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Braaf, B.

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The principles of Optical Coherence Tomography for posterior eye imaging

Background

Optical interference arises from the wave nature of light and describes how the superposition of light waves can create a resultant wave with a different amplitude. The most famous demonstration of optical interference is Young’s double-slit experiment, in which light from a single source radiates through two slit (or hole) openings and together project an alternating pattern of bright and dark bands on a screen [1]. The bright and the dark bands are caused by constructive and destructive interference respectively. Constructive interference occurs when the light waves from the two slits are in phase and add up to a resultant wave with a higher amplitude, whereas destructive interference occurs when the light waves are out of phase and add up to a lower amplitude. As such, both effects are dependent on the optical path length difference (OPD) between the slits to the same location on the projection screen. These basic principles of optical interference form the foundation for low-coherence interferometry techniques such as OCT.
2.1 Low coherence interferometry

In low-coherence interferometry (LCI) controlled optical interference is used to obtain high resolution depth information from an object with micrometer accuracy. The core of LCI instrumentation is the Michelson interferometer [2] which is schematically represented in Fig. 2-1(A). In a Michelson interferometer the light from a light source is split in two directions by a beam splitter and is thereby divided over two separate optical paths that form the interferometer arms. In one optical path, the reference arm, the light is reflected back by a reference mirror; while in the other optical path, the sample arm, the light is reflected back by the sample under test. The reflected light from both arms is recombined at the beam splitter and measured by a photodetector. The intensity of the light $I_{\text{int}}$ at the detector is subject to interference and depends on the reflectivity and optical path length of both interferometer arms [3]:

$$I_{\text{int}}(k, z_R, z_S) = S(k) \cdot \left\{ R_R + R_S + 2 \cdot \sqrt{R_R \cdot R_S} \cdot \cos\left(2k (z_R - z_S)\right)\right\},$$

(2.1)

where $S(k)$ is the intensity of the light source as a function of wavenumber $k$, $R_R$ and $R_S$ are the reflectivities of the reference mirror and sample, respectively, and $z_R$ and $z_S$ are the optical path lengths of the reference and sample arm, respectively.

Fig. 2-1. (A) Schematic drawing of a Michelson interferometer. Light from a light source is divided over two interferometer arms by a beam splitter. The reflected light from both arms is recombined at the beam splitter and an interference pattern is measured at the detector as a function of OPD $(Z_R - Z_S)$. (B) Monochromatic light with a long coherence length creates a repetitive interference signal as a function of OPD. (C) The interference pattern for polychromatic light includes the interference for many monochromatic light sources as shown in the left subfigure for three distinct wavenumbers with $k_a < k_b < k_c$. The monochromatic interference patterns are all in phase only for an OPD of zero (indicated by the vertical red dashed line) and in practice the simultaneous detection over the full spectrum therefore generates a localized interference pattern around this OPD (right subfigure).
$R_s$ are the reflectivity of the reference and sample arm respectively, and $z_R$ and $z_S$ are the single pass optical path lengths of the reference and sample arm respectively. For a monochromatic light source a repetitive sinusoidal modulation (fringe) will be measured at the detector as a function of the OPD $(z_R - z_S)$ between the interferometer arms as shown in Fig. 2-1(B). The modulation frequency of this signal depends on the wavenumber of the light source and is given by the $2k$ term in Eq. (2.1), while the amplitude is given by $2\sqrt{(R_R \cdot R_S)}$. In case of a polychromatic light source many monochromatic interference patterns are measured simultaneously, for which each has its own modulation frequency as shown in the left subfigure of Fig. 2-1(C). These monochromatic interference patterns are all in phase for an OPD of zero and in practice the simultaneous detection over the full polychromatic spectrum therefore results in a localized interference peak when the OPD between the interferometer arms reaches (nearly) zero. An example of this interference pattern is shown in the right subfigure of Fig. 2-1(C) for a Gaussian spectral source bandwidth and is mathematically given by integrating equation (2.1) over $k$:

$$I_{\delta z}(z_R, z_S) = S_0 \cdot \left( R_R + R_S + 2 \cdot \sqrt{R_R \cdot R_S} \cdot e^{-(z_R - z_S)^2 \Delta k^2} \cdot \cos(2k_0(z_R - z_S)) \right), \quad (2.2)$$

with $S_0 = \int_0^\infty S(k)dk$ as the spectrally integrated power from the source, $k_0$ as the source center wavenumber and $\Delta k$ as the Gaussian source bandwidth in wavenumber. By scanning the reference arm length, LCI measures the interference for a range of OPDs and is thereby able to determine the distance to the sample. The OPD range over which interference occurs for a single reflector is given by the light source coherence length and defines the axial resolution $\delta z$ of the sample localization [4]:

$$\delta z = \frac{2 \ln(2) \cdot \lambda_0^2}{n \pi \cdot \Delta \lambda}, \quad (2.3)$$

with $\lambda_0$ as the center wavelength, $\Delta \lambda$ as the Gaussian source full width at half maximum bandwidth in wavelength, and $n$ the refractive index of the medium. Equation (2.3) shows that for a true monochromatic light source $\delta z$ is infinitely large and no localization accuracy is achieved. Increasing the source bandwidth will subsequently improve the LCI axial resolution.

