Three-Dimensional Adaptive Optics–Assisted Visualization of Photoreceptors in Healthy and Pathologically Aged Eyes

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Purpose. To visualize and characterize photoreceptor (PR) layers on a subcellular level in healthy and pathologically aged eyes using adaptive-optics optical coherence tomography technology (AO-OCT).

Methods. AO-OCT scanning was performed within a 2 × 2° field of view, focused on the PR layer at different retinal regions in healthy aged eyes (n = 32 eyes/16 subjects; 62- to 85-years old), eyes with early to intermediate AMD (n = 16 eyes/8 subjects, 65- to 83-years old), and eyes with advanced nonneovascular AMD (n = 16 eyes/8 subjects, 61- to 84-years old). Areas of interest were determined by AO-fundus camera, which is part of the AO-OCT system and spectral-domain (SD)-OCT. PR integrity was evaluated in AO-OCT en face and cross-sectional images at the level of inner/outer segment junction (IS/OS) and cone/PR end tips (COST/ETPR).

Results. AO-OCT in healthy eyes showed clearly distinguishable and regular IS/OS and COST patterns and an inverse relation between cone density and eccentricity. In early to intermediate AMD, PR in between drusen showed more irregular patterns (P < 0.001) and slightly lower PR density (P ≤ 0.002). Drusen caused attenuated and distorted signals and a loss of PR mosaic. In advanced AMD, IS/OS and COST patterns were affected to different degrees between surrounding area, junctional zone, and atrophic area (P < 0.001), ranging from reduced PR densities to total signal loss.

Conclusions. AO-OCT imaging allows the three-dimensional visualization of different PR layers in patients with AMD and age-matched healthy subjects. Thereby, AO-OCT provides a unique insight into PR morphology and shows potential to fill the gap between conventional OCT and histologic examination of the retina.

Keywords: adaptive optics, AO-OCT, AMD, photoreceptor visualization, ultrahigh resolution

Integrity of structure and function of human photoreceptors (PR) is essential for normal vision and continuous advances in optical coherence tomography (OCT) have improved the capacity to visualize signals from PR and the retinal pigment epithelium (RPE) in vivo. Four separate bands of the outer retina can be resolved in healthy individuals with conventional OCT, which are distorted and disrupted in the presence of retinal disease and thus serve as a landmark for PR assessment. An improvement in visualizing PR structures was achieved recently using adaptive optics (AO), which is not an imaging modality on its own but a supporting technology and was originally introduced for telescopic imaging by the astronomer Babcock. A Shack Hartman sensor and a deformable mirror are combined to measure and compensate in real time for wavefront distortions, which vastly enhances the transverse and/or axial resolution of fundus camera (FC), scanning laser ophthalmoscopy (SLO), and OCT. Adaptive optics–enhanced (AO) OCT depicts retinal structures on a subcellular level and resolves the outer retinal bands seen in OCT into signals from individual PR. As light is coupled into the PR segments similar to a wave guide, the refractive index changes cause distinct reflections at different PR interfaces. These signals are visible as bright spots in AO-OCT B-scans (as shown in Fig. 1A). In healthy individuals, the bright spots at the height of the PR inner segment–outer segment (IS/OS) junction and from the cone OS tips (COST) result in a dense pattern in AO-OCT en face images, which actually represents the PR mosaic. This mosaic is similar to that obtained with AO-SLO and AO fundus camera (AO-FC). In contrast to the latter modalities, AO-OCT has the advantage of a significantly higher axial resolution (more than an order of magnitude) and the ability to create full three-dimensional volumetric image data of the retina.

Similarly to OCT, depiction of intact outer retinal bands in AO-OCT suggests an intact morphology, whereas retinal disease may cause alterations in the bands’ signal intensity and structure. Pathological aging, namely AMD, is a major cause of structural changes and loss of function of the outer retina and is a growing burden for healthcare systems due to the global rise in elderly populations.

In AMD, the balance of cellular preservation and degeneration and the homeostasis between pro- and anti-inflammatory factors are heavily disturbed and result in morphologic changes of the outer retina, which can be seen in OCT, such as the formation of drusen in early and intermediate AMD or retinal atrophy in advanced AMD. With AO, visualization of changes
in healthy and pathological aging has become possible in vivo on the subcellular level. However, up to now only a few patients have been imaged with AO-OCT31–33 and no assessment of the detailed PR composition in patients with AMD is available. The following study presents insights into pathologically and healthy aged retinas as imaged with AO-OCT in 55 eyes of 32 participants. The aim was to assess which pathologically and healthy aged retinas as imaged with AO-OCT en face images between healthy retinas and retinas with different stages of nonneovascular AMD. The secondary goal was to examine if density and regularity of the PR mosaic differed at the height of the IS/OS and COST between healthy and diseased eyes.

