Human Macula Investigated In Vivo with Polarization-Sensitive Optical Coherence Tomography

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PURPOSE. To investigate a depolarizing layer that is visible in polarization-sensitive optical coherence tomography (PS-OCT) images of the retina. To identify this layer and characterize its depolarizing effect quantitatively.

METHODS. Ten healthy human subjects (mean age, 31 ± 8 years) and two patients with RPE diseases participated in the study. The macular region of one eye of each subject was investigated with a phase-resolved PS-OCT system. The instrument measured backscattered intensity (standard OCT), phase retardation, and (cumulative) birefringent axis orientation, simultaneously. For a quantification of the depolarizing layer, plots of the distributions of retardation and axis orientation within and above this layer were analyzed.

RESULTS. A polarization-scrambling layer (PSL) was observed at the posterior boundary of the retina in PS-OCT images of all volunteers. It was identified in PS-OCT images by determining random retardation and axis orientation in a transverse direction. Measurements in patients with neurosensory retinal detachment, retinal pigment epithelium (RPE) detachment, and RPE atrophy suggest that the PSL is the RPE. The statistical analysis provided objective discrimination of the RPE from the other retinal structures.

CONCLUSIONS. PS-OCT represents a powerful tool for increasing image contrast in ocular tissues. The observed polarization-scrambling nature of the RPE may be used in diseased eyes to locate the RPE or remains of the RPE definitively in OCT images. (Invest Ophthalmol Vis Sci. 2006;47:5487–5494) DOI: 10.1167/iovs.05-1589

The retinal pigment epithelium (RPE) plays an important role in the maintenance of photoreceptor function. Many diseases (e.g., age-related macular degeneration [AMD], macular dystrophies, central serous chorioretinopathy [CSCR]) that become manifest at the level of the retina are caused by a primary RPE malfunction or effect the RPE itself. A noninvasive technique that enables direct assessment of the RPE in vivo may be a useful step toward a better understanding of diseases with RPE involvement, an improved follow-up of disease progression, and an optimized evaluation of new treatment modalities.

Optical coherence tomography (OCT) represents a well-established imaging tool in ophthalmology. The technique offers the possibility of performing a noninvasive optical “biopsy” of tissue (e.g., the human retina) in vivo. Several studies have shown the diagnostic value of the method for investigating retinal diseases. Recently, new developments in the field have led to an increased imaging speed with the use of Fourier- or spectral-domain OCT. High-speed imaging of the retina has been achieved with this novel technology, which was subsequently followed by in vivo three dimensional (3D) imaging of normal and diseased retina.

In contrast, as known from microscopy, images that represent only the backscattered light intensity (as is the case in standard OCT images) often yield poor image contrast. The possibility of using polarized light to increase image contrast and to gather additional information about the sample is well known and has been used to investigate the polarization properties of the human retina. Probably the most common and commercially available (e.g., GDx; Carl Zeiss Meditec GmbH, Oberkochen, Germany) imaging tool that uses polarized light for retinal imaging is scanning laser polarimetry (SLP). This technique measures the amount of retardation introduced by the retinal nerve fiber layer (RNFL) to obtain information on thickness changes associated with glaucoma.

However, SLP cannot provide depth-resolved information on the polarization properties of the retina. Polarization-sensitive (PS)-OCT combines the ability of OCT to retrieve birefringent information. A prototype instrument used this technique to measure birefringence introduced by the RNFL. Recently, retardation introduced by Henle’s fibers in the foveal region and the discovery of a polarization-scrambling layer (PSL) within the retina have been presented. In this study, we used PS-OCT to investigate the healthy human macula in vivo. A depolarizing layer was visible in PS-OCT images of all volunteers who participated in the study. For a better identification of the anatomic layer responsible for the depolarization, we performed measurements on patients with macular diseases. Furthermore, we sought to develop an analysis method for quantitative description of the depolarizing layer.

