementioned morphological features paved the way for the clinical application of OCT, limiting the use of OCT to these features does not fully exploit the potential of this imaging modality. The high imaging speeds of current commercially available OCT systems enable the acquisition of volumetric scans that contain much more information than just morphological descriptions of the various retinal layers. The strength of the OCT signal provides information on the scattering properties of these layers. Because interpreting the signal strength is not straightforward, quantitative analysis of this additional information requires more sophisticated approaches. The signal is related to the scattering properties of the tissue at the location corresponding to a certain pixel, but in addition, it depends on many other factors, including media opacities, strength of the incident light beam, and scattering properties of tissue the light travels through before reaching a specific depth. Quantitative analysis of OCT signals, directly or after simple scaling,4–7 need to take these factors into account to reduce artifacts and errors in accuracy and precision.

It was recently shown that, ignoring these factors and just using the absolute OCT signal data, there is a difference between the signal strength of healthy and glaucomatous nerve fiber layer tissue (Vermeer KA et al. Invest Ophthalmol Vis Sci. 2011;52:ARVO E-Abstract 3666). Because of the earlier mentioned problems associated with these absolute signal strengths, we developed a different method to measure a property of the RNFL from the OCT data. By relating the absolute signal strength of the RNFL to the signal of the retinal pigment epithelium (RPE) and assuming spatially invariant RPE scattering properties, the attenuation coefficient of the RNFL was calculated.8,9 The attenuation coefficient is not affected by the aforementioned factors and represents a tissue property that can be quantitatively analyzed. For manually labeled OCT data, a significant decrease of the RNFL's attenuation coefficient was reported recently for increasing glaucoma severity and a significant structure-function relationship was shown for the attenuation coefficient and the visual field’s mean defect.9

In this article, we present spatial maps of the RNFL's attenuation coefficient derived from automatically segmented volumetric OCT data. The RNFL attenuation coefficient was analyzed on healthy and glaucomatous eyes, at a distance...
between 1.5 and 2.5 mm from the optic nerve head (ONH) on healthy and glaucomatous patients, as a function of radial distance from the ONH, and as a function of the angular sector around the ONH. The diagnostic value of the RNFL’s attenuation coefficient and the attenuation coefficient maps was evaluated by quantifying the separation between data derived from healthy and glaucomatous patients for these three metrics.

METHODS

Data

The data used to test the presented methods consisted of 10 images of normal eyes and 8 images of glaucomatous eyes. All eyes were selected from an ongoing study into glaucoma imaging at the Rotterdam Eye Hospital, which was approved by the institutional review board and adhered to the tenets of the declaration of Helsinki. One eye was selected randomly per subject. For inclusion, a healthy subject needed to have a normal visual field and an intraocular pressure below 22 mm Hg. A normal visual field had, by definition, a Glaucoma Hemifield Test within normal limits and a mean deviation (MD) and pattern SD within the 95% confidence intervals. For glaucomatous eyes, only those eyes with moderate glaucoma, as defined by a reproducible MD between −12 and −6 dB on visual fields (Humphrey Field Analyzer, white-on-white, 24-2 SITA program; Carl Zeiss Meditec, Inc., Dublin, CA) were considered for inclusion.

Subjects were excluded from participation in the presence of any coexisting ocular or systemic disease known to possibly affect the visual field (eg, diabetes mellitus), a history of intraocular surgery (except for uncomplicated cataract surgery or glaucoma surgery in glaucoma patients), uncontrollable arterial hypertension, and secondary glaucoma in the case of glaucoma. All participants had a best-corrected Snellen visual acuity of at least 20/40 in both eyes, a spherical equivalent refractive error between −10.0 D and +5.0 D, and had unremarkable findings on slit-lamp examination, including open angles on gonioscopy.

All OCT scans of included eyes were obtained by a Spectralis OCT system (Heidelberg Engineering, Dossenheim, Germany). Per volume, 193 (averaged) B-scans were acquired, each consisting of 512 A-lines containing 496 pixels. The system was set to average five B-scans obtained at the same location, by using the built-in eye tracker. The field-of-view was 20 × 20°, corresponding to an area of approximately 6 × 6 mm.

Raw measurement data were exported by the manufacturer’s supplied software. This resulted in averaged and registered B-scans after conversion from spectral data to image data but before nonlinear scaling. In accordance with the manufacturer’s recommendations, the image data were transformed by calculating its fourth root (\( \sqrt[4]{s} \)) for display purposes. All analyses were performed on the data before the scaling was applied.