MATERIALS AND METHODS

This prospective study using multimodal imaging on three groups of patients, classified by AMD status, was approved by the Medical University of Vienna institutional review board and conducted according to the Guidelines for Good Clinical Practice. The study adhered to the tenets of the Declaration of Helsinki.

Study Cohort

Sixteen patients with AMD, grouped into either early to intermediate AMD or advanced nonneovascular AMD, were recruited from the outpatient clinic for macular diseases of the Medical University of Vienna and 16 age-matched healthy participants were recruited by public advertisement. All participants received a full explanation of the study and gave written informed consent for participation compliant with the Declaration of Helsinki.

Exclusion criteria for the healthy participants were a current or past history of any eye disease and ocular surgery except senile cataract or cataract surgery. The grade of senile cataract had to be below nuclear color (NC) 3.0, nuclear opalescence (NO) 3.0, cortical (C) 2.0, and posterior subcapsular (P) 0.5 using the Lens Opacities Classification System III (LOCS III). The cataract surgery had to be performed at least 12 months before the study, using an acrylic mono-focal lens without capsular support. Pseudophakic eyes had to be free from anterior capsule contraction syndrome and/or posterior capsule opacification within a central diameter of 5.0 mm. Analogous criteria were used for patients in the AMD group, who had to be diagnosed by a retina specialist and be free of any past or current history of neovascularization. Eyes were excluded if they had ametropia with a spherical error above 3.0 diopters (D) or a cylindrical error above 2.0 D. Additionally, a minimum pupillary diameter of 5.0 mm with a regular pupil shape had to be achieved in every participant after instillation of 1.0% tropicamide (Mydriaticum Agepha; Vienna, Austria) and 2.5% phenylephrine hydrochloride. Finally, any participant who showed signs of poor fixation during visual acuity testing, slit-lamp examination, or spectral-domain (SD)-OCT imaging was excluded.

Visual Acuity Testing and Imaging

Visual acuity testing (ARK-1s; Nidek Co. Ltd., Gamagori, Japan) and measurement of axial length, anterior chamber depth, and keratometry (IOL Master 500; Carl Zeiss Meditec, Jena, Germany) were performed before medical mydriasis with tropicamide and phenylephrine. After achieving a pupil dilatation above 5.0 mm and a thorough slit-lamp examination including LOCS III grading, wavefront analysis (KR-1W; Topcon Medical, Tokyo, Japan), infrared fundus (IR), and SD-OCT (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany) were performed. OCT scans on the Spectralis imaging platform were performed with the following settings: size 20 × 20°, 49 B-scans, high-resolution mode, and automatic real-time tracking with 20 averaged images. AO-OCT and AO-FC were performed using the system described below.

Adaptive-Optics Optical Coherence Tomography System

AO-OCT images were acquired with a system developed at the Centre for Medical Physics and Biomedical Engineering of the Medical University Vienna, described previously. In brief, the system consists of two subsystems in a compact design, combining a FC with OCT and using a deformable mirror (Mira502 e RC; Imagine Eyes, Orsay, France) and a high-speed Shack Hartmann wavefront sensor (Haso first; Imagine Optics, Orsay, France) for AO enhancement. AO-FC images are obtained with the FC of the system that is similar to a commercially available AO fundus camera (RTX 1; Imagine Eyes, Orsay, France) and provides a field of view of 4° × 4°. AO-OCT images are obtained with a superluminescent diode operating at 841 nm and provide a field of view of 2° × 2°, achieving an optical axial resolution of 4.5 μm (assuming a refractive index of 1.4) and an optical transverse resolution of approximately 3.2 μm in the retina. To optimize image quality, the focus can be adjusted manually by the operator on a displayed live stream of the B-scan, which can also be used to estimate the image quality. Volume acquisition of 400 B-scans takes under 2 seconds and is possible within a radius around the fovea of approximately 16°.