METHODS

PS-OCT Technology

PS-OCT imaging was performed with a system that is based on a setup published previously in detail. In brief, the system is capable of measuring backscattered intensity (as measured in standard OCT systems), phase retardation, and birefringent axis orientation simultaneously. The technique uses circular polarized light incident on the cornea. A polarization and phase-sensitive two channel detection unit is used to retrieve all three parameters. Note that the anterior segment birefringence somewhat distorts the retardation and axis orientation values obtained with this method from the retina. However, this influence can be disregarded, because this study focused on the difference between polarization-preserving and depolarizing structures of...
the retina, and the randomization of the polarization state caused by depolarizing effects are not affected by anterior segment birefringence. Nevertheless, a quantitative analysis of retinal birefringence would be possible by compensating for the influence of the anterior segment (e.g., by introducing an appropriate retarder into the sample beam, as in the GDx VCC [Carl Zeiss Meditec, GmbH], or by a numerical compensation algorithm). The fast (or priority) scanning axis of the system is parallel to the retinal surface which differs from standard (or A-scan–based) OCT setups (fast scanning axis perpendicular to the retinal surface). One advantage of this technique is that the light power incident on the cornea can be increased because of the very short exposure times at a certain location on the retina.\(^\text{17}\) This advantage can be used to increase the sensitivity of the setup compared with standard time-domain OCT systems. The power incident on the cornea was measured with 1.2 mW, which is below the ANSI (American National Standards Institute) standard limits for repeated light exposure of the retina within measuring time.\(^\text{31}\) The axial (depth) resolution of the system is determined by the optical bandwidth of the used light source (superluminescent diode: Superlum Diodes Ltd., Moscow, Russia; \(\lambda_0 = 841 \text{ nm and } \Delta \lambda = 51 \text{ nm}\) and was measured with \(-6.1 \mu\text{m}\) in air which corresponds to \(-4.5 \mu\text{m}\) within the retina (assuming a group refractive index of the retina of 1.38). The system records a B-scan image (OCT tomogram) consisting of 3400 \(\times\) 500 pixels corresponding to \(-3.4 \times 1 \text{ mm (x-z image plane)}\) in 0.5 second.

An additional detection channel was implemented from the previous setup, which allowed the recording of scanning laser ophthalmoscope (SLO) images (with similar detection optics as those used for the OCT images) at a frame rate of 5 frames per second (fps) for alignment purposes. The SLO image, which was recorded immediately before (delay of \(-100\) ms) the OCT measurement, was stored to determine the position of the B-scan on the retina.

### Control Subject and Patient Selection and Imaging Procedure

All investigations were performed in a protocol that adhered to the tenets of the Declaration of Helsinki and was approved by the ethics committee of the Medical University of Vienna. For this study, 10 healthy, white volunteers (mean age, 31 ± 8 years) without any detectable ocular disease or history of an ocular disease were selected. Apart from a routine eye examination including visual acuity testing, color fundus photography and red-free fundus photography were performed before the measurement with the PS-OCT system. All eyes were rated as normal, with a refractive error of \(-2.2 \pm 2.0\) D. One eye of each volunteer was investigated with the PS-OCT instrument. Cross-sectional (B-scan) images of the foveal region were recorded and used for this study. No pupil dilation was necessary for this measurement.

To test our hypothesis that the PSL is the RPE, images of two patients (one patient with RPE atrophy, one patient with RPE detachment) were included in the study.

### Data Analysis

A combination of anterior segment birefringence and birefringence of Henle’s fiber layer results in an asymmetric retardation pattern in PS-OCT images of the foveal region.\(^\text{28}\) This effect is similar to that giving rise to the well-known ‘bow-tie’ pattern in SLP.\(^\text{32,35}\) To minimize the influence of this effect on the statistical analysis of the PSL, we divided all PS-OCT images at the position of the foveola into two parts: nasal and temporal. The analysis was performed for the nasal part.