The advantage of LCI over other optical detection methods is the interferometric amplification of a weak sample signal by a much stronger reference (arm) signal to make the detection of the former much easier. In addition LCI can achieve an incredible axial resolution of several micrometers using the broadband light sources that are available nowadays [5].
2.2 Optical Coherence Tomography

Optical coherence tomography (OCT) uses LCI for investigation of composite samples with multiple sample reflectors in depth. When a sample is illuminated, its scattered light is utilized by OCT to determine a one dimensional reflectivity profile of the sample structures in depth: the A-scan (also called A-line). In this section the measurement of the A-scan signals is described for several OCT techniques that can be used for imaging of the in vivo posterior eye.

2.2.1 Time-domain OCT

Time-domain OCT (TD-OCT) was the first implementation form of OCT and directly adapted from LCI [6]. In TD-OCT the Michelson interferometer consists usually of a 2 × 2 fiber optic coupler which divides the light from a broadband light source over the two interferometer arms as schematically visualized in Fig. 2-2(A). In one arm the light falls on a movable reference mirror that is scanned in distance to vary the OPD with the other arm that holds the sample under test. While scanning the reference mirror the interference between the reflected light from both arms is recorded by a photodetector. During this scan interference fringes are detected when the path length in the reference arm matches with the path length of a reflector in the sample arm. This process is illustrated in Fig. 2-2(B) where a set of three partial reflectors at different depths, thus at different OPDs, reflect light and generate interference fringes, so-called fringe bursts, when the reference arm matches their position. The detected interference signal for TD-OCT is math-

![Fig. 2-2. (A) Schematic drawing of a time-domain OCT system. A 2 × 2 fiber optic coupler is used to construct a Michelson interferometer. A moving reference arm mirror varies the OPD between the interferometer arms in order to detect interference fringes from different depths within the sample. (B) Schematic example of the TD-OCT signal detection for a sample consisting of three partial reflectors at different depths. Interference fringe bursts are detected when the path length of the reference mirror matches the path length for a sample reflector.](image-url)
Principles of OCT

Mathematically described as a function of the reference arm path length $z_R$ and defines the A-scan as [7]:

$$I_{TD-OCT}(z_R) = S_0 \cdot \left\{ R_R + \sum_{n=1}^{N} R_{Sn} + 2 \cdot \sum_{n=1}^{N} \sqrt{R_R \cdot R_{Sn}} \cdot e^{-\left(z_R-z_{Sn}\right)^2} \cdot \cos\left(2k_0\left(z_R-z_{Sn}\right)\right) \right\}$$

$$+ S_0 \cdot \left\{ 2 \cdot \sum_{n \neq m=1}^{N} \sqrt{R_{Sn} \cdot R_{Sm}} \cdot e^{-\left(z_{Sn}-z_{Sm}\right)^2} \cdot \cos\left(2k_0\left(z_{Sn}-z_{Sm}\right)\right) \right\}, \quad (2.4)$$

where $R_{Sn}$ is the reflectivity of the $n$-th reflector with a sample arm path length corresponding to $z_{Sn}$. Similar to Eq. (2.2) in (2.4) the first two terms are offsets due to the reflectivity of the reference and sample arms respectively, and the third term describes the interference between the reference arm and the sample reflectors from multiple depths. The fourth term (the second line of Eq. (2.4)) describes the autocorrelation signal, i.e. the interference between the different sample reflectors. In Eq. (2.4) the autocorrelation signal is independent of $z_n$ and leads to an additional signal offset. In practice the TD-OCT signal is demodulated for its interference carrier frequency $k_0$ to obtain the amplitude envelopes of the fringe bursts. Afterwards the signal amplitude envelope is squared to get $R_R R_S$ as a measure of the sample reflectivity, which is often denoted as the OCT intensity of the A-scan.

### 2.2.2 Fourier-domain OCT

Fourier-domain OCT (FD-OCT) is an alternative to TD-OCT which uses LCI with spectrally resolved detection and does not require scanning of the reference mirror. FD-OCT can be implemented in two forms: spectral-domain OCT (SD-OCT) [8,9] and swept-source OCT (SS-OCT) [10,11]. In SD-OCT the photodetector of the TD-OCT setup is replaced with a spectrometer as is visualized in Fig. 2-3(A). In its simplest form a spectrometer uses a diffraction grating for the angular separation of wavelengths and a lens to image them as multiple small wavelength bands on a charge-coupled device (CCD) detection array. An entire SD-OCT A-scan is acquired by a “single shot” measurement of the spectrometer over the full CCD array. In SS-OCT, also known as optical frequency domain imaging (OFDI) [12], the light source of a TD-OCT setup is replaced with a narrow band laser that is rapidly swept in time over a broad spectral bandwidth. In Fig. 2-3(B) a schematic drawing of a typical SS-OCT setup is given that incorporates a reference arm in transmission and a balanced detector. In SS-OCT balanced detection is highly advantageous to suppress common mode signals, in particular the relative intensity noise of the swept-source, which is in general much stronger than for SD-OCT light sources. In SS-OCT an entire A-scan is acquired by a single wavelength sweep over the full spectral width of the swept-source.
In both the FD-OCT implementations the interference signals are acquired spectrally resolved and are described as a function of $k$ as [7]:

$$I_{FD-OCT}(k) = S(k) \cdot \left\{ \sum_{n=1}^{N} R_{Sn} + 2 \cdot \sum_{n=1}^{N} \sqrt{R_{R} \cdot R_{Sm}} \cdot \cos(2k(z_R - z_{Sm})) \right\} + S(k) \cdot \left\{ 2 \cdot \sum_{n \neq m=1}^{N} \sqrt{R_{Sn} \cdot R_{Sm}} \cdot \cos(2k(z_{Sn} - z_{Sm})) \right\}. \quad (2.5)$$

For the spectrally resolved interference signal the modulation frequency is set by the OPD $(z_R - z_{Sm})$ between the interferometer arms, and will be different for reflectors from different depths. This effect can be exploited by Fourier analysis which transforms Eq. (2.5) directly to the depth domain:

$$I_{FD-OCT}(z) = \mathcal{F}^{-1}\{I_{FD-OCT}(k)\} = \Gamma(z) \otimes \left\{ R_{R} + \sum_{n=1}^{N} R_{Sn} + \sum_{n=1}^{N} \sqrt{R_{R} \cdot R_{Sn}} \cdot \delta \left(z \pm 2(z_R - z_{Sn})\right) \right\} + \Gamma(z) \otimes \left\{ \sum_{n \neq m=1}^{N} \sqrt{R_{Sn} \cdot R_{Sm}} \cdot \delta \left(z \pm 2(z_{Sn} - z_{Sm})\right) \right\}. \quad (2.6)$$

**Fig. 2-3.** Fourier-domain OCT. (A) Schematic drawing of a spectral-domain OCT setup which uses a spectrometer for the detection of spectrally resolved interference signals. (B) Schematic drawing of a swept-source OCT setup which uses a rapidly wavelength swept light source and balanced detection for the detection of spectrally resolved interference signals. (C) An example of the FD-OCT signal detection for an SD-OCT fringe corresponding to the threefold partial reflector of Fig. 2-2(B). Fourier decomposition of the spectral fringe transforms the signal to the depth (OPD) domain in which three peaks are observed at the locations of the three partial reflectors.
where the reflectors are modeled by delta functions $\delta$ at a depth corresponding to an OPD of $2(z_r - z_s)$, denotes convolution, and $\Gamma(z)$ represents the envelope of the coherence function as given by the Fourier transform of $S(k)$. Consequently path length scanning within the reference arm is not necessary anymore. Note that the spectrally resolved FD-OCT signals of Eq. (2.5) are real and result in complex conjugate signal pairs for the positive and negative OPDs in Eq. (2.6) over which the signal power is divided. An example of the FD-OCT signal detection is given in Fig. 2-3(C) where a spectrally resolved interference fringe corresponding to the three partial reflectors of Fig. 2-2(B) is Fourier transformed. The result shows three distinct reflectivity peaks at different OPDs in the sample for which only the positive half of the complex conjugate signal pair is shown. In practice the amplitude of the Fourier transformed signal is squared to get $R_r R_s$ as a measure of the sample reflectivity, which defines the FD-OCT intensity of the A-scan.

In FD-OCT the autocorrelation term is not a constant signal offset and can obscure the A-scan. However, the modulation frequency of the interference between the reference arm and the sample is normally higher than for the autocorrelation term since the distances within the sample are small compared to the OPD with the reference arm. The autocorrelation signal therefore in practice hardly ever obscures the sample interference information. In addition the sample reflectivity is usually several orders of magnitude lower than the reference arm which makes the autocorrelation amplitude low. In SS-OCT the balanced detection further reduces the autocorrelation signal and the reflectivity offsets $R_r$ and $R_{sn}$ since they are common mode signals.

### 2.2.3 FD-OCT advantage over TD-OCT

In 2003 it was recognized that FD-OCT has a fundamental signal-to-noise ratio (SNR) advantage over TD-OCT with a typical sensitivity improvement of 2 to 3 orders of magnitude [13-15]. The SNR improvement of FD-OCT arises from the distribution of the photonic shot noise over multiple separately detected spectral bands, instead of a single detection over the full spectral width as done in TD-OCT.

In both TD-OCT and FD-OCT the electrical power (in $A^2$) of the interference term at the detector can be defined as:

$$S_{\text{sample}} = 2q^2 e^2 P_{\text{ref}} P_{\text{sample}} / E_v^2,$$

(2.7)

with $q$ as the detector quantum efficiency, $e$ the elementary charge, $P_{\text{ref}}$ the reference arm optical power, $P_{\text{sample}}$ the sample arm optical power, and $E_v$ as the photon energy [16]. The noise in an optimal OCT measurement is dominated by photonic shot noise which has a white noise characteristic, and thus has the same noise power for all signal frequencies [16]. In an OCT instrument the generation of shot
noise is normally dominated by the optical power of the reference arm, since its reflectivity is several orders of magnitude higher compared to the weakly reflecting biological samples in the sample arm [17]. In this case the instantaneous shot noise power is defined (in $A^2/Hz$) as:

$$N_{\text{shot}} = 2q e^2 P_{\text{ref}} / E_v.$$  \hspace{1cm} (2.8)