An example OCT frame is shown in Figure 1. Because it is difficult to appreciate differences in signal strength for different A-lines, an integrated en-face view of the full volume, corresponding to a scanning laser ophthalmoscopy image, is shown in Figure 2. Note the horizontal lines in the image and the apparent floater in the inferior part (denoted by yellow arrows). These intensity fluctuations reflect the different OCT signal strengths of the corresponding A-lines and indicate that the OCT signal should not be quantitatively analyzed as such.

Calculation of RNFL Attenuation Coefficient

Straightforward analysis of the raw data has been done previously4 (Vermeer KA, et al. IOVS 2011;52:ARVO E-Abstract 3666), but in these approaches absolute or simple ratiometric measurements were assumed and these measurements can therefore not be interpreted quantitatively. Instead, the signal should in some way be normalized to correct for spatial fluctuations of the incident light intensity and ocular opacities. Because an external reference object is not available, we used an internal layer for normalization. Following recent work,8,9 we use the RPE as the reference layer, which is assumed to be a uniformly scattering layer. In addition, the intensity of the incident light beam is affected by the retinal tissue itself. Therefore, instead of analyzing the raw OCT intensity values, which represent the amount of backscattered light that reaches the detector, the attenuation coefficient was computed. This attenuation coefficient is an optical property of the tissue and is therefore not affected by the amount of incident light and media opacities.

For every A-line, the attenuation coefficient of the RNFL (\( \mu_{RNFL} \)) was calculated according to the following equation that was presented earlier8,9:

\[
\mu_{RNFL} = \frac{\log \left( \frac{\rho}{\beta + 1} \right)}{2d}
\]
Here, $R$ denotes the ratio of the integrated OCT signal of the RNFL over the integrated OCT signal of the RPE, $d$ denotes the thickness of the RNFL, and $\beta$ is a constant. The optimal fit of a model of the RNFL reflectivity as a function of RNFL thickness for healthy eyes resulted in an estimate for $\beta$ of 2.3. Note that this parameter acts like a fixed, nonlinear scaling factor. To calculate $R$ and $d$, the RNFL and the RPE needed to be segmented. We applied a previously published method to fully automatically segment the RNFL and RPE. This method provides the posterior and anterior surface of the RNFL and the anterior surface of the RPE. To obtain the posterior boundary for calculating the integrated OCT signal of the RPE, the anterior surface of the RPE was displaced by a fixed amount.

**Visualization and Analysis**

Based on the automated segmentation, $R$ and $d$ were calculated for every A-line. The corresponding $\mu_{\text{RNFL}}$ for every A-line was mapped to an image to spatially represent the RNFL's attenuation coefficient. These images are referred to as attenuation coefficient maps.

The resulting images provide the most comprehensive view of the local RNFL attenuation coefficient and provide a visual representation of the data that can easily be interpreted by human operators. For quantitative analyses and comparison between patients and patient groups, the data were further processed in several ways to provide concise descriptors of the RNFL's attenuation coefficient.

First, a simple spatial average (determined by the median) was calculated from the attenuation coefficient map. The area that was included in the average was delineated by two concentric circles, manually centered on the ONH, with a radius of 1.5 mm for the inner circle and 2.5 mm for the outer circle (see the red lines in Fig. 2). To obtain the average, the median was calculated over all values within the resulting 1-mm-wide band.

To determine the spatial variation of the attenuation coefficient, the dependency of the attenuation coefficient on the distance to the center of the ONH was investigated. The 1-mm-wide band was subdivided into 20, 0.05-mm-wide bands and the median value per band was calculated, resulting in 20 concentric RNFL attenuation coefficient averages per eye.

To capture local defects, which are roughly radially oriented, segments with matching radial orientation were analyzed. Twenty-four of these segments were defined, each covering 15 degrees of the full circular band, and ordered in a temporal, superior, nasal, inferior, and again temporal sequence (see the green lines in Fig. 2). The median attenuation coefficient of a segment was denoted by the segment attenuation coefficient.

The distribution of the averages (overall, concentric, and per segment) was analyzed. The separation of examples of normal and glaucomatous eyes based on these parameters was assessed. Statistical analyses were based on the Mann-Whitney $U$ test, a nonparametric test to compare two unpaired groups.

