High speed spectral domain polarization sensitive optical coherence tomography of the human retina

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Abstract

We developed a high-speed polarization sensitive optical coherence tomography (PS-OCT) system for retinal imaging based on spectral domain OCT. The system uses two spectrometers, one for each polarization channel, that operate in parallel at 20000 A-lines/s each. It provides reflectivity, retardation, and cumulative optic axis orientation simultaneously. We present our instrument and discuss the requirements for the alignment of the two spectrometers specific for our setup. We show 2D spectral domain PS-OCT images and – to the best of our knowledge – the first 3D spectral domain PS-OCT data sets in form of fly-through movies and volume rendered data sets recorded in human retina in vivo.

1. Introduction

Optical coherence tomography (OCT) is a noninvasive imaging technique that generates high-resolution cross-sectional images of transparent and translucent samples [1,2,3]. Conventional OCT measures spatially resolved backscattered intensity with a resolution on the order of a few μm. The development of polarization sensitive (PS) OCT takes advantage of the additional polarization information carried by the reflected light [4,5]. Thereby, PS-OCT can reveal important information about biological tissue that is unavailable in conventional OCT. Tissue can change the polarization state of light by several mechanisms that have already been studied by PS-OCT: by birefringence [5,6,7], by diattenuation [8,9,10], and by polarization scrambling [11,12]. Based on these mechanisms, several possible applications of PS-OCT to medical diagnostics have been suggested, e.g., birefringence measurements can be useful for burn depth estimation in skin [13], caries diagnostics [7], glaucoma [14] and keratoconus [15] diagnostics in ophthalmology, while measurement of polarization scrambling has recently been suggested for diagnosing the retinal pigment epithelium (RPE) in age related macula degeneration (AMD) [16].

While early work on PS-OCT [4,5] measured only reflectivity and retardation of a sample, in recent years many proposals have been made to extract more information. These improved techniques require phase sensitive recording of interferometric signals and were used to measure and image Stokes vectors of the backscattered light [17], Müller [18] and Jones matrix [19] distribution, fast axis orientation [17,20], and diattenuation [8,9,10]. Parameters with straightforward physical interpretation are retardation, diattenuation, and orientation of the fast birefringent axis of a sample. These parameters are inherently contained in Müller and Jones matrices, so a possible way of accessing them would be to...
acquire either of these matrices and derive these parameters from the matrix elements. If
diattenuation is neglected – it is very low in most biological tissues [8,10] – the three most
important parameters: reflectivity, retardation, and axis orientation can also be determined
by other methods based on the PS low coherence interferometry setup first devised by Hee
et al. [4] in combination with a phase sensitive recording of the interferometric signals in the
two orthogonal polarization channels [17,20]. These methods have the advantage of probing
the sample with only a single input polarization state, thus requiring only a single
measurement per sample location. We demonstrated the latter method [20] successfully in
skin [11], cornea [21], anterior chamber [22] and retina [12].

While the original OCT technique was based on mechanically scanning a reference mirror to
perform so called A-scans in time domain (TD), spectral domain (SD) OCT [23] has gained
considerable interest since it has been shown that SD OCT has huge advantages in terms of
sensitivity and acquisition speed [24,25,26]. Most of the SD OCT work reported so far was
limited to intensity based imaging, with some applications also demonstrating flow imaging
[27,28]. Implementation of polarization sensitivity to SD OCT was demonstrated previously
with a slow scan system [29], while a very recent paper demonstrated high speed scanning at
a wavelength of 1300 nm [30].

Retinal imaging is one of the most promising applications of PS-OCT [14,12]. Several
retinal structures can change the light’s polarization state: in the retina, the nerve fiber layer
[31] and Henle’s fiber layer [32] are birefringent which is of great importance for glaucoma
diagnostics [33] and anterior segment compensation in scanning laser polarimetry [34].
Furthermore, a polarization scrambling layer is located near the RPE which might be useful
for AMD diagnostics [16,12]. While most of the work on retinal PS-OCT has been
performed with time domain systems, a first conference report on SD PS-OCT of the retina
has been presented recently [35]. More information on this work can be found in ref. [36].

It is the purpose of this work to implement polarization sensitivity into SD OCT and to
develop an instrument that simultaneously provides intensity, retardation, and axis
orientation images at a state of the art scan rate of 20000 A-scans/s. We present the
instrument and demonstrate 2D and 3D data sets recorded in human retina in vivo.