In a first step, we extracted the PSL from the OCT images by image segmentation. The PSL represents the last (posterior) strongly reflecting layer in retinal OCT images. Therefore, we used a peak detection algorithm operating on the intensity images to locate the position of the PSL. After this peak detection, we fitted a second-order polynomial through the detected peaks, to exclude erroneous peak locations caused by noise. The fitted polynomial was regarded as the location of the PSL. From the two dimensional (2D) B-scan images, depth lines (corresponding to A-scans) of intensity, retardation, and axis orientation were derived and aligned with respect to the fitted polynomial. The result was a corrected B-scan image in which the PSL appeared as a horizontal line.

A horizontal evaluation line (single pixel width corresponding to a depth extension of 2 \(\mu\text{m}\)) parallel to the PSL was interactively shifted in depth by the operator through each image. Distributions of retardation and axis orientation along this evaluation line were plotted at the location of the PSL and at two locations above the PSL. Note that data points with a corresponding intensity below a certain intensity threshold were excluded from this analysis to avoid erroneous data caused by noise.\(^\text{20}\) The peak of each distribution was regarded as the corresponding retardation and axis orientation of each line. To evaluate the degree of polarization-scrambling, we determined the number of data points (\(n\)) with retardations (axis) outside an interval of \(-10°\) to \(10°\) from the peak of the distribution. This number was divided by the total number of data points, to normalize the data, and thus obtain a comparable depolarization value.

### RESULTS

#### Healthy Subjects

Figure 1 shows an example of PS-OCT images obtained in the foveal region of subject 1. In the intensity image (Fig. 1A) all retinal layers that are known from ultrahigh resolution OCT (UHR-OCT) are observed.\(^\text{7,34–36}\) The enlarged (by a factor of 2) subsection of the foveola region (Fig. 1D) shows four distinct strong reflecting layers in the outer retina, which are labeled in Figure 1D from interior to posterior with the numbers 1 to 4. Note that a clear separation between layers 3 and 4 is not always visible in intensity-based UHR-OCT images.\(^\text{7,34–36}\) This unclear separation may be the reason for the controversy in labeling of layers 3 and 4 in the literature, in which the following layer associations are found: 1 is the external limiting membrane (ELM), 2 is the interface between the inner and outer segments of the photoreceptor layer (IPLR), 3 + 4 is the retinal pigment epithelium (RPE),\(^\text{5,34–55}\) 1 is the ELM, 2 is the IPLR, 3 is the RPE, 4 is the choriocapillaris (CC),\(^\text{56}\), or 1 is the ELM, 2 is the IPLR, 3 is Verhoeff’s membrane (VM), and 4 is the RPE.\(^\text{37}\)

Figure 1E shows an SLO image that was recorded immediately before the OCT scan. The arrow marks the position of the OCT image.

More information can be obtained from the polarization-sensitive images. The (single-pass) retardation image (Fig. 1B) shows rather constant and low retardation throughout the retina (with the exception of layer 4).\(^\text{28}\) The observed retardation was induced by birefringence of the anterior segment of the eye (mainly corneal birefringence). The fact that retardation remained constant with depth indicates that these areas of retinal tissue were nonbirefringent. A slight asymmetry of retardation between the left and right sides of the image can be observed at layers 1 to 3. This can be explained by a combined birefringence of the anterior segment and Henle’s fiber layer (Fig. 1D).\(^\text{28}\) Within layer 4, rather random retardation is observed. This randomness is clearer in the enlarged image of a subsection of the foveola region (Fig 1F). Figure 1C shows the (cumulative) fast birefringent axis orientation of this region. Similar to the retardation image, a fairly constant axis orientation is observed throughout the retina, with the exception of layer 4. (There is more noise in the axes of the inner retinal layers due to the lower intensity obtained from these areas; the range of axes observed in the inner layers was restricted, however, to yellow-orange colors [left] and green-yellow colors [right], whereas the entire range from blue to red is observed in layer 4.) The difference in axis orientations within layers 1 to 3 between foveola and surrounding fovea (which was more pronounced than the difference in the retardation in...
this region) can be explained by the birefringence of Henle’s fiber layer. This layer changes the cumulative axis orientation in the region around the foveola; however, because this layer is not present in the foveola, the cumulative axis does not change in this region and remains the same as in the interior layers. Within layer 4, rather random axis orientations are observed.