In healthy eyes, volumes were recorded and analyzed at an eccentricity of 2.5° and 6.5° superior to the fovea. In eyes with early to intermediate AMD, images were acquired above drusen at various eccentricities between 2.0° and 8.0°. In patients with advanced AMD, images were acquired above areas of full outer retinal atrophy and above areas that appeared not affected by atrophy at various eccentricities between 2.0° and 8.0°. Due to the various locations of drusen and atrophy, volumes of diseased eyes were not location matched with healthy individuals. Several volumes were acquired at each location. An imaging session with our AO system lasted approximately 20 to 30 minutes. Patients who complained of dry eye symptoms or showed signs of mild dry eye syndrome during slit-lamp examination, received lubricant drops containing Povidon K25 (Oculotect Fluid; Thea, Clermont-Ferrand, France) prior and during imaging. We did not perform extensive assessment of tear film quality, such as Schirmer’s
Pattern Irregularity

<table>
<thead>
<tr>
<th>Pattern Irregularity</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>No pattern irregularity</td>
<td>Regular PR pattern over the whole image</td>
</tr>
<tr>
<td>Light pattern irregularity</td>
<td>Irregular PR pattern up to one-third of the imaged area</td>
</tr>
<tr>
<td>Medium pattern irregularity</td>
<td>Irregular PR pattern between one- and two-thirds of the imaged area</td>
</tr>
<tr>
<td>Severe pattern irregularity</td>
<td>Irregular PR pattern above two-thirds of the imaged area</td>
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Signal Loss

<table>
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<tr>
<th>Signal Loss</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>No signal loss</td>
<td>No signal loss from PR apart from areas underneath vessels</td>
</tr>
<tr>
<td>Light signal loss</td>
<td>Signal absence from PR in up to one-third of the imaged area excluding areas underneath vessels</td>
</tr>
<tr>
<td>Medium signal loss</td>
<td>Signal absence from PR between one- and two-thirds of the imaged area excluding areas underneath vessels</td>
</tr>
<tr>
<td>Severe signal loss</td>
<td>Signal absence from PR above two-thirds of the imaged area excluding areas underneath vessels</td>
</tr>
<tr>
<td>Total signal loss</td>
<td>Total absence of signal from PR over the imaged area</td>
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Table 1. Grading System Used for Assessment of PR Patterns in the En Face Imaging Plane

**Results**

**Participant Data**

The mean age was 70.4 years (standard deviation [SD] 7.5, minimum [min] 62, maximum [max] 85) for the 16 healthy participants, 74.9 years (SD 7.5, min 65, max 85) for the eight patients with early to intermediate AMD and 73.0 years (SD 6.7, min 61, max 84) for the eight patients with advanced non-neovascular AMD.

Fifty-five of 64 eyes imaged (both eyes of each participant) could be included for image analysis, whereas nine eyes had insufficient image quality in all the AO-OCT volumes acquired. The PR could not be resolved in both eyes of one healthy participant and of one patient with intermediate AMD due to low signal strength despite adequate pupil size, no advanced cataract, and a low root mean square (RMS) wavefront error. Another patient in the early to intermediate AMD group and two patients with advanced AMD showed strong axial or transversal movements that resulted in partly missed or cropped B-scans in all volumes. Altogether, approximately 340 locations were imaged with AO-OCT and at least three volumes were acquired per participant. Of the roughly 1100 AO-OCT volumes, 381 could not be used due to insufficient imaging quality including the cases mentioned above.

**AO-OCT Assessment of Healthy Eyes (n = 30)**

The Outer Retinal Bands in AO-OCT B-Scans. In healthy eyes, the external limiting membrane (ELM), the IS/OS, the COST, the ROST, and the RPE could be visualized. Compared with SD-OCT, AO-OCT B-scans showed much more details of the outer retinal bands (compared with Fig. 2). The ELM presented as a discontinuous dotted line with low signal strength. Although barely visible in nonaveraged, single B-scans (as can be seen in Fig. 2C), it could be identified in each of the 60 locations from the 30 eyes. The IS/OS junction was clearly visible in all eyes and consisted of bold, bright, hyperreflective dots, similar to a string of beads (see Fig. 2C). The signal brightness of the dots oscillated throughout B-scans, ranging from bright white to dark gray (Fig. 2C). The FWHM of the dots from the IS/OS (IS/OSd) was measured in a small sample (one dot per patient) and was on average 4.9 μm (range, 4.6–6.1 μm), which corresponded to the axial resolution of the AO-OCT system. The COST was formed by hyperreflective dots in a similar manner to the IS/OS and showed a FWHM of 4.7 μm (range, 4.6–5.9 μm). Hyperreflective IS/OSd and dots of the COST (COSTd) were distinguishably separated from neighboring dots (as seen by the gaps between dots in Figs. 2C, 2E). An individual IS/OSd was usually aligned exactly above an individual COSTd (examples are shown by blue rectangles in Fig. 2C), indicating that both dots originated from the same PR. Paired dots showed slight variation in size and brightness in B-scans (compare the dots of the IS/OS and COST in Fig. 2C). Dots located between the IS/OS and COST, probably indicating irregular disc arrangement or defects within the OS, &35; were found in every eye. While IS/OSd showed less variety in their vertical alignment, occasionally some COSTd would reach distinctly deeper
than others (examples are shown by the yellow rectangle in Fig. 2C).