In TD-OCT each wavenumber encodes for a unique signal frequency and the total shot noise power (in $A^2$) for a measurement over the full spectral width is therefore obtained by integration over the signal bandwidth $BW$:

$$N_{\text{TD-OCT}} = N_{\text{shot}} \cdot BW = \left(2q e^2 P_{\text{ref}} / E_v\right) \cdot BW.$$  \hspace{1cm} (2.9)

The SNR for TD-OCT can now be defined as:

$$\text{SNR}_{\text{TD-OCT}} = S_{\text{sample}} / N_{\text{TD-OCT}} = \left(q P_{\text{sample}}\right) / (E_v BW).$$  \hspace{1cm} (2.10)

In FD-OCT the signal bandwidth is distributed over multiple spectral bands that are either measured by separate detector elements (SD-OCT) or on different time-points (SS-OCT). In either case the detected signal bandwidth reduces corresponding to the number of separately detected spectral bands $M$:

$$N_{\text{FD-OCT}} = N_{\text{TD-OCT}} / M = \left(2q e^2 P_{\text{ref}} / E_v\right) \cdot \left(BW / M\right).$$  \hspace{1cm} (2.11)

In the corresponding A-scan consisting of $M$ samples, the signal bandwidth $BW$ is equal to the inverse of two times the (proportional) acquisition time for a single spectral band: $\tau_{\text{acq}}$ [18]. In addition, the multiplication of $\tau_{\text{acq}}$ with $M$ gives the total acquisition time of the A-scan: $\tau_{A\text{-scan}}$. Eq. (2.11) can now be rewritten as:

$$N_{\text{FD-OCT}} = \left(2q e^2 P_{\text{ref}} / E_v\right) \cdot \left(1 / (M \cdot 2 \cdot \tau_{\text{acq}})\right) = \left(2q e^2 P_{\text{ref}} / E_v\right) \cdot \left(1 / (2 \cdot \tau_{A\text{-scan}})\right).$$  \hspace{1cm} (2.12)

As noted in section 2.2.2 FD-OCT only measures the real part of the complex spectral interference signal and therefore detects only half the signal power. The FD-OCT SNR is then defined as:

$$\text{SNR}_{\text{FD-OCT}} = 0.5 \cdot S_{\text{sample}} / N_{\text{FD-OCT}} = \left(\eta P_{\text{sample}} \tau_{A\text{-scan}}\right) / E_v.$$  \hspace{1cm} (2.13)

From this analysis it can be concluded that the SNR for FD-OCT is proportional to the SNR for TD-OCT with a factor that scales linearly with the amount of separately detected spectral bands:

$$\text{SNR}_{\text{FD-OCT}} = \left(M / 2\right) \cdot \text{SNR}_{\text{TD-OCT}}.$$  \hspace{1cm} (2.14)

In FD-OCT a high number for $M$ is realized by a high number of pixels on the SD-OCT CCD detection array, or by a high-speed sampling of the wavelength sweep in SS-OCT. In both FD-OCT implementations $M$ easily surpasses 1000, and leads to an over 500 fold improved SNR compared to TD-OCT.

The improved SNR of FD-OCT can be traded against faster acquisition speeds
and opened the door for (faster than) video-rate OCT imaging of the posterior eye with A-scan acquisition rates ranging from several tens of kHz up to several MHz [19-21]. In addition, the narrow instantaneous linewidth of swept-source lasers makes SS-OCT less sensitive to fringe washout due to sample movement [22,23] and gives a lower signal decay with depth [24] compared to SD-OCT. SS-OCT is therefore rapidly becoming the dominant FD-OCT form in experimental research, and was the technique of choice for the scientific work presented in this thesis.

2.3 Functional OCT

In OCT cross-sectional reflectivity profiles of sample structures are measured for which the image contrast is based on the amount of scattered light. In functional OCT additional contrast mechanisms are extracted from the OCT signal to obtain clinically important physiological information of the tissue. In this section two widely used functional OCT forms are introduced that formed the main research topics for the work presented in this thesis: Doppler OCT and polarization-sensitive OCT.

2.3.1 Doppler OCT

The Doppler effect describes the change in frequency of a wave for which the source is moving relative to an observer. In OCT the Doppler effect is encountered when light is backscattered by moving particles in a fluid flow, and is caused for example by erythrocytes in flowing blood. Doppler OCT analyzes the corresponding Doppler frequency shift in the OCT signal which is defined as [25]:

\[
\Delta f_{\text{Doppler}} = \frac{1}{2\pi} (\Psi_s - \Psi_i) \cdot \mathbf{V},
\]

where \(\Psi_s\) and \(\Psi_i\) are the wave vectors of the incoming and the scattered OCT light respectively, and \(\mathbf{V}\) is the velocity vector of the moving particle. Assuming the backscattered light follows the reverse path of the incoming OCT light a Doppler angle \(\alpha\) can be defined between the OCT beam and the flow velocity vector to simplify Eq. (2.15) accordingly to:

\[
\Delta f_{\text{Doppler}} = \frac{2nV \cos(\alpha)}{\lambda_0},
\]

where \(n\) is the refractive index of the medium, \(V\) is the flow velocity, and \(\lambda_0\) is the center wavelength of the light source. This condition is schematically visualized in Fig. 2-4 in which the OCT light is incident on a blood vessel. Eq. (2.16) shows with its cosine term that only the axial velocity component is represented in the Doppler frequency shift, which reaches its maximum when the OCT light is in parallel with the flow velocity vector. Vice versa, the observed Doppler frequency shift becomes zero for perpendicular Doppler angles. In biological samples the Doppler
angles can become nearly perpendicular and the flow velocities are relatively low. The observed Doppler frequency shifts are therefore often much smaller than the OCT axial resolution and only give a phase shift in the OCT signal over time. This phase shift can be measured between successively acquired A-scans from the same sample location as [26]:

\[ 2\pi f_{\text{Doppler}} = \frac{\Delta \phi}{\tau}, \]  

(2.17)

in which \( \Delta \phi \) denotes the OCT phase shift and \( \tau \) describes the time interval between the successive A-scan measurements. Together equations (2.16) and (2.17) can be rewritten to describe the OCT phase shift between A-scans in terms of the setup and sample parameters:

\[ \Delta \phi = \frac{4\pi nV \cos(\alpha)}{\lambda_0}. \]  

(2.18)

The phase shifts obtained with Doppler OCT can be used to calculate the flow velocity under controlled circumstances with a known Doppler angle [27], or can simply be used to discriminate blood flow from static tissues for angiographic purposes [28,29]. The latter is presented in chapters 3, 4 and 5 of this thesis for non-invasive in vivo angiography of the posterior eye.

Fig. 2-4. Schematic drawing for a Doppler OCT measurement on a blood vessel. The Doppler angle \( \alpha \) is defined between the OCT light and the flow velocity vector \( \mathbf{V} \). Light backscattered by moving particles in the blood flow causes a Doppler frequency shift between the wave vectors \( \psi_i \) and \( \psi_s \) for the incoming and the scattered light respectively.

2.3.2 Polarization-sensitive OCT

Polarization-sensitive optical coherence tomography (PS-OCT) determines the optical polarization properties of a sample by depth-resolved polarimetry (polarization analysis) of the backscattered light. The polarization state of the light changes when passing through birefringent structures, which hold a (slightly) different refractive index for different polarization orientations. In crystals the regular array of atoms in the material causes intrinsic birefringence which is often utilized by hardware components to modify and control the optical polarization. In tissue
the aligned orientation of fibrous structures causes *form birefringence* in which the refractive index difference between fibers and their surrounding medium causes an orientation dependent and therefore a polarization dependent refractive index [30]. In Fig. 2-5(A) the effect of form birefringence is shown on polarized light that traverses through a group of parallel aligned fiber bundles. The horizontal polarization component (the red wave) and the vertical polarization component (the yellow wave) are respectively in parallel with and orthogonal to the fiber bundles and experience a different refractive index. This results in a different path length for both polarization components through the fiber bundles and causes a phase delay between both components which is denoted as phase retardation. The relation of the phase retardation $\eta$ between the orthogonal polarization components and the birefringence $\Delta n$ is described by [31]:

$$\eta = \frac{2\pi \cdot \Delta n \cdot x}{\lambda_0},$$

(2.19)

where $x$ is the distance traveled through the birefringent medium and $\lambda_0$ is the center wavelength of the OCT light source. The goal of PS-OCT is to measure the phase retardation and the orientation of birefringent tissue structures.

**Fig. 2-5.** (A) The effect of form birefringence on polarized light for a group of fiber bundles. The fiber bundles hold a difference in refractive index with their surrounding medium and their highly ordered alignment therefore generates an orientation dependent and thus a polarization dependent refractive index. This causes different optical path lengths for the horizontal and vertical polarized light components and results in a phase delay which is denoted in PS-OCT as phase retardation. (B) The schematic drawing of a free-space polarization-sensitive TD-OCT system. Component abbreviations: LP: linear polarizer, BS: polarization independent beam splitter, QWP: quarter-wave plate, PBS: polarizing beam splitter. The LP generates vertical linearly polarized light out of a light source which is rotated by a QWP at 45° to circular polarized light on the sample. The interference of the backscattered light with the reference arm is measured by a polarization dependent detection arm in which the horizontal and vertical polarization components are separated by a PBS and detected simultaneously.
Although several different PS-OCT implementations have been developed over the years in this section the very first implementation is discussed because of its simplicity. Early PS-OCT implementations were based on free space TD-OCT systems as visualized in Fig. 2-5(B) [32-34]. Light from a light source is vertically linear polarized by a linear polarizer (LP) and divided by a beam splitter (BS) over the reference and sample arms. In the reference arm the light double passes a quarter-wave plate (QWP) oriented at 22.5° via reflection on the reference mirror. This provides equal reference light intensity in both channels of the polarization-sensitive detection arm that separately measures the horizontal and vertical linear polarized light components using a polarizing beam splitter (PBS). In the sample arm the light passes through a QWP oriented at 45° and provides a circular polarization state onto the sample. This ensures the measurement of the sample polarization properties independent from its birefringence axis orientation. The backscattered light from the sample is recombined with the reference arm light at the beam splitter and their interference is detected separately for both polarization components in the polarization-sensitive detection arm. The analytical representation of the detected signals describes the horizontal $E_h(z)$ and vertical $E_v(z)$ electric field components of the OCT signal [35] and can be written as a complex 2-element vector in accordance with the Jones calculus [36]:

$$
\overline{E}_{\text{det}}(z) = \begin{bmatrix} E_h(z) \\ E_v(z) \end{bmatrix} = \begin{bmatrix} A_h(z)e^{i\phi_h(z)} \\ A_v(z)e^{i\phi_v(z)} \end{bmatrix},
$$

(2.20)

where $A_h(z)$ and $A_v(z)$ are the amplitudes, and $\phi_h(z)$ and $\phi_v(z)$ are the phases of the horizontal and vertical electric field components of the so-called Jones vector for scattered light from a depth $z$ in the sample. The detected field $\overline{E}_{\text{det}}(z)$ can be mathematically described as the combined effect of the polarization effects of all components in the setup. The (non-depolarizing) properties of every component are represented by their individual Jones matrix $J$: a complex $2 \times 2$ matrix that mathematically describes their change on the polarization state. $\overline{E}_{\text{det}}(z)$ can then be described as a chain of Jones matrix multiplications that act upon the electric field $\overline{E}_{\text{source}}$ emitted by the light source in the same order as the light passes the sample arm optics [35]:

$$
\overline{E}_{\text{det}}(z) = J_{\text{QWP45°}}^T \cdot J_{s}(z) \cdot J_{\text{QWP45°}} \cdot J_{\text{LP}} \cdot \overline{E}_{\text{source}},
$$

(2.21)

where $J_{\text{LP}}$ is the Jones matrix of the linear polarizer, $J_{\text{QWP45°}}$ is the Jones matrix of the QWP at 45° in the sample arm, and $J_{s}(z)$ is the Jones matrix that describes the round-trip polarization properties of the sample. Here the reference arm light is assumed to have a perfect 45° linear polarization state for which the contribution in Eq. (2.21) reduces to a complex scalar. This scalar can be included in $\overline{E}_{\text{source}}$. 
together with the common sample signal amplitude and phase to simplify further analyses. The Jones matrix for a vertical linear polarizer is described as [36]:

\[
J_{LP} = \begin{bmatrix} 0 & 0 \\ 0 & 1 \end{bmatrix},
\]

(2.22)

while a QWP at 45° is given by:

\[
J_{QWP45°} = R(45°) \cdot \begin{bmatrix} e^{i \pi/4} & 0 \\ 0 & e^{-i \pi/4} \end{bmatrix} \cdot R(-45°),
\]

(2.23)

with \( R \) as a standard rotation matrix in the form \( R(\theta) = [\cos(\theta), -\sin(\theta); \sin(\theta), \cos(\theta)] \).

The Jones matrix of the sample is defined similarly to the QWP as:

\[
J_s(z) = R(\theta_s(z)) \cdot \begin{bmatrix} e^{i \eta(z)/2} & 0 \\ 0 & e^{-i \eta(z)/2} \end{bmatrix} \cdot R(-\theta_s(z)),
\]

(2.24)

with \( \eta(z) \) as the phase retardation and \( \theta_s(z) \) as the birefringence optic axis orientation angle of the sample. It can be shown that Eqs. (2.20) - (2.24) can be used to calculate the phase retardation as [32,35]:

\[
\eta(z) = \arctan \left( \frac{A_H(z)}{A_V(z)} \right),
\]

(2.25)

and the optic axis orientation angle as [37]:

\[
\theta_s(z) = (180° + \phi_H(z) - \phi_V(z)) / 2.
\]

(2.26)

The PS-OCT system as discussed above can be modified for equivalent implementation with FD-OCT [38]. In order to facilitate optical alignment, PS-OCT systems are nowadays often built with single-mode optical fibers (SMFs) [39]. In SMFs stress-induced birefringence and imperfections in the fiber core symmetry pose a problem by changing the polarization state of the OCT light to an unknown state. This problem has been solved by polarization modulation and multiplexing methods that simultaneously probe the sample with several distinct polarization states [39]. In this approach the separate incident polarization states experience the same SMF birefringence in the fiber-based interferometer and their polarization state changes equally. Consequently a more sophisticated modeling and post-processing is necessary to extract the sample phase retardation and optic axis orientation angle. In chapter 6 of this thesis an elaborate description, modeling and processing approach for such a SMF-based PS-OCT setup is presented for depth-resolved polarimetric imaging of the \textit{in vivo} posterior eye.
2.4 Ophthalmic OCT implementation

In this section several implementation aspects are discussed that are specific for OCT imaging of the posterior eye.