For a quantitative analysis, the procedure described in the Data Analysis section was implemented. Figure 2 shows an example of the distribution of retardation (obtained from Fig. 1) in the different layers. The peak (obtained by a Gaussian fit) of each distribution was regarded as the corresponding retardation in each layer. Note that the retardation measured in layer 4 was higher than that measured in layer 3, probably because of the rather random retardations obtained in layer 4 (Fig. 2C) which broadens the distribution of retardation. Because the retardations provided by the algorithm used are always positive, the distribution is distorted and the center of gravity, which is determined by the fit, is shifted to larger values.

Furthermore, we calculated the full width at half maximum (FWHM) of each distribution. On the one hand, a clear difference between the distributions obtained at layers 2, 3, and 4 can be observed in Figure 2. At layers 2 and 3 the retardation was well defined (FWHM = 10°; Figs. 2A, 2B). On the other hand, a rather wide distribution (FWHM = 24°), which was caused by depolarization, was observed in layer 4 (Fig. 2C). A complete depolarization should show equally distributed retardations. A similar result was obtained for axis orientation (Fig. 3). An FWHM of 40° was measured in layers 2 (Fig. 3A) and 3 (Fig. 2C) which broadens the distribution of retardation. Because the retardations provided by the algorithm used are always positive, the distribution is distorted and the center of gravity, which is determined by the fit, is shifted to larger values.

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indicate the good reproducibility of the system. To test the reproducibility of our system, we evaluated retardations and axis orientations and corresponding DPRs and DPA in one subject from 10 different measurements at different days spread over a period of 3 months. At layer 3, we measured a retardation $\delta = 17 \pm 3^\circ$ with a corresponding DPR = 17% ± 7% and an axis orientation $\theta = 25 \pm 11^\circ$ and a corresponding DPA = 7% ± 3%. At layer 4, we measured a retardation $\delta = 26 \pm 5^\circ$ with a corresponding DPR = 56% ± 4% and an axis orientation $\theta = 28 \pm 11^\circ$ and a corresponding DPA = 52% ± 5%. Layer 2 showed results essentially similar to those of layer 3. The small errors (SD) of the different values can be disregarded because it is similar for both layers.

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Figure 3. Distributions of axis orientation values ($\theta$) obtained at retinal layers 2 (A), 3 (B), and 4 (C) in a healthy subject. See Figure 1 for the labeling of the layers. Rel. count, number of data points per angular interval (2°) divided by the total number of points.

Patients
To identify the anatomic structure that corresponds to the depolarizing layer, we performed PS-OCT imaging on patients with different macular diseases. Figure 4 shows images obtained close to the foveal region in a patient with neurosensory retinal and RPE detachment (Fig. 4A). Note that the normal appearance of the posterior retinal layers (three strongly reflecting bands, layers 2–4 in Fig. 1A) is not the case in Figure 4A. A diffuse backscattering layer (Fig. 4A, arrow) and only one strongly reflecting layer are visible. Therefore, a comparison of these with the layers of a healthy retina is rather difficult in the intensity image. The polarization-sensitive images (Figs. 4B, 4C) contain more information. The depolarizing layer (layer 4) was not detached at the location of the neurosensory detachment. At the center of the image (RPE detachment) a detachment of the depolarizing layer is visible, indicating that the depolarizing layer is the RPE. The faint line below the RPE detachment visible in the images may be Bruch’s membrane.