Regarding PR rods, the ROST were not clearly distinguishable as a separate band in single AO-OCT B-scans in over two-thirds of the eyes, but several oval-shaped, reflective structures could be distinguished positioned in between and underneath the COSTd in all volumes (marked by pink squares in Fig. 2C). In 23% of volumes a separate band in between the COST and RPE-Bruch’s membrane (BM) complex was implied in single AO-OCT B-scans (see the green line Fig. 1A), which became clearly visible if several B-scans were averaged (as shown in Fig. 1B). The RPE-BM complex was present as a thick layer in all eyes and showed a dense structure but not a distinctive pattern as compared with the IS/OS and COST (compare the different layers in Fig. 2C).

The Outer Retinal Layers in AO-OCT En Face Images. The following en face images were created from the data sets: a depth-integrated en face image, including the signals from the ELM to COST layer (“full en face image”), segmented en face images of the different layers (ELM, IS/OS, COST, ROST, and RPE-BM complex), and false-color composite images of the IS/OS and COST and ROST and COST (an overview of the different types of en face images is given in Fig. 3).

The full en face images showed a regular mosaic of reflective dots in healthy individuals (see Fig. 3A). Analogous to the dots seen in B-scans (Fig. 2C), the dots in “full en face” view showed slight variations in size and brightness and formed a dense pattern.

The ELM layer showed very weak and sparse signals in all volumes that did not form a distinct pattern. The segmented IS/OS and COST layers both presented with regular, bright dot patterns, although the pattern of the COST layer appeared generally finer and better demarcated than the IS/OS (compare the patterns in Figs. 3B, 3C). Both, the IS/OS and COST patterns showed no loss of signals in healthy eyes and were mainly regular (see Table 2). Mean IS/OS density at 2.5° super peripheral eccentricity from the fovea (sec) was 20,735/mm² (SD 2450, min 16,600, max 25,100). At 6.5° sec the IS/OS density decreased to 16,035/mm² (SD 1840, min 13,400, max 22,000). Mean COST density was slightly lower at 2.5° sec the density was 20,365/mm² (SD 2440, min 16,400, max 24,900, signed-rank test P < 0.001) and at 6.5° sec the density was 15,690/mm² (SD 1870, min 13,000, max 21,500, P < 0.001).

The false-color composite images of the IS/OS and of the COST layer were used to analyze the overlap of PR signals. Qualitative assessment of these composite images revealed that IS/OSd and COSTd overlapped in many but not all dots (compare the magenta dots as a sign of signal overlap with the blue and red dots in Fig. 3F). Regarding the analysis of rods, visualization of the segmented ROST layer was more challenging due to its proximity to the COST and RPE and only possible in 14 of 30 eyes. The ROST images revealed a branched network of small, reflective signals forming ring-like patterns (Fig. 3D), but individual rod signals could not be clearly resolved. The false-color composite images of the COST and ROST showed that COSTd were exclusively fitting into the “gaps,” which were formed by the ring-like patterns of the ROST (seen by the red COSTd centered within the green ROST and barely any yellow signal overlap in the red-green composite images of Figs. 3G, 3H). The RPE-BM complex could be visualized in all eyes and showed a denser and less branched pattern, when compared with ROST signals (compare Figs. 3D, 3E).

Regarding artefacts from retinal vessels, larger veins and arteries caused clearly visible, river-shaped areas of signal loss throughout all outer retinal layers (see Fig. 3), whereas small vessels formed thin lines of signal reduction that were sometimes just hinted.