**RESULTS**

All image data were automatically processed to obtain volume data sets with segmented RNFL and RPE. For every A-line in every volumetric scan, values for $R$ and $d$ were derived and the local attenuation coefficient $\mu_{\text{RNFL}}$ was calculated. In Figures 3 and 4, two examples of the resulting image are shown, for a healthy and a glaucomatous eye respectively. Similar patterns were observed in the other normal and glaucomatous eyes. The calculation of the RNFL attenuation coefficient was
hardly affected by common artifacts in the raw OCT data. The horizontal lines and the floater that are present in Figure 2 are not visible in the RNFL attenuation coefficient map of the same eye (Fig. 3, left image). Note that the reduced attenuation coefficient at the edges of the image in Figure 3 does not result from vignetting: the same data were used to construct Figures 2 and 3, and Figure 2 does not show any vignetting. In addition, any vignetting would have affected the signal strength of the RNFL and the RPE in the same way. This would, however, not have changed the ratio \( R \) in equation 1 and consequently the calculated attenuation coefficients would have been unaffected.

Figure 5 shows the distribution of the average attenuation coefficient for every included eye, calculated by taking the median within a 1-mm-wide band centered on the ONH. The corresponding \( P \) value of the Mann-Whitney \( U \) test was less than \(.0001\), indicating that the probability of the distributions having equal median is very small.

The average RNFL attenuation coefficient in 20, 0.05-mm-wide bands was determined for every eye. The results are shown in Figure 6 and illustrate a strong negative correlation between radial distance and attenuation coefficient. However, this applies to both normal and glaucomatous eyes and consequently the Mann-Whitney \( U \) test showed a significance level smaller than \(.001\) for all radial distances.

Angular dependence was tested by analyzing segments of 15 degrees width. Data within every segment were averaged (by calculating the median) and the results are depicted in Figure 7. Although the average attenuation coefficient of the glaucomatous eyes is smaller than the attenuation coefficient of the normal eyes almost everywhere, there is a lot more variation between locations. The significance of the Mann-Whitney test is shown in Figure 8, indicating that some segments show larger differences between healthy and glaucomatous eyes. The difference was significant at the 5% level in 17 of the 24 segments and at the 1% level in 11 of the 24 segments.
DISCUSSION

The proposed method to locally estimate RNFL attenuation coefficients based on using the RPE layer as a reference layer was used to produce RNFL attenuation coefficient maps of both healthy and glaucomatous eyes. These RNFL attenuation coefficient maps showed many fewer artifacts than the raw OCT data. Both horizontal lines, presumably due to fluctuating power of the incident OCT laser beam on the retinal surface, and the shadowing effect of floaters were virtually removed. This illustrates both the usefulness of looking at tissue properties rather than at the raw OCT signal and the effectiveness of the algorithm.

The RNFL attenuation coefficient maps in normal eyes was relatively constant in the peripapillary region, whereas the RNFL thickness shows considerable local variation due to the concentration of nerve fibers in arcuate bundles. This variation is especially large because both the location and the thickness of the arcuate bundles vary. The homogeneous appearance of attenuation coefficient maps makes interpretation of these data much easier. We speculate that it also reduces the range of normative values that may be used for diagnosis and it enables better detection of wedge-shaped defects.

The results show that the RNFL attenuation coefficient for glaucomatous eyes was considerably smaller than for healthy eyes. This indicates that, apart from a change in the thickness of the RNFL, the remaining nerve fiber tissue has different optical properties. Assessing the RNFL attenuation coefficient in addition to the thickness may thus provide useful information in the clinical management of glaucoma.

The $P$ value of the Mann-Whitney $U$ test for the average RNFL attenuation coefficients is very small, meaning that the difference of the medians is very significant. For use of this feature in diagnosis, however, the most important property is the separability of normal and glaucomatous eyes based on the attenuation coefficient. The distributions of normal and glaucomatous eyes in our data set did not show any overlap. Although the number of included eyes was relatively small, this is an encouraging result that warrants a larger study, including a comparison with RNFL thickness measurements.

The attenuation coefficient decreased with increasing distance to the ONH in both normal and glaucomatous eyes. No correlation between the distance and the significance of the difference of the medians was found. This implies that the radial distance for measuring the attenuation coefficient is not very critical, although it should be constant. The attenuation coefficient changed considerably with varying angle around the ONH, as did the significance of the difference of the medians. This agrees with previous findings that glaucomatous damage occurs more often in some segments than in others.11,12

Physiologically, the RNFL attenuation coefficient may correspond to the density of nerve fibers. When nerve tissue is lost, as in glaucoma, the remaining fibers may take up part of the resulting space, resulting in the observed lower attenuation coefficient for glaucomatous eyes. In healthy eyes, the RNFL attenuation coefficient is higher for the superior and inferior locations. This implies that both the thickness of the RNFL and the density of the fibers within that layer are higher at those locations, to accommodate for the higher number of nerve fibers.