Figure 5 shows an example of PS-OCT images obtained from a patient with RPE atrophy secondary to AMD. In the intensity image (Fig. 5A) an enhanced penetration depth into the choroid and sclera is visible in the atrophy region. Note that at the left hand side of the image and at two other positions (Fig. 5A, arrow) dark vertical bands (shadows) obscure the structure of the choroid and sclera. Probably at these locations the RPE is still present, blocking the probing light from penetrating deeper into the tissue, but it is rather difficult to locate remnants of the RPE in the intensity image. In the polarizationsensitive images (Figs. 5B, 5C) remnants of the depolarizing layer can be easily identified (Figs. 5B 5C, arrows). A comparison with the intensity image (Fig. 5A) shows that at locations with an intact depolarizing layer, structures of deeper layers are obscured.

### Table 1. Retardation ($\delta$) and Depolarization (DPR) Obtained from Posterior Retinal Layers of Healthy Subjects

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<th>DPR/°%</th>
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Data were obtained from layers 3 and 4. $\delta_3$ ($\delta_4$), retardation at layer 3 (4); DPR$_3$ (DPR$_4$), depolarization at layer 3 (4).

### Table 2. Axis Orientation ($\theta$) and Depolarization (DPA) Obtained from Posterior Retinal Layers of Healthy Subjects

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<th>Pt.</th>
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Data were obtained from layers 3 and 4. $\theta_3$ ($\theta_4$), axis orientation at layer 3 (4); DPA$_3$ (DPA$_4$), axis depolarization value at layer 3 (4).
Furthermore, Figures 5B and 5C show additional information at deeper layers of the retina. In Figure 5B, an increase in retardation is visible (black arrow), whereas the axis orientation (Fig. 5C) remains rather constant. Note the difference between depolarization (random retardation and axis orientation values) and retardation (increasing retardation and constant axis orientation). Because the sclera is birefringent, we believe that the polarization changes demarcate the boundary of the sclera.

**DISCUSSION**

In this study, we investigated the human macula in vivo with a PS-OCT system. A depolarizing layer in the posterior outer retina (Fig. 1) was visible in PS-OCT (retardation and axis orientation) images of all subjects who participated in the study. Only light backscattered directly from this layer was depolarized; light transmitted through this layer was not depolarized, since the images show that light backscattered from deeper layers had a defined polarization state. We think that the term polarization-scrambling or depolarization may be appropriate to describe this observation. Furthermore, in PS-OCT images a separation between two layers that are sometimes indistinguishable in intensity images (Fig. 1, layers 3 and 4) was always possible. Therefore four distinct layers (Fig. 1) were observed in OCT images of the outer retina.

Because the degree of polarization cannot be directly measured by OCT (as a coherent imaging technique, it always provides a measured degree of polarization equal to 1), we introduced a measure based on the local variability of the measured polarization state. Depolarization leads to a random polarization state in PS-OCT images (i.e., the polarization state varies between neighboring speckles in a random manner; therefore, the term polarization-scrambling is sometimes preferred instead of depolarization in the context of PS-OCT). To quantify this effect, we introduced the depolarization values DPR and DPA, which indicate the fraction of pixels in a certain area (in our study, a certain retinal layer) that exhibit a polarization state (quantified by retardation and an axis orientation) outside the range observed normally in the investigated area (layer). The DPR and DPA enable an objective differentiation between polarization-preserving or birefringent structures from depolarizing structures. Our system shows a good reproducibility of the polarization parameters \( \delta \) and \( \theta \) and the depo-
larization parameters DPR and DPA, tested over an extended period of 3 months. The summarized depolarizations in all volunteers (Tables 1 and 2) showed a clear difference between depolarizations obtained from the depolarizing layer and other retinal layers, indicating the usefulness of these parameters.