AO-OCT Assessment of Eyes With Early and Intermediate AMD (n = 13)

The Outer Retinal Bands in AO-OCT B-Scans. RPE irregularities, which were present in all imaged locations (n = 41), altered the appearance of the outer retinal bands compared with healthy eyes (compare Figs. 4I, 4L with 4O, 4P), the bright dots of the IS/OS band were present above small RPE irregularities but became thinned out and less distinctive above drusen (see the yellow arrow in Fig. 4L) or vanished completely (see the IS/OS band in Fig. 4I). The COSTd showed the same alterations. A distinctive COST band was not found in any volume. The RPE-BM complex appeared disturbed above drusen (compare the thinner and weaker RPE signal above the drusen in Figs. 4I and 4L). The distortion of the outer retinal bands in AO-OCT B-scans correlated with SD-OCT B-scans, although the latter showed generally less details as a result of lower resolution and averaging of images (compare the irregular but still continuous IS/OS in Figs. 4G, 4H, 4J, 4K with the IS/OS in Figs. 4I, 4L).

The Outer Retinal Layers in AO-OCT En Face Images. The full en face images showed a mosaic of hyperreflective dots similar to healthy eyes in areas without RPE irregularities whereas drusen were depicted as variously shaped masses with diffuse, dense signal patterns (seen in Fig. 4C and outlined in Figs. 4D-F). The dot mosaic disappeared partially or fully above drusen (compare the distorted patterns in Fig. 4C with the regular pattern in Fig. 4M).

The IS/OS layer could be assessed in all eyes. Distinct IS/OSd were found above areas without drusen, corresponding to the patterns found in full en face images. Regarding the
qualitative grading, the IS/OS patterns in areas in-between drusen were less regular (Fisher’s exact test, $P < 0.001$) and showed more signals loss ($P = 0.002$) compared with healthy eyes at a similar eccentricity (ecc; see Table 2). IS/OSd density of these areas was slightly lower compared with healthy eyes ($13,111 / \text{mm}^2$, SD 600, min 11,300, max 15,000, $t$-test $P = 0.002$). Above drusen, the IS/OS signals were distorted and attenuated, the dot mosaic was partially or even fully missing (shown in Fig. 4D within the orange outlined drusen) and only some IS/OSd (e.g., the yellow and white arrows in Fig. 4D) were found. A small sample of 20 randomly selected drusen from five patients showed an IS/OSd mean density of 2270/ mm$^2$ (SD 1120, min <1000, max 3000; no further analysis performed).

Due to the irregular RPE underneath, the COST and ROST layer could not be separated, hence a layer combining both signals from the end tips of the PR (ETPR) was analyzed. Above drusen, the ETPR en face images showed diffuse dense signals from the protruding RPE instead of the regular patterns of COST and ROST (see the orange outlined drusen in Fig. 4E). In between drusen, COSTd patterns were visible but highly irregular compared with healthy eyes (Table 2, Fisher’s exact test, $P < 0.001$) and showed a lower density of 10,911/ mm$^2$ (SD 2110, min 7700, max 15,300) compared with healthy individuals ($t$-test $P < 0.001$). False-color composite images of the IS/OS and ETPR revealed a patchy, highly uneven signal distribution between the two layers (e.g., shown by the mainly red signals above drusen in Fig. 4F) compared with the equally distributed signal appearance of healthy individuals (compare...
The Outer Retinal Bands in AO-OCT B-Scans. In eyes with advanced AMD, we acquired volumes from zones with different degrees of structural distortion, namely from the atrophic lesions, the border or junctional zone, which is the immediate region before the atrophic border and the surrounding region, which is the region without visible atrophy.

The ELM band was barely visible in single B-scans of the surrounding region, similar to healthy individuals (subtle ELM signals can be seen directly below the first 2 yellow arrowheads in Fig. 6I). These signals became even less distinct above the junctional zone (Fig. 6I, left from the yellow arrow) and some ELM signals are still visible) and fully disappeared above the atrophic lesions (Fig. 6I, right from the yellow arrow).

The IS/OS band was visible in the surrounding region (Fig. 5G), but often distorted (compare the irregular bands in Fig. 5G with healthy bands in Fig. 5L) due to the presence of RPE irregularities similar to eyes with intermediate AMD (a drusen is visible between the first 2 arrowheads in Figs. 6H, 6I). At the junctional zone, the IS/OS band was severely distorted and thinned out throughout all volumes (compare the loss of IS/OS signals between the third yellow arrowhead and the arrow in Fig. 6I with a healthy IS/OS band in Fig. 6L). Above the atrophic lesions, the IS/OS signals were completely gone, except for some singular dots that could be found in some disorganized isles of RPE remnants (such a single dot at height of the IS/OS band can be seen in the yellow square in Fig. 6I).