2. Methods

PS-OCT and SD-OCT have been extensively reported in previous literature. Therefore, this
chapter provides only a short overview of the methods on which our system is based, and of
how these techniques are combined (chapter 2.1). We then provide a detailed description of
the experimental setup (chapter 2.2) and discuss specific alignment problems of our
implementation of SD PS-OCT (chapter 2.3).

2.1 Polarization sensitive spectral domain OCT

Theory and experimental implementation of the time domain version of our PS-OCT
technique have been reported in previous papers [12,20,21,22]. In short, the method is based
on the initial configuration devised by Hee et al. [4] that illuminates the sample with
circularly polarized light, augmented by a phase sensitive detection of the interferometric
signals in the two channels of a polarization sensitive detection unit [20]. In this way,
analytic signals are obtained in both, the horizontal (H) and the vertical (V) polarization
channels:

\[ \tilde{A}_{H,V}(z) = A_{H,V}(z) \exp[i\Phi_{H,V}(z)] \]  (1)

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where z is the depth coordinate, A is the amplitude of the interference signal (proportional to the intensity measured by a photodetector), and \( \Phi \) is the phase of the signal. From these analytic signals the three quantities: reflectivity \( R \), (single pass) retardation \( \delta \), and (cumulative) fast axis orientation \( \Theta \) can be obtained:

\[
R(z) \propto |A_n(z)|^2 + |A_r(z)|^2 \quad (2)
\]

\[
\delta(z) = \arctan \left( \frac{A_r(z)}{A_n(z)} \right) \quad (3)
\]

\[
\Theta = \frac{180^\circ - \Delta \Phi}{2} \quad (4)
\]

with \( \Delta \Phi = \Phi_V - \Phi_H \). The unambiguous measurement ranges are 90° for \( \delta \) and 180° for \( \Theta \).

The principle of SD-OCT is based on the fact that the spectral amplitude of the backscattered wave equals the Fourier transform of the spatial distribution of the object scattering potential [3,23]. An inverse Fourier transform of the spectral amplitude would therefore directly provide the object structure. Since amplitude data are not directly accessible, an inverse Fourier transform of the spectral intensity \( I(k) \) (wavenumber k) of the interfering beams is usually taken in SD-OCT. This yields the autocorrelation of the object structure instead of the true object structure [23]. If the reference mirror is placed at a distance well away from the sample interfaces (i.e. reference arm several coherence lengths shorter or longer than sample arm), DC terms, autocorrelation terms, structure terms and their mirror terms will be well separated and the structure term can be directly extracted from the total signal. This works well in cases where the measured object is thinner than 1 – 2 mm, as in the case of retinal imaging discussed in this paper. Therefore, we omit the DC, autocorrelation, and mirror terms in the following discussion, and obtain the structure term \( \Gamma \) directly from the inverse Fourier transform of the measured spectral intensity \( I(k) \):

\[
FT^{-1} \{ I(k) \} \rightarrow \Gamma(z - \Delta z) = A(z - \Delta z) \exp[i\Phi(z - \Delta z)], \quad (5)
\]

where \( \Delta z \) is the (optical) path length difference between reference and sample beams. For polarization sensitive SD-OCT, we need two spectrometers in a polarization sensitive detection unit, one recording the horizontally and one the vertically polarized spectral interferograms \( I_H(k) \) and \( I_V(k) \). Since the Fourier transform of the real-valued intensity data provides directly a complex signal (cf. eq. 5), we have the amplitude and phase information required to calculate \( R, \delta, \) and \( \Theta \) (eqs. 2-4) directly at hand.

### 2.2 Experimental setup

Figure 1 shows a sketch of the optical setup. It is based on the original polarization sensitive low coherence interferometer first described by Hee et al. [4]. Light emitted from a SLD with a center wavelength at 840 nm and a bandwidth of 50 nm illuminates, after being vertically polarized, a Michelson interferometer, where it is split by a non polarizing beam splitter (NPBS) into a sample and a reference beam. The reference light transmits a variable density filter, a glass plate for dispersion compensation, a quarter wave plate (QWP) oriented at 22.5°, and is reflected by a mirror. After double passage of the QWP, the orientation of the polarization plane is at 45° to the horizontal, providing equal reference power in both channels of the polarization sensitive detection unit. The sample beam passes a QWP oriented at 45°, which provides circularly polarized light to the sample. The scanning device consists of a x-y galvanometer scanner, and via a telescope (consisting of
two lenses, the first with a focal length of 30 mm and the second with a focal length of 50 mm) the pivot point of the scanning device is imaged into the pupil plane of the eye.

