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Detailed Terms
Polarization sensitive optical coherence tomography of melanin provides tissue inherent contrast based on depolarization

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ABSTRACT

Polarization sensitive optical coherence tomography (PS-OCT) was used to investigate the polarization properties of melanin. Measurements in samples with varying melanin concentrations revealed polarization scrambling, i.e. depolarization. The results indicate that the depolarizing appearance of pigmented structures like, for instance, the retinal pigment epithelium (RPE) is likely to be caused by the melanin granules contained in these cells.

Keywords: Optical coherence tomography, polarization sensitive devices, imaging, melanin, retinal pigment epithelium, depolarization, polarization scrambling

1. INTRODUCTION

Polarization sensitive (PS) optical coherence tomography (OCT) provides depth-resolved imaging of light polarizing properties of transparent and translucent samples with micrometer scale resolution.¹ Other than standard, intensity-based OCT, which provides image contrast based on the amount of light backscattered from different sites inside the sample, PS-OCT opens an additional contrast channel: By illuminating the sample with light in a well-defined polarization state and by using a polarization sensitive detection unit, not only reflectivity but also the polarization state of light can be assessed three-dimensionally. From the detected polarization state, phase retardation, optic axis orientation as well as Stokes vectors can be calculated.¹⁻⁴

In the human eye, PS-OCT imaging allows to distinguish structures with birefringent (e.g., cornea, retinal nerve fiber layer), polarization preserving (e.g., stroma of the iris, photoreceptor layer), and depolarizing (e.g., pigment epithelia of iris and retina) properties.³⁻⁵ It was shown that the unique depolarizing characteristics of the retinal pigment epithelium (RPE) can be used for segmenting that layer.⁸ In diseased retinas, this enables to assess retinal disorders – such as fluid volumes in exudative age-related macular degeneration (AMD) and the areas of atrophic zones in dry AMD – in a quantitative way.⁹

Recently, we investigated the depolarizing character of the RPE in the macula region of healthy volunteers.¹⁰ The RPE’s depolarization appeared most pronounced close to the center of the fovea and decreased in the periphery (Fig. 1). However, the origin of the polarization scrambling was still unclear and could be caused by the outer morphology of the RPE cells or by their structural composition. In addition to regular cell organelles like mitochondria or cell nuclei, the human RPE cells contain different pigments in granular shape: melanin, lipofuscin, and composites of both.¹¹ While the lipofuscin concentration has a minimum in the fovea and increases in the periphery of the posterior pole, the concentration of melanin shows the inverse characteristic with a peak concentration in the fovea (Fig. 2).¹²⁻¹⁴ Since the
macular distribution of melanin corresponds to the depolarization pattern measured with PS-OCT, one could suggest that melanin could be the origin of the RPE’s polarization scrambling property. In order to verify this hypothesis, measurements in phantoms are necessary.

Fig. 1. Macular depolarization pattern. (A) For the left eyes of 10 healthy volunteers, the minimum degree of polarization uniformity (DOPU) value was plotted for each depth profile. En face maps showing depolarization were generated by plotting these values DOPUmin for each transverse position of the scanned macular regions. A map showing the average macular DOPUmin distribution of the 10 eyes is shown in (A). A decrease of the RPE’s depolarizing character in the periphery can be observed. The polarization scrambling character is most pronounced close to the center of the fovea, which manifests in the lowest DOPUmin values there. (B) Horizontal DOPUmin profiles extracted from the macular depolarization maps are shown along with the mean profile (black line). The grey band indicates the range of ±1 standard deviation around the mean DOPUmin value. Like in the en face map of (A), also in the horizontal profiles a minimum of DOPUmin, i.e. most pronounced depolarization, is visible in the center of the fovea. A more detailed discussion of the depolarization patterns can be found in ref. [10]. (color online)

Fig. 2. Distribution of RPE pigments after ref. [13]. While lipofuscin shows a local minimum in the central fovea, the melanin concentration has a maximum there. (color online)
2. MATERIALS AND METHODS

Here, we present the results of PS-OCT measurements in melanin models. From raw eumelanin (Sigma Aldrich), ellipsoid melanin particles were produced by dissolving and re-precipitating. A narrow size distribution of the melanin particles with a maximum at ~530 nm was observed by dynamic light scattering (DLS) measurements (which corresponds well to the size of melanin granules in the human RPE cells reported in literature). Scanning electron micrographs of the powder revealed particles in the respective sizes and shapes (cf. Fig. 3). Using distilled water, solutions with melanin concentrations \( C_{\text{mel}} = V_{\text{mel}}:V_{\text{H}_2\text{O}} \) in the range from 1:1 to 1:100 were prepared. The solutions were treated with ultrasound for 30 s in order to prevent from and to break up particle agglomerates.

