Supplementary Material

In the following sections complementary details on the in-house built polarization sensitive OCT system and its improved post-processing of the acquired data specifically designed for this study, are described.

Overlay of the depth multiplexed polarization state images

In order to determine the birefringence parameters of a sample, the sample is probed with two orthogonal polarization states and in the detection the signal is split into two orthogonal detection channels. The different input states are created in a polarization delay unit where, after being separated by a polarization beam splitter, the states are delayed in time before being sent to the sample (the retina). The time delay between the different input polarization states creates a depth displacement between their images. During the PS-OCT data processing this displacement needs to be determined and compensated. The depth displacement calculation was done by computing the autocorrelation from the Fourier transform of the squared real valued OCT signal in spectral domain: $AC = FFT\{S(k)^2\}$. The autocorrelation was averaged over a full B-scan yielding a clear peak representing the displacement between the input polarization states. Sub-pixel accuracy was achieved by computing the center of mass of the autocorrelation peak p. To compensate this depth delay, the image of the delayed polarization state was shifted to the same depth position as the image from the non-delayed polarization state by applying a complex phase ramp to the spectral domain signal: $S(k) \cdot \exp[i2\pi \cdot p/n]$ with n the index number of the wavenumber samples.

PS-OCT post-processing improvements

The correction of system polarization distortions including PMD of the PS-OCT measurements has been described previously[1]. Here improvements for an automated judgement on the quality of the correction functions are described for a more robust use in clinical practice. Correction functions were determined across 15 spectral bins. Automated feedback of the correction was used to help the user choose an optimal B-scan from which the corrections could be computed and was based on criteria described subsequently. In order to eliminate polarization distortions in the optical path from the retina to the detector, the surface polarization state at the vitreous humor-retina interface must be determined. It has been found that the detection of the interface is more robust if the degree of polarization uniformity (DOPU)[2] from uncorrected data is used in comparison to intensity images. In addition, areas where the signal is weak (e.g. by beam clipping) were excluded from surface state detection through thresholding of the skewness of the distribution of intensity data of the A-lines. The skewness was found to be a good indicator whether a signal was recorded which can be discriminated from the noise floor. Next, a sufficient amount of pixels with significant phase retardation (>0.1 rad) is necessary to build up statistical ensembles to determine the correction functions for the remaining system distortions. It has been found empirically that pixel numbers above 20 000 suffice and thus, neighboring B-scans are included in the ensemble if less pixels are counted in a single B-scan. The correction functions are extracted from the eigenvector elements for each pixel which can be described as[1]:

$$v(k) = \begin{bmatrix} v_{11} & v_{12} \\ v_{21} & v_{22} \end{bmatrix} = \begin{bmatrix} \alpha(k) \cdot e^{i(k\gamma' + \rho(k))} & 0 \\ 0 & 1 \end{bmatrix}^{-1} \begin{bmatrix} x(k) & -y(k)^* \\ y(k) & x(k)^* \end{bmatrix} \begin{bmatrix} s_1 & 0 \\ 0 & s_2 \end{bmatrix} \tag{1}$$

where $\alpha(k)$ is the amplitude ratio between the two input states, k the wavenumber, γ' the residual displacement after overlap of the depth-multiplexed images, $\rho(k)$ the phase difference between vertical and horizontal field components before the PDU, $\left[x(k), -y(k)^*; y(k), x(k)^*\right]$ is a Unitarian matrix including the incoming path and a standard rotation matrix, and s_1 and s_2 are complex scalars needed for eigenvector normalization. Equation (1) was used to determine $\alpha(k)$ and the amplitudes of $s_1, s_2, x(k)$ and $y(k)$ using histograms of the amplitudes of the eigenvector components under the assumption that each is represented by a monomodal distribution where the maximum reflects the expectation value. Depending on the selected B-scan it can deviate from this ideal case, e.g. if the relative optical axis orientations are diverse and induce multimodal distributions. This effect is checked by a fit of a Gaussian distribution to the histograms and the R^2

values are computed for those fits and displayed to the user. It was found empirically that R^2 values above 0.95 indicated that the histograms from the selected B-scan(s) could be considered monomodal distributions and that they were suitable for a robust determination of the correction parameters. In addition the phase functions $\varphi_v(k) = \arg(x(k) \cdot y(k)^* \cdot e^{-i(k\gamma' + \rho(k))})$ and $\varphi_{XY}(k) = \arg(x(k) \cdot y(k))$ were determined. For physically meaningful results those functions should only show low to moderate oscillations across the spectrum, which was checked with the tortuosity parameter $T = L / C$ with the length L of the curve and the distance C between first and last point. If T was below a value of 2 the correction functions were found to be reliable. These criteria were used to choose a suitable B-scan for the calibration of the polarization distortion compensation which was then applied to all B-scans in a full volume.

Visualization of local birefringence

The visualization of local birefringence was implemented according to Villiger *et al.*[3] in which the original measurements in the Jones space are converted to Mueller space. In this method spatial averaging (3 in depth by 7 lateral pixels $\triangleq$ 16.2 µm by 21 µm) of the Mueller matrices was used and birefringent parameters were extracted from the differential Mueller matrices over one pixel in depth (5.4 µm in air) and converted to a birefringence vector. This vector was averaged with a second spatial filter of 10 by 20 pixels. For OAxU[4, 5] calculation a two-dimensional kernel of 10 by 20 pixels was used.

References

1. Braaf B, Vermeer KA, de Groot M, Vienola KV, de Boer JF. Fiber-based polarization-sensitive OCT of the human retina with correction of system polarization distortions. *Biomed Opt Express* 2014;5:2736-2758.
2. Gotzinger E, Pircher M, Geitzenauer W, et al. Retinal pigment epithelium segmentation by polarization sensitive optical coherence tomography. *Opt Express* 2008;16:16410-16422.
3. Villiger M, Lorenser D, McLaughlin RA, et al. Deep tissue volume imaging of birefringence through fibre-optic needle probes for the delineation of breast tumour. *Sci Rep-Uk* 2016;6.
4. Willemse J, Gräfe MGO, van de Kreeke JA, Verbraak FD, De Boer JF. Optic axis uniformity as a metric to improve the contrast of birefringent structures and analyze the retinal nerve fiber layer. *in preparation*.
5. Feroldi F, Willemse J, Davidoiu V, et al. In vivo multifunctional optical coherence tomography at the periphery of the lungsIn vivo multifunctional optical coherence tomography at the periphery of the lungs. *Biomed Opt Express* accepted.