After recombination of the two beams at the NPBS, light is directed towards a polarization sensitive detection unit, where it is split into orthogonal polarization states by a polarizing beam splitter. The two orthogonally polarized beam components are coupled into two polarization maintaining fibers (PMFs) and directed towards two separate spectrometers which are designed identically and consist of similar components, therefore only one spectrometer is described. (We preferred a setup with two separate spectrometers and external polarizing beam splitter over an approach where a single spectrometer with internal polarizing beam splitter is used to avoid possible aberation related focusing problems that might occur with the large-area beam splitter cubes necessary in the latter case). The birefringent axis of the PMF was aligned parallel to the polarization state of the incoming beam to excite only one polarization mode in the PMF, thus propagating linear polarized light to the spectrometer, which is necessary to avoid polarization mode dispersion and to maximize the diffraction efficiency of the reflection grating. The light emitted from the fiber tip was collimated by a fiber coupler with a focal length of 50 mm. In front of the spectrometer a half wave plate was added and oriented to optimize the polarization angle of the light beam entering the spectrometer with respect to the diffraction grating orientation for maximum diffraction efficiency.

The spectrometer consists of a reflection grating (1200 lines/mm), a camera lens with a focal length of 200 mm, and a 2048 element line scan CCD camera with a pixel size of 14 × 14 \( \mu \text{m}^2 \) (Atmel Avivia M2 CL 2014). The maximum line rate of the camera is 29 kHz, and via camera link and a high speed frame grabber board (PCI 1428 National Instruments) data could be transferred continuously to a personal computer. The resolution of the camera is 12 bit per pixel. The sensitivity of our system was 98 dB with an integration time of 50 \( \mu \text{s} \) and a power of 700 \( \mu \text{W} \) onto the sample. The sensitivity decay, due to the finite spectrometer resolution, was 14 dB (equal in both channels) along the measurement range of 3 mm. The signal-to-noise-ratio SNR within retinal intensity tomograms (defined as the ratio between the strongest signal obtained from the tissue and the mean noise) was ~ 40 dB.

Our system was operated at an A-scan rate of 20k lines/sec, and each B-scan consists of 1000 A-lines. For alignment purposes necessary for in vivo measurements, a real time display of a reduced dataset of 2k A-scans/sec was implemented. To reduce the amount of data, only 1024 pixels of each camera where read out. After data collection the following data processing steps were performed: At first fixed pattern noise, originating from the camera readout, was eliminated. This procedure consists of subtracting a mean spectrum (averaged over 1000 A-scans) from each spectral data set, inverse Fourier transforming the data set, removing two remaining sharp frequencies generated by the camera, and Fourier transforming the data back to obtain a spectrum free of fixed pattern noise. Afterwards zero padding was performed, the data were rescaled into k-space, and the inverse FFT of both signals was calculated. From these two signals, reflectivity, retardation, and fast axis orientation were calculated by equations 2-4.

2.3 Requirements to spectrometer alignment

As mentioned above, SD PS-OCT requires two identical spectrometers to record the horizontally and vertically polarized spectral interferograms. It is important to carefully align the two spectrometers with respect to each other. In a setup based on Stokes vector measurements, it has been shown that a slight translational misalignment of the two spectrometer cameras along the length of the CCD array can cause image artifacts that mimic retardation which cannot be distinguished from real retardation caused by sample birefringence [30]. Since our setup is somewhat different, we discuss the effects of
misalignment based on the Fourier shift and similarity theorems [37]. We distinguish two types of misalignment: translational shifts and tilts.

Ideally, there should be a pixel-to-pixel correspondence between the CCD cameras of the horizontal and vertical channel, i.e., a certain wavenumber \( k_n \) should be imaged onto the \( n \)-th pixel of the horizontal and vertical channel CCD camera. A translational shift of, e.g., the vertical channel CCD camera with respect to the other along the length of the array causes wavenumber \( k_n \) to be imaged on the \((n+\delta n)\)-th pixel of this camera, i.e., there will be a shift of one spectrum with respect to the other by an amount \( \delta k \). According to the Fourier shift theorem, this causes a phase change in the A-line signal:

\[
FT^{-1} \{ I(k - \delta k) \} \equiv \exp(i2\pi \delta k z) FT^{-1} \{ I(k) \} \rightarrow \exp(i2\pi \delta k z) \Gamma(z - \Delta z). \tag{6}
\]