PS-OCT imaging was performed using our recently reported clinical PS-OCT instrument. In brief, we used a system design based on a free-space Michelson interferometer with additional polarization optics. A broadband superluminescent diode (center wavelength: 839 nm, FWHM bandwidth: 58 nm) was employed as a light source. Circularly polarized light was used to illuminate the sample. At the interferometer exit, the OCT signal was split into two orthogonal polarization components, which were detected by two identical spectrometer units. Each readout of the spectrometer linescan cameras yielded the spectral data necessary to compute not only depth-resolved reflectivity, but also phase retardation, optic axis orientation and Stokes vectors for one depth profile.

Using this PS-OCT system, 3D data sets (64×1024×1024 voxels, 12×12×3.3 mm³) of melanin solutions were recorded. The liquid solutions were imaged inside a cylindrical glass container. Evaluation of a PS-OCT data set recorded in the empty container confirmed the polarization preserving characteristics of the container material.

3. RESULTS AND DISCUSSION

Fig. 4 shows results of PS-OCT measurements of solutions with melanin concentrations of 1:3 and 1:60. Reflectivity B-scan images are shown on the left hand side (Figs. 4(A) and (B)). In order to evaluate polarization scrambling, degree of polarization uniformity (DOPU) B-scan images were computed. For that purpose, the Stokes vector elements \((I, Q, U, V)\) were calculated from the amplitudes and phases of the two polarization channels’ OCT signals for each image pixel. The Stokes vector elements were then normalized and averaged in a sliding evaluation window. DOPU(\(z\)) was then computed as

\[
\text{DOPU}(z) = \sqrt{\frac{Q^2}{Q_{\text{mean}}^2}(z) + U^2}{U_{\text{mean}}^2}(z) + V^2}{V_{\text{mean}}^2}(z)\]

For polarization preserving and birefringent structures, DOPU values close to 1 will be observed, whereas polarization scrambling will result in lower DOPU values.

In Figs. 4(C) and (D), DOPU B-scans are shown for the concentrations of 1:3 and 1:60. Clearly, a polarization scrambling characteristic can be observed, which manifests in low DOPU values for both concentrations. Also, polarization scrambling appears more pronounced in the solution with the higher melanin concentration (Fig. 4(C)), where the DOPU images are mostly bluish whereas the mostly green color in Fig. 4(D) indicates less polarization scrambling for the lower concentration.

The polarization scrambling characteristic of melanin was investigated for concentrations from \( C_{\text{mel}} = 1:1 \) to \( C_{\text{mel}} = 1:100 \) by evaluating the distribution of DOPU values. The average DOPU value in the respective solutions is plotted in Fig. 5 as a function of melanin concentration. The polarization scrambling character is decreasing as the concentration of melanin granules is decreasing.

The depolarizing characteristics observed in the melanin samples are consistent with the polarization scrambling character of pigmented ocular structures. Therefore, the polarization scrambling appearance of the RPE (and that of the iris pigment epithelium) is likely to be caused by the melanin granules.

4. CONCLUSION

In this study, we investigated the polarization properties of melanin samples. Ovoid melanin particles were produced and solutions of concentrations in the range from 1:1 to 1:100 were prepared. PS-OCT data sets were recorded in these...
solutions. In order to investigate the polarization scrambling characteristics of the acquired PS-OCT data sets, an evaluation of DOPU values was performed. Depolarization (manifesting in low DOPU values) was more pronounced for higher concentrations of melanin, and decreased for lower melanin concentrations. The polarization scrambling character of the melanin solutions was in analogy to that of pigmented ocular structures like the RPE, which contain melanin granules of similar size and shape. Therefore, it is likely that the depolarizing appearance of the pigment epithelia of iris and retina be caused by the melanin granules contained within.

**Fig. 3.** Melanin samples. (A) Size distribution as measured using DLS. (B) Melanin solution. (C) – (F) Scanning electron micrographs of melanin sample granules at magnifications between 10,000× and 40,000×. (color online)
Fig. 4. PS-OCT B-scan images of melanin samples. (A) – (B) Reflectivity images for melanin concentrations of 1:3 and 1:60, respectively. (C) – (D) DOPU images showing increasing average DOPU for decreasing melanin concentration. (color online)

Fig. 5. DOPU vs. melanin concentration. For melanin solutions ranging from 1:1 to 1:100, average DOPU values measured within the melanin solutions are plotted. Clearly, a trend from low to high DOPU values can be observed when the concentration is decreasing. (color online)
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