Several results of this study can lead to the conclusion that the depolarizing layer is the RPE. First of all, as can be seen in images of a healthy human fovea (Fig. 1), the depolarizing layer represents the last strongly reflecting layer. Only a little light reaches deeper layers in healthy eyes, due to light absorption and scattering by the pigments of the RPE. Second, images of a patient with retinal and RPE detachment (Fig. 4) show that the depolarizing layer was detached at the location of RPE detachment (a similar observation was made in three other patients). This detachment excludes a possible association of the depolarizing layer with the choriocapillaris. Furthermore, the images indicate that the depolarizing layer cannot be associated with the photoreceptor layer, because this layer is detached from the depolarizing layer in areas of neurosensory retinal detachment (Fig. 4). Third, images of a patient with RPE atrophy secondary to AMD (Fig. 5) show an enhanced penetration depth at locations where the RPE is missing. These locations coincide with locations of a missing depolarizing layer, which further confirms our associations. A similar effect was observed in another patient with RPE atrophy. Based on our findings, we suggest revising the associations in OCT images of the outer retinal layers with histologic structures. Although further investigations are needed, we think that the following association of the posterior layers is the most plausible: 1 is the ELM, 2 is the IPRL, 3 is the outer segment-RPE junction (OS/RPE), and 4 is the RPE. This description is supported by recently reported high transverse resolution B-scan images which were obtained with adaptive optics. These images show clearly a constant spacing between reflections within layer 2, which were attributed to retinal cones. The same spacing can be observed within layer 3 which suggests that layer 3 is part of the cones.

At the current state of investigation we can only speculate about the origin of the depolarization of light within the RPE. A possible explanation that needs further investigation, could be multiple scattering on large nonspherical particles (e.g., melanosomes) of the RPE. An observation that could support this hypothesis is the slightly more diffuse and thickened appearance of the RPE in the intensity image (Fig. 1D) compared with the appearance of layers 2 and 3. Because the thickness of the RPE is on the order of the resolution of our instrument, it should appear as a very sharp boundary in the case of single backscattering events. The other interior layers of the retina...
are rather transparent which can be observed by a weak backscattering signal of these layers (Fig. 1). Therefore, multiple scattering should not occur within these layers. Note that the strong signal observed from layers 2 and 3 results from a specular reflection within the photoreceptors (which can only be seen if the light is coupled into the photoreceptors) and therefore yields a sharp boundary.

Our results could have impact on other polarization-sensitive imaging methods (such as SLP). SLP maps the retardation of the retina in a depth-integrated manner, assuming that light is backscattered from a posterior retinal layer that maintains the polarization state of reflected light.\cite{5} As we have clearly shown, a nonnegligible part of the total backscattered light intensity is backscattered from the RPE and depolarized. Although in eyes with intact posterior retina the fraction of depolarized light may be constant across the image (although this remains to be investigated) and a variability across the population is probably included in the normative database used in a diagnostic glaucoma scanning system (e.g., GDx; Carl Zeiss Meditec, GmbH), care has to be taken in patients with any RPE changes. In this case the fraction of depolarized light will vary across the image, possibly distorting the measured retardance and the measured nerve fiber layer thickness.

The capability of PS-OCT to identify the RPE in OCT images opens a new application for PS-OCT imaging. With this method, it is now possible to locate the RPE or its remains in macular diseases (e.g., AMD, CSC) in a depth-resolved manner, or to identify different kinds of detachments (inner retinal detachment, RPE detachment). This can be of importance in cases with distorted retinal structures, particularly in cases where an identification on the basis of conventional intensity based OCT (including UHR-OCT) is not possible. Although further investigations are needed, we think that PS-OCT may be capable of distinguishing between the sclera and choroid (Fig. 5) and may therefore be an important tool in measuring the thickness of the choroid.

Furthermore, PS-OCT combined with fast 3D sampling, as made possible by newly developed Fourier-domain OCT,\cite{8} could become an essential tool in monitoring RPE diseases and in evaluating new treatment strategies.

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References