The amount of this phase shift is proportional to the shift \( \delta k \) and to the depth \( z \). The amplitude of the signal, however, remains unchanged. Since the retardation calculated by eq. 3 depends only on amplitudes, a translational misalignment will not distort the retardation measured by our method. There will, however, be a distortion in the measured axis orientation \( \Theta \) since this is encoded in the phase difference between the two channels (cf. eq. 4). Since the phase shift is proportional to depth \( z \), an image artifact mimicking an axis change linearly varying with depth will be observed. Because of the \( 2\pi \) ambiguity, this can lead to the impression of an axis orientation oscillating with depth.

We can estimate the magnitude of this image artifact: a phase shift of 180° will be observed at the maximum imaging depth \( z_{\text{max}} \), if the translational shift equals one full pixel width \( PW = 14 \mu m \), since in this case the phase shift equals half the spectral oscillation length. \( z_{\text{max}} \cong 3 \text{ mm} \) in our case, however, due to signal attenuation at large depths, the practically usable imaging depth is \( \sim 2 \text{ mm} \), corresponding to a phase shift of 120° upon a translational misalignment \( \Delta_{tr} = PW \). To keep the axis orientation drift \( \Delta \Theta \) below 5° within the range \( 0 \leq z \leq 2 \text{ mm} \), \( \Delta_{tr} \) should be kept smaller than \( PW/(120°/(2\times5°)) \cong 1.2 \mu m \) (where we kept in mind that \( \Delta \Theta \) is only half of the phase shift \( \Delta \Phi \)). This indicates that the translational camera alignment has to be performed quite carefully.

A second type of misalignment is a tilt of one CCD camera with respect to the other, about an axis perpendicular to both, the optic axis of the spectrometer, and the direction of the CCD array. Such a tilt causes a distortion of the spectrum, equivalent to stretching the spectrum by a factor \( a = 1/\cos(\alpha) \), where \( \alpha \) is the tilt angle. The effect can be calculated with the Fourier similarity theorem:

\[
FT^{-1} \{ I(k \cdot a) \} = \frac{1}{|a|} \left[ FT^{-1} \left\{ I \left( \frac{k}{a} \right) \right\} \right] \rightarrow \frac{1}{|a|} \Gamma \left( \frac{z}{a} - \Delta z \right). \tag{7}
\]

We see that the amplitude of the A-line is changed, as well as the scale, i.e., the position where a signal will be observed along the A-line. The amplitude change can be regarded as negligible, given the rather insensitive dependence of the cosine function on small tilt angles. The scale change, on the other hand, should be kept well below the width of the coherence function \( l_c \) throughout the measurement range used, otherwise the signals corresponding to a given reflection site in the sample would not or only partly overlap in the two channels. A total decorrelation of signals would prevent the acquisition of the polarization parameters, a partial decorrelation would cause a chirp in the retardation signal corresponding to a single reflection site. An angular misalignment of \( \alpha = 1° \), which is not difficult to maintain, would cause a distortion of \( \sim 0.3 \mu m \) at a desired maximum imaging depth of 2 mm. This should be well tolerable at a coherence length \( l_c \cong 6 \mu m \).
The practical alignment procedure to achieve the necessary high precision translational camera alignment consists of the following steps: In a first step the sample arm is blocked and the spectra obtained in the two channels from the reference light are coarsely aligned to equal shape and position. In a second step a sample consisting of a wave plate and a mirror is used (the wave plate may have any retardation ≠ 90° or multiples of 90°). This ensures that a coherence signal can be observed in both channels. In an online display of the Fourier transform of the spectrum, equal width and shape of both coherence functions is checked. The phase difference of the two Fourier transformed signals is continuously measured while the path length difference is changed from close to zero to increasingly larger values. If a deviation of the phase difference from that recorded initially is observed, one of the spectrometer cameras is translationally shifted by a translation stage until the original phase difference is regained. This step is iteratively repeated until the phase difference stays constant within the usable measurement range of 2 mm.