COST bands were generally even more distorted than IS/OS bands. As in early to intermediate AMD, they disappeared partially or fully above drusen. In the junctional zone, the COST band was usually no longer clearly visible, as the signals from the PR end tips and RPE were thinned out and merged. COST signals were fully absent above the atrophy. As in eyes with less advanced AMD, the ROST band was not detectable but some signals could be occasionally found in areas that were several degrees away from the atrophic lesions. The RPE-BM complex was visible in the surrounding zone but continuously thinned out in the junctional zone (see the gradually fading RPE-BM band in Fig. 6I). In atrophic lesions, only a thin line of highly reflective diffuse signals remained (Fig. 6I, right from the yellow arrow). If drusen were present in eyes with advanced AMD, they showed the same attributes as in eyes with early to intermediate AMD, such as thin and less reflective isles of RPE remnants (such a single dot at height of the IS/OS band can be seen in the yellow square in Fig. 6I).

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The Outer Retinal Layers in AO-OCT En Face Images. The surrounding regions showed remarkably distorted cone patterns even at distances over 3° away from the atrophic border (Fig. 5R). En face images of the IS/OS and COST, which could be analyzed in all 12 eyes, showed thinned out and patchy patterns of IS/OSd and COSTd (compare Figs. 3J–L with 3A–C). Regarding the qualitative grading (see Table 2), the IS/OS patterns of the surrounding regions were more irregular (Fisher’s exact test < 0.001) and showed more signal loss ($P < 0.001$) than healthy eyes. Mean IS/OSd density of surrounding

### Table 2. PR Pattern Grading of Healthy Eyes, Eyes With Early to Intermediate AMD and Advanced AMD

<table>
<thead>
<tr>
<th>Pattern Irregularity</th>
<th>No Pattern</th>
<th>Light Pattern</th>
<th>Medium Pattern</th>
<th>Severe Pattern</th>
<th>Total Signal Loss</th>
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<td>Healthy (n = 30)</td>
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<tr>
<td>IS/OS between 5.0 and 8.0</td>
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<tr>
<td>COST between 5.0 and 8.0</td>
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<tr>
<td>IS/OS junctional zone</td>
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<td>IS/OS and ETPR atrophic lesions</td>
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</table>

The table shows the percentage distribution of signal loss for different pattern irregularity grades in healthy eyes, eyes with early to intermediate AMD, and advanced AMD. The data indicate a higher frequency of severe pattern irregularity and signal loss in advanced AMD compared to healthy and early to intermediate AMD eyes.
regions between 5.0º and 7.0º ecc (n = 7) was 7014/mm² (SD 2530, min 2300, max 9800), which was significantly lower than IS/OSd density between 5.0º and 7.0º in eyes with early to intermediate AMD (t-test P < 0.001) and healthy eyes at 6.5º ecc (P < 0.001). The patterns of the COSTd showed the same changes regarding irregularity and signal loss (Table 2). COSTd density was 6428/mm² (SD 2770, min 1100, max 9600) between 5.0º and 7.0º ecc (n = 7) and significantly lower when compared with healthy eyes (t-test P < 0.001) and those with early to intermediate AMD (P < 0.001). In composite images of the IS/OS and COST, the signals were unequally distributed and there was less overlap of IS/OSd and COSTd (as seen by the color distributions of Fig. 3O compared with 3F).

The ROST layer (visible in 5 eyes) of the surrounding region was thinned out (compare Fig. 3M with 3D) and appeared especially distorted in regions that also showed a hypointense signal in IR fundus images, which was the case in two eyes (Fig. 3, compare the mottled signal appearance in the upper right corner in Figs. 3M and 3P, which correlates to the hypointense IR signal in the upper right corner of the white rectangle in Fig. 3R). Composite images of the COST and ROST revealed signal gaps and enlarged but disfigured PR signals (compare the patterns in Figs. 3G and 3H with 3P and 3Q).

Above drusen, the relation of the ROST signals to the COSTd was disturbed by the RPE, which lead to signal overlap (shown by the yellow masses in Fig. 3Q). Volumes of the junctional zone (n = 8) were characterized by highly attenuated PR signals and a severely irregular RPE (Table 2). As in patients with less advanced AMD, the ROST layer could no longer be clearly separated from the COST signals, thus the ETPR layer as a combination of COST and ROST signals had to be used for analysis. In full en face view, it was challenging to identify the border between the junctional zone and the atrophic lesions, as the IS/OS and ETPR mosaic of the junctional zone were usually severely thinned out and distorted (see the junctional zone in Figs. 6D–F in between the dotted line of the atrophic border and the dashed line of the beginning surrounding region, where the PR mosaic becomes visible again). The junctional zone was also visible in AO-FC as a blur around the atrophic border (marked by white arrows in Fig. 6B).