FIGURE 6. Median RNFL attenuation coefficient for normal (blue) and glaucomatous (red) eyes as a function of the distance to the ONH center.

FIGURE 7. Median segment RNFL attenuation coefficients for healthy and glaucomatous eyes as a function of the angular location. T, temporal; S, superior; N, nasal; I, inferior.

FIGURE 8. Significance of the difference of the median of the segment RNFL attenuation coefficient between normal and glaucomatous eyes (blue line). The horizontal lines indicate the 5% (red line) and 1% (green line) significance levels. T, temporal; S, superior; N, nasal; I, inferior.
An interesting comparison can be made with polarization-sensitive OCT (PS-OCT). In PS-OCT, the retardation and the birefringence of the RNFL can be assessed. Like the attenuation coefficient, the birefringence is an optical property of the tissue. Both RNFL attenuation coefficient and birefringence show similar properties. The angular dependence for healthy eyes (see Fig. 7), with high values superiorly and inferiorly and low values nasally and temporally, has been shown in a small number of healthy subjects as well. With a similar difference between high and low values. Comparison of a healthy and a glaucomatous eye showed reduced birefringence determined by PS-OCT in one study, matching our findings of reduced attenuation coefficients in glaucomatous eyes. Another study showed a less clear double-hump pattern for the birefringence of a glaucomatous eye, but no clear reduction of the birefringence. A comparison between 8 normal eyes and 12 glaucoma suspects showed a significantly reduced birefringence of the RNFL (Götzinger E et al. IOVS 2009;50:ARVO E Abstract 5823). Changes in attenuation coefficient and birefringence may thus both be the result of the same process, where the density of the RNFL changes due to glaucoma. This correlation between birefringence (as measured with PS-OCT) and reflectance (related to our attenuation coefficient) may, at least partly, be explained by the microtubules of the RNFL. It has been shown that treatment of retinal nerve fiber bundles with a colchicine solution reduces the birefringence of the nerve fiber bundles significantly. The same treatment also resulted in a reduced RNFL reflectance, albeit to a lesser extent.

The presented processing method for separation of normal and glaucomatous eyes based on attenuation coefficient maps is very straightforward and provides promising results; however, various improvements and refinements are possible. Blood vessels are currently included in the analysis, while their attenuation coefficient obviously does not reflect the attenuation coefficient of the RNFL. Use of the median as a robust alternative for the mean alleviates this issue somewhat, but especially in smaller areas, such as the 15-degree-wide segments, the vessels may still dominate the output. Explicitly excluding the vessels would make the method more robust and would possibly further improve the results. The different significance levels for each segment indicate that combining only selected segment attenuation coefficients in the average may further improve the results. Also, algorithms for specifically detecting wedge-shaped defects may be developed, as was previously done for other imaging modalities. These improvements should, however, be made and evaluated on a larger data set.

The procedure that was used to determine the attenuation coefficient from the OCT volume scans requires that a segmentation of the RNFL and the RPE is available. These segmentations are performed based on the OCT data and will contain some errors. Especially in glaucomatous eyes, the contrast between the RNFL and the ganglion cell layer (GCL) may be very small and consequently the most prominent segmentation errors are expected at the posterior RNFL interface. In eyes with low RNFL/GCL contrast, the attenuation of the RNFL and GCL will be similar. The estimated average attenuation coefficient, calculated from RNFL and partially from GCL, will therefore be close to the RNFL attenuation coefficient.

The present method relies on the RPE to act as a reference layer. This assumption may not hold in all cases, such as in age-related macular degeneration. In the proposed application of glaucoma, the assumption of uniform RPE scattering properties may be violated by the presence of peripapillary atrophy (PPA). The minimum diameter of the area included in our analysis was relatively large (1.5 mm), which means that the results would have been affected in case of extensive PPA. In the included eyes, with moderate glaucoma, the observed PPA did not extend into the measured area.

In conclusion, the RPE-normalized OCT-derived RNFL attenuation coefficient maps provide a new means of assessing the health of the RNFL. Clinically, these maps may be relevant for diagnosis and monitoring. For diagnosis, the attenuation coefficient may prove to outperform or at least complement RNFL thickness measurements, because the attenuation coefficient shows less spatial variation. For monitoring of glaucoma patients, attenuation coefficients may detect the deterioration of the RNFL's health before further tissue loss. Finally, the attenuation coefficient may be used as an additional modality in structure-function research.

References