Figure 2 shows the result obtained after the final step of the calibration procedure. The wave plate was set with its optic axis oriented at −35° to the horizontal. The measured axis orientation is plotted as a function of path length difference. Within the desired measurement range of 2 mm the mean measured axis orientation is −27°. The deviation of ~8° from the set value is an offset that has already been observed in time domain setups [20] and is probably caused by imperfect properties of the optical components. There is a slight drift of θ over a total of 6° within the range up to 2 mm, indicating that the camera alignment is not perfect, but the drift stays within the desired range of ±5°. Figure 2 also shows that the measured retardation values are very constant with depth. The mean value of 74° (range: 72 – 76°) is in good agreement with the value of 72° obtained by our time domain system.

3. Results

The retina of the left eye of a healthy human volunteer was imaged after informed consent was obtained. Two-dimensional images were obtained in the fovea centralis region, from the nerve head two- and three dimensional data sets were obtained. In recording sessions for 2D imaging typically 20 to 40 horizontal cross sectional images were recorded in 1 – 2 seconds, in a 3D imaging session, 60 2D images were obtained in 3 sec., while the y-scanner was slowly scanning the horizontal sectioning plane in the vertical direction. To stabilize the subject’s head and minimize motion artifacts to obtain smooth 3D data sets, a bite-bar was used.

Figure 3 shows images of intensity (a), (single pass) retardation (b), and (cumulative) fast axis orientation (c) recorded across the fovea centralis. In the intensity image (Fig. 2(a)) the individual retinal layers known from ultrahigh resolution OCT [38] can be observed. The retardation image (Fig. 3(b)) shows low retardation, indicated by dark blue color, in most of the retinal layers (the small retardation offset is caused by the corneal birefringence). The very last strongly reflecting layer (last bright layer in Fig. 3(a)), however, shows retardation values changing randomly in transverse direction, yielding an average green color in the retardation image. This result is similar to our recent findings of a polarization scrambling (or depolarizing) layer by time domain PS-OCT [12]. First clinical trials on patients with certain retinal pathologies indicated that this layer is probably the RPE [16]. This possibility to localize the RPE via its polarization properties might be of great interest for diagnosis of RPE pathologies.

Figure 3(c) shows an image of the fast axis orientation. The anterior retinal layers show rather constant green color. Since these layers are not birefringent (the nerve fiber layer is very thin in the imaged area), the observed axis corresponds to that of the cornea (slight
variations of the observed color between individual measurement sessions can be caused by
decentration of the measurement beam with respect to the corneal apex because the optical
axis is not constant across the cornea [21]). In the posterior layers, a color change to light
blue can be observed, probably caused by the birefringent Henle’s fiber layer that is located
within the weakly backscattering area marked by H. Near the center of the fovea, where no
Henle fibers are, this color change from green to light blue is not observed. The polarization
scrambling effect of the last layer (probably RPE) is also observed in Fig. 3(c) by the
random color distribution across this layer.

Figure 4 shows 2D images of intensity (a), retardation (b), and axis orientation (c), recorded
across the center of the optic nerve head. In the intensity image (Fig. 4(a)), well-known
features can be observed: the layers of the retina, the excavation of the optic disk, cross
sections through vessels, and structures of the lamina cribrosa. In Figs. 4(b) and (c),
polarization changing features that have not yet been reported, can be observed: A small,
strongly birefringent structure temporal to the nerve head (marked by arrows), probably the
rim of the scleral canal; retardation and axis orientation changes in parts of the lamina
cribrosa, indicating that this structure consists of birefringent tissue with varying orientation.

Figure 5 shows images of intensity (a), retardation (b), and axis orientation (c), recorded
superior to the nerve head. In the retardation image (5(b)) the increase of retardation with
depth caused by the birefringence of the retinal nerve fiber layer (RNFL) can be observed.
This retardation increase is strongest at the thickest nerve fiber bundles. In the axis
orientation image (5(c)) the horizontally varying axis orientation of the nerve fibers which
emerge approximately radially from the nerve head can be observed. The most superficial
green color on the left hand side of the image corresponds to the corneal axis orientation (the
cornea’s birefringence is not compensated for), only in deeper areas the birefringence of the
RNFL dominates, and the cumulative fast axis orientation approaches the true orientation of
the RNFL fast axis (perpendicular to the orientation of the nerve fibers).