The atrophic lesion was characterized by a total loss of the PR mosaic in full en face images and showed multiple, bright, small structures instead, which did not form a distinctive pattern (compare the lower right corner in Figs. 6C, 6F and the healthy retina in Figs. 6J, 6K). While most of these hyper-
reflective structures had diffuse shapes, occasionally some reflective dots could be found within the atrophy in AO-FC (orange squares in Fig. 6B) and corresponding AO-OCT full en face images (orange square in Fig. 6C). Although these dots appeared similar to the IS/OS and ETPR signals found above drusen (blue circle and green rectangle Figs. 5D, 5E), additional depth information from AO B-scans and composite images revealed that these dots did not appear at the height of the IS/OS layer or ETPR but were located solely at the height of the degenerated RPE-BM complex (compare the 2 dots within the orange square in Fig. 6C with 6F, where signals originate at the height of the atrophied RPE-BM complex and are colored green).

The segmented IS/OS and ETPR en face images showed a total loss of signal in 80% of images (Table 2) and occasionally some diffuse, unorganized signals of protruding RPE remnants. Thus, no PR density was calculated. Adjacent to lesion borders, mound-like masses were occasionally seen, which formed dune or sandbank-like formations in segmented en face images (marked by the blue arrowheads in Figs. 6D, 6E). Within these masses, some remaining reflective dots could be found, which appeared at the height of the IS/OS layer or ETPR and were similar in diameter and signal intensity to IS/OSd and COSTd (examples are shown in the yellow square in Fig. 6D and the corresponding B-scan in Fig. 6I and the red square in Fig. 6E), indicating the presence of cones with disturbed waveguiding properties. A branched vessel causes signal reduction (white asterisks, see also D, E).

**DISCUSSION**

This study is the largest application of AO-OCT in a clinical setting up to date and provides a novel insight in the changes of the PR mosaic between healthy aged eyes, eyes with early to intermediate AMD, and eyes with advanced nonneovascular AMD. Our results show that distinct changes of PR can be detected between healthy and pathologically aged eyes with
These changes include irregular patterns of the IS/OS and ETPR as well as a reduced density of PR signals. However, slight variations in appearance and density of the IS/OS and COST mosaic were also found between healthy individuals and especially between different eccentricities, which correlates with post mortem tissue examinations.40–42 The counted cone densities at IS/OS and COST at 2.5° and 6.5° eccentricity were in agreement with AO-FC/SLO examinations of healthy eyes although these modalities are incapable of separating IS/OS from COST signals and show a mixture of signals from all outer retinal layers.43–45 The oscillation of IS/OS and COST signals, which were especially visible in our IS/OS+false-color composite images, were also found with other AO-OCT systems and are most likely a sign of PR segment renewal.38,39,46 Apart from morphologic factors, technical aspects, such as the wavelength or the type of light source, could contribute to this variation in reflectance properties.47 Regarding the depiction of rods, which are known to encircle and outnumber cones, the ROST signals found in our AO-OCT B- and en face scans are in agreement with histologic sections and a small AO-OCT assessment on young adults.23,40,41,48

In patients with early to intermediate AMD, the reduced IS/OS and ETPR density and the distorted signal patterns, which were present in areas around and especially above drusen, may be explained by waveguiding properties of PR segments. Cones and rods waveguide light like a fiber and cause reflections due to changes in the refractive index and possible backscatter of RPE processes.49,50 The light-coupling efficiency of the tightly packed PR depends strongly on the direction of incident light and the alignment of their outer receptor segments, which is usually perpendicular to the outer shell of the eyeball and directed toward the center of the pupil, contributing to the Stiles-Crawford effect.51–53 Misalignments of the receptor segments prevents coupling of incident light, causing a reduced or lost IS/OS or COST/ETPR signal. In stained histologic sections, it has been shown that drusen and subretinal drusenoid deposits compress PR segments, causing shortening and deflection.54,55 These morphologic changes may be responsible for the sharply demarcated areas of attenuated and distorted IS/OS signals and the loss of the PR mosaic in our IS/OS en face images. In contrast to the signal attenuation in the IS/OS images, the ETPR layer showed diffuse but dense signal accumulations above drusen, which originat-
ed from the elevated RPE as seen in the corresponding AO-OCT B-scans. Above the elevated RPE, distorted IS/OS or COST signals could be found, indicating that cones are still present above drusen. The severely irregular cone mosaic above drusen is therefore not necessarily a result of PR destruction but rather implicates a different orientation of receptor segments, which prevents efficient light coupling into the PR. This concept is supported by functional measurements that show an influence of drusen and subretinal drusenoid deposits on PR-mediated neuroretinal function.56–58 The loss of the PR mosaic above the dense RPE signals of drusen frequently appears like a halo in full en face view, which has also been described for subretinal drusenoid deposits in AO-SLO and AO-FC.58,59 The presence of the halo-like signal attenuation varied between drusen, which can be explained by the varying degree of mechanical PR distortion.

In advanced AMD, several regions with varying degree of IS/OS and ETPR signal distortion were depicted by AO-OCT, namely the regions surrounding the atrophy, the junctional region adjacent to the atrophic lesions and the atrophic lesion itself. Such regions have also been described in histopathological sections of patients with outer retinal atrophy as follows: a “zone of moderate degeneration,” which shows densely packed but irregular shaped RPE cells is adjacent to a “zone of severe degeneration,” which shows conflating subretinal deposits and extremely attenuated RPE and PR and is adjacent to a “junctional zone” with only a few surviving, “grossly abnormal photoreceptors” and shortened inner and fragmental outer PR segments.60 In the junctional zone, all AO-OCT images showed massive disfigurement of the RPE-BM band and extensive loss of RP signals. In contrast to attenuated and distorted photoreceptor patterns above drusen or RPE irregularities, where cones are present but do not waveguide properly, the atrophic lesions itself presented with a subtotal to total loss of signals at the level of IS/OS, COST, and ROST as a result of photoreceptor atrophy. At height of the BM however, the atrophic lesions showed dense, diffuse signals. Depth encoding of AO-OCT was particularly important when studying atrophic areas, as the hyperreflective dots at the height of RPE-BM may be misinterpreted as PR signals on AO-FC images. These dots within the atrophy are likely of phagocytic origin or pigment-containing membrane bodies.61–63 The reflective, mold-like masses of RPE remnants close to the atrophic border, potentially containing cone fragments, have also been described in histologic samples.60

There are several limitations of our study. Despite rigorous exclusion criteria for the imaged subjects, 35% of all acquired volumes could not be used for image analysis due to motion artefacts, low signal-to-noise ratio, or inadequate focusing in the outer retinal layers during volume acquisition. Motion artefacts appeared to be the main issue for excluding recorded images, data despite the fast volume acquisition times (compared with commercial systems) of our system. Over two-thirds of the motion artefacts appeared in patients with advanced AMD. To reduce such artefacts, an eye tracking system can be implemented, which, however, is a technically difficult task due to a required tracking accuracy in the order of the size of a single cell and has not yet been demonstrated with sufficient performance for AO-OCT. Alternatively, faster A-scan rates can be used to reduce acquisition time, which has the downside of lower system sensitivity. The reason for the low-signal quality of the photoreceptor band in three eyes with good RMS values was a wrongly adjusted focus during image acquisition. This issue can be avoided by providing a better real time view of the recorded image data. Currently, image processing is performed on the central processing unit (CPU), which has a limited evaluation speed and only a reduced number of A-scans within the B-scan can be streamed on the live display. Thus, the perfect location of the focus is difficult to be determined in some cases even if the operator is sufficiently trained. To overcome this issue, data evaluation using the graphic processing unit (GPU) that provides massive parallel evaluation can be implemented, which would significantly reduce the amount of not usable data, as only volumes with sufficient quality would be saved.

Regarding the resolution of our system, cones in the central 1° and individual rods cannot be resolved. This is a result of the system design that is optimized for pupil diameters of 5 mm and represents a compromise between achievable resolution and applicability to a wider range of patients, as a higher resolution requires a system that is optimized to pupil diameters of over 7 mm, which cannot be achieved in all patients.

In conclusion, our results show that three-dimensional visualization of PR with AO-OCT is feasible in healthy elderly individuals as well as in patients with AMD and that the obtained images are congruent with histopathological reports, thus showing the potential of AO-OCT to fill the gap between conventional OCT and histologic examination of the retina in the future.

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