2.4.1 Ophthalmic interface design for OCT imaging of the posterior eye

In Figs. 2-2, 2-3 and 2-5 the measurement of the eye (the sample) by the OCT instrumentation was schematically visualized by projection of a collimated beam on to the cornea that is consecutively focused onto the retina. In these figures the actual optical interface which provides raster scanning functionality was left out for simplicity. In Fig. 2-6 the complete optical design is given for the OCT ophthalmic interface that was used for the scientific work presented in chapters 3 to 6 of this thesis. The component parameters of the ophthalmic interface were adapted for the specific needs of each study.

The sample arm optical fiber from the OCT interferometer was connected to the ophthalmic interface via a collimator (C) which consists of a small lens to create a collimated bundle with a Gaussian intensity profile (red paths). The collimated bundle was reflected by the X- and Y-galvanometer (galvo) mirrors which were used for high frequency raster scanning of the OCT beam. Afterwards the collimated OCT bundle was demagnified using a telescope consisting of lenses L1

![Image of the optical design of the OCT ophthalmic interface]

**Fig. 2-6.** The optical design of the OCT ophthalmic interface. The OCT light is shown in red and is supplied by the sample arm optical fiber from the OCT interferometer to a collimator (C) that projects a collimated bundle on the galvanometer scanners X-galvo and Y-galvo. A telescope consisting of lenses L1 and L2 demagnified the OCT bundle and projected it through the pupil of the eye. The optics of the eye focused the OCT light onto the retina. A dichroic mirror (DM) was used to couple the optical paths of a webcam and an LED grid with the OCT light for respectively alignment and subject fixation purposes. A glass slide (GS) was used to combine the optical paths of the webcam and the LED grid.
and L2 on its way to the cornea. In OCT literature L1 is often denoted as the *scan lens* while L2 is denoted as the *ophthalmic lens*. The ophthalmic lens directed the demagnified OCT bundle for different galvo scan angles (light red paths) through the pupil of the eye. Afterwards, the optics of the eye focused the OCT light onto the retina. In order to improve the subject’s fixation stability and facilitate instrument alignment respectively a fixation target LED grid (green path) and a pupil camera (webcam, blue path) were coupled together via a glass plate (GP), and were combined with the OCT light using a dichroic mirror (DM). The DM was placed under 45° in between L1 and L2 to ensure a constant incidence angle on the DM during scanning of the OCT beam with negligible (angle dependent) polarization effects. The pupil camera consisted of a standard webcam including its focusing optics to image the anterior part of the eye with visible light provided by the room illumination. Its field of view (FOV) was demagnified using telescope L3 - L2 and limited the FOV to the pupil and iris. The fixation target stabilized the subject’s gaze during OCT measurement and allowed a controlled tilting of the eye by changing the location of the on-switched LED in the grid. This provided the ability to shift the imaged retinal area and to aim the OCT imaging on specific retinal structures. The lens L4 was used to image the fixation target onto the retina.

In Fig. 2-7 the different modes of raster scanning of the posterior eye are visualized. As explained before, the OCT measurement of the tissue structures in depth at a single location is called an A-scan (or A-line). When the OCT light entering the eye is scanned over an angle through the pupil, several A-scans can be taken

![Fig. 2-7. The different modes of raster scanning for OCT imaging of the posterior eye. A single OCT measurement gives a tissue depth-profile called an A-scan. Multiple A-scans measured along a line over the retina surface together create a cross-sectional OCT image called a B-scan. A three-dimensional data volume is acquired by obtaining multiple B-scans side by side in a grid. From the three-dimensional volume a single slice in depth is called a C-scan, while a summation over multiple C-scans is called an *en face* image. The schematic figure of the eye is the courtesy of the National Eye Institute, National Institute of Health.](image)
successively while tracing a line over the retinal surface. This collection of A-scans forms the two-dimensional cross-sectional OCT image which is called a B-scan. Note that since the B-scan is acquired by an angular scan, B-scans of the posterior eye are normally given with pixel locations according to a polar coordinate system. When several B-scans are acquired side by side in a grid a complete three-dimensional data volume is obtained. From the three-dimensional data volume the information from a single depth can be taken to obtain a transversal tissue slice called a C-scan. Often it is interesting to compile the information from a complete tissue layer, by summation of multiple C-scans, into a single depth slice which is denoted as an en face image.

The human eye is an integrated part of the OCT system and its optics should therefore be considered when evaluating the lateral resolution and FOV. The eye is often represented in optics by a reduced eye model [40] in which a single lens describes the combined optical power of both the cornea and the crystalline lens and focuses the light onto the retina. This simplification is possible since the nodal points for both ocular components are located close to each other at the back of the crystalline lens. The power of this simplified eye model is 60 D and has a corresponding focal length \( f_{\text{eye}} \) of 16.7 mm. Although the axial resolution for OCT is determined by the spectral bandwidth of the light source (Eq. 2.3), the lateral resolution is purely depending on the optical system. The OCT lateral resolution is calculated from the bundle size on the cornea for which the radius is defined as:

\[
r_{\text{cor}} = N A_{\text{fiber}} \cdot f_C \cdot (1/f_{L2}/f_{L1}),
\]