Figure 6 shows a fly-through movie of B-scans across the nerve head obtained from a 3D
data set. The movie shows intensity (top), retardation (middle), and fast axis orientation
(bottom). The movie starts with cross sections inferior to the nerve head, moving up through
the nerve head, and ends with cross sections superior to the nerve head. In the intensity
movie, the evolution of retinal layers, the excavation of the optic disk, and vessels can be
observed in detail. Furthermore, details of the lamina cribrosa structure are visible. In the
retardation movie, the birefringence of the RNFL (increasing retardation with depth) inferior
and superior to the nerve head is clearly visible, as well as the temporal rim of the scleral
canal. The axis orientation movie shows clearly that the horizontal axis orientation gradient
inferior to the nerve head is oppositely oriented as compared to locations superior to the
nerve head, as expected from nerve fibers emerging approximately radially from the nerve
head.

From the 3D data set shown in the movie of Fig. 6, 3D volume rendered data sets were
derived [39]. Figure 7 shows a movie of such an animated volume rendered 3D data set. The
movie combines information on the backscattered intensity (corresponding to the opacity)
and on the retardation (corresponding to the color coding). For better visualization, areas
below a certain intensity threshold are displayed totally transparent. At the beginning of the
movie the nerve head is viewed from inferior towards superior. The depolarizing layer can
be seen (due to the data reduction necessary to generate a movie of sufficiently small file
size, the contrast of this layer is somewhat weak), as well as the increasing retardation with
depth caused by the birefringence of the RNFL. In the views from the bottom, the strongly
birefringent temporal rim of the scleral canal is clearly visible. Retardation is also observed
in parts of the lamina cribrosa.
Figure 8 shows a movie of an animated 3D volume rendered data set providing information on axis orientation (color coding) while backscattered intensity corresponds again to opacity. The animation pattern is approximately similar to that in Fig. 7. The varying axis orientation caused by the RNFL can best be seen in the perspective from the bottom, which also provides good views of the variable axis of the lamina.

4. Discussion

We have successfully implemented our PS-OCT approach into a spectral domain OCT instrument, thus demonstrating the first high-speed 3D retinal SD PS-OCT imaging system. The system operates at $2 \times 20000$ A-lines/s, providing an image acquisition speed of 20 intensity, retardation, and axis orientation images per second simultaneously. The acquisition of a 3D data set consisting of 60 intensity, retardation and axis orientation images takes 3 seconds. Compared to our previous transversal time domain PS-OCT instrument that recorded one B-scan in 0.5 sec, this is an improvement in imaging speed of one order of magnitude. This improved imaging speed should now enable 3D PS-OCT imaging in patients which was, due to motion artifacts, not feasible with our time domain system.

3D PS-OCT of the retina might be important for several diagnostic applications. We have shown that RPE atrophy in AMD patients can be discernible via the loss of the depolarizing layer before it is visible in intensity images [16]. However, small areas of missing depolarizing layer might easily be missed in 2D sections, while the 3D data sets should be able to provide the necessary information. Similarly, the detection of disease progression in follow-up studies requires re-imaging of exactly the same location which is very difficult in 2D cross sectional images but should be easily achieved with 3D imaging. The detection of RNFL thinning caused by early glaucoma should also benefit from 3D imaging because the rather large noise of retardation data requires averaging [14], and 3D imaging would provide the possibility of averaging in two transversal directions which might further decrease the noise. A further interesting feature that might be used for glaucoma diagnostics is the strongly birefringent structure at the temporal rim of the nerve head. It seems to be related to the rim of the scleral canal, a structure whose exact location is sometimes difficult to determine, which is, however, necessary to determine optic nerve head parameters used by scanning laser ophthalmoscopic glaucoma diagnosis [40]. 3D PS-OCT might provide a better, more reliable means to determine this structure, potentially improving glaucoma diagnostics.

Although spectral domain PS-OCT has the advantage of higher sensitivity and/or higher imaging speed, as compared to the time domain version of the technology, it has to be noted that the use of two parallely operating spectrometers for the two polarization channels requires special care regarding alignment of these spectrometers, a problem that does not occur with point detectors of TD PS-OCT. In a setup based on the Stokes vector approach [30] it has already been shown that a translational shift of the two spectrometer cameras with respect to each other can generate an artifact mimicking retardation. We have shown that misalignments of the cameras used in our approach can cause related artifacts, however, the situation is somewhat different. Because of decoupling of retardation and axis information in our approach, the measured retardation is insensitive to translational misalignments. Only a tilt between the cameras can affect the measured retardation. However, because of the insensitivity of the cosine function to small tilt angles, such artifacts can be easily avoided. On the other hand, the axis orientation measurement is very sensitive to translational misalignments. Figure 9 shows an example of what happens in case of such a misalignment. The axis orientation of the fovea centralis region of a healthy volunteer is shown. Since this part of the retina is essentially non-birefringent, the measured axis orientation should be that...

Opt Express. Author manuscript; available in PMC 2010 November 12.
of the cornea and should not change with depth in the retina. However, \( \sim 2/3 \) of one full color oscillation, mimicking an axis change of \( \sim 120^\circ \), is observed over a rather small depth of \( \sim 400 \mu m \). This is caused by an intentional translational camera misalignment of \( \sim 70 \mu m \) (cf. eq. 6). Figure 9 clearly shows the necessity of exact camera alignment which is, however, feasible with our iterative alignment procedure (cf. Fig. 2).

Our implementation of PS-OCT illuminates the sample with circularly polarized light and requires only a single A-scan per measurement location to acquire reflectivity, retardation, and axis orientation. The advantage of this method is the faster image acquisition and the decoupling of retardation and axis information, as compared to the Stokes vector method [14]. However, the drawback of our method is (similar to the case of its time domain implementation [12]), that results are influenced by the birefringence of the anterior segment through which the retina is measured (as a differential technique, the Stokes vector method does not suffer from this problem). This effect presently prevents quantitative measurements of the RNFL birefringence, only qualitative information is available. For some applications this qualitative information should be sufficient: imaging of the depolarizing layer and disturbances of it just require the detection of a scrambled polarization state, absolute information on retardation and axis is not required. Furthermore, the position of the rim of the scleral canal can be obtained from qualitative PS-OCT images. But also quantitative information, necessary for glaucoma detection via RNFL birefringence measurements, should be possible. A solution might be an individual compensation of anterior segment birefringence with a variable retarder, as demonstrated successfully in scanning laser polarimetry [34]. Another solution might be an appropriate software algorithm that uses the retardation and axis information measured at the retinal surface to correct for the anterior segment birefringence.

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Fig. 1.
Schematic drawing of spectral domain PS-OCT instrument. SLD, super luminescent diode; FC, fiber coupler; POL, polarizer; NPBS, non-polarizing beam splitter; VDF, variable density filter; QWP, quarter wave plate; M, mirror; SC, galvo scanner; L, lens; S, sample; PMF, polarization maintaining fiber; HWP, half wave plate; DG, diffraction grating; LSC, line scan camera.
Fig. 2.
Results of instrument calibration. A sample consisting of a wave plate and mirror was measured. Axis orientation and retardation are plotted as a function of path length difference. Within the desired measurement range of 2 mm, the axis drift stays within $\pm 3^\circ$. 

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Fig. 3. B-scan images of human fovea in vivo. (a) Intensity (log scale); (b) retardation; (c) fast axis orientation. Image size: 3 mm (horizontal) × 0.75 mm (vertical). Values on color bars: degrees (to avoid erroneous birefringence data, areas below a certain intensity threshold are displayed in grey).
Fig. 4.  
B-scan images of human optic nerve head in vivo. (a) Intensity (log scale); (b) retardation; (c) fast axis orientation. Image size: 3 mm (horizontal) × 1.75 mm (vertical). Values on color bars: degrees. Arrow: temporal rim of scleral canal.
Fig. 5.
B-scans superior to human optic nerve head in vivo. (a) Intensity (log scale); (b) retardation; (c) fast axis orientation. Image size: 3 mm (horizontal) × 1 mm (vertical). Values on color bars: degrees.
Fig. 6.
(2.5 MB) Frame no. 31 of fly-through movie of 3D dataset of human nerve head in vivo. Top: intensity; middle: retardation; bottom: axis orientation (color scales similar to Figs. 2-4). Image size: ~3mm (x) × 3mm (y) × 1.75mm (z).
Fig. 7. (1.2 MB) Frame no. 4 of animation of a 3 dimensional volume rendered data set from a human nerve head in vivo. The opacity corresponds to the backscattered intensity, the retardation corresponds to the color coding (color coding similar to Figs. 2-4).
Fig. 8.
(2.2 MB) Frame no. 3 of animation of a 3 dimensional volume rendered data set from a human nerve head in vivo. The opacity corresponds to the backscattered intensity, the fast axis orientation corresponds to the color coding (color coding similar to Figs. 2-4).
Fig. 9.
PS-OCT axis orientation image of fovea centralis in vivo. The image illustrates the effect of translationally misaligned spectrometer cameras. Image size: ~ 3 mm (horizontal) × 0.75 mm (vertical).