where \( N A_{\text{fiber}} \) is the numerical aperture of the sample arm optical fiber, and \( f_C, f_{L2} \) and \( f_{L1} \) are the focal lengths for \( C, L1 \) and \( L2 \) respectively. Experimentally, the value for \( r_{\text{cor}} \) can be confirmed with a knife-edge experiment that measures the bundle width by evaluating its Gaussian intensity distribution [41]. Assuming diffraction limited focusing by the eye the size (diameter) \( d_{\text{ret}} \) of the focused spot on the retina can then be calculated using the equations for the focusing of a Gaussian laser beam [42]:

\[
d_{\text{ret}} = \frac{2 \cdot \lambda_0 \cdot f_{\text{eye}}}{\pi \cdot r_{\text{cor}}},
\]

with \( \lambda_0 \) as the center wavelength of the OCT light source. In practice ocular aberrations will limit the minimum achievable spot size on the retina and lead to increased values for \( d_{\text{ret}} \). The angular FOV \( \theta_{\text{FOV}} \) for imaging the retina is defined by the angle \( \theta_{\text{galvo}} \) over which the galvo mirrors scan and the angular magnification of the L1-L2 telescope:

\[
\theta_{\text{FOV}} = \theta_{\text{galvo}} \cdot 2 \cdot (f_{L1}/f_{L2}).
\]

The angular FOV can be converted into an approximate distance \( l_{\text{FOV}} \) on the retina
to describe the imaged retinal surface area which is often reported in the OCT literature:

\[ l_{\text{FOV}} = 2 \cdot f_{\text{eye}} \cdot \tan(\theta_{\text{FOV}}/2). \]  

(2.30)

### 2.4.2 Retinal OCT wavelength ranges, laser safety, and SNR estimation

The wavelength ranges that can be used for OCT imaging of the posterior eye are mainly restricted by three parameters: the available broadband light sources, light absorption by water in the ocular media, and the maximum permissible exposure (MPE) that ensures a safe level of laser radiation.

The broadband light sources used in early OCT systems were developed for fiber-optic telecommunication applications and operated in the 850 nm and 1310 nm ranges [43]. Light that double passes the human eye however traverses through ~48 mm of water containing ocular media and can be significantly absorbed. This restricted OCT imaging of the posterior eye to the 850 nm range. In Fig. 2-8 the transmission of near infrared light between 700 nm and 1400 nm is shown for double passing the eye as function of wavelength by a blue dashed curve [44]. In this figure the nowadays commonly used OCT wavelength ranges are marked by light red areas. It can be clearly seen that the transmission is high for the 850 nm range, while for the 1310 nm range nearly all the light is absorbed. In between both wavelength ranges a peak in the transmission spectrum can be seen around 1070 nm. The improved transmission through water in this wavelength area triggered the development of new OCT light sources in the 1050 nm range. The reduced

Fig. 2-8. The double-pass transmission of the eye is visualized as a function of wavelength by the dashed blue curve. The transmission was calculated from optical absorption through 48 mm of water. The maximum permissible exposure as a function of wavelength for ocular exposure to laser radiation is given in accordance with ANSI Z136.1-2007 by the green curve. The three commonly used wavelength ranges for OCT imaging are marked in the figure in light red.
optical scattering of tissue at longer wavelengths [45] allows 1050 nm OCT to penetrate deeper into the posterior eye tissues and can better visualize the choroid and sclera compared to 850 nm OCT [46]. The penalty of switching to this longer wavelength range is a small increase in the OCT axial resolution as can be seen from Eq. (2.3).

In order to protect the eye from damage induced by laser irradiation only safe power levels should be used. In the work presented in this thesis the laser power levels were in accordance with the American National Standard Z136.1-2007 [47]. This standard provides guidelines on how to calculate the MPE for ocular exposure that are at least 10 times lower than the tissue damage threshold. In the most strict interpretation of the standard, the MPE for OCT imaging of the posterior eye is calculated according to Rule 2 and protects against cumulative injury for a single retinal location that is irradiated longer than 10 s with a spot diameter of 25 µm or smaller [48]. In Fig. 2-8 the MPE according to this definition is plotted as a function of wavelength by a solid green line. It can be seen that the MPE is low when the ocular transmission is high and abruptly increases after 1150 nm where the ocular transmission becomes negligible. It can further be seen that the MPE for 850 nm OCT (0.78 mW) is 2.5 times lower than for 1050 nm OCT (1.93 mW) and even 20 times lower than for 1310 nm OCT (15.58 mW).

Based on the known ocular transmission and the MPEs for the three OCT wavelength ranges the SNR can be estimated for in vivo FD-OCT measurements of the posterior eye. In this calculation a perfect FD-OCT system was assumed without optical losses for which the SNR is described by Eq. (2.13). Three OCT system configurations were evaluated with typical spectral bandwidth specifications of 850 ± 25 nm [49], 1040 ± 50 nm [24] and 1310 ± 50 nm [50]. The optical power returning from the sample was described as:

$$P_{\text{sample}}(\lambda) = \text{MPE}(\lambda) \cdot T_{\text{ocular}}(\lambda) \cdot \chi,$$

(2.31)

where $T_{\text{ocular}}(\lambda)$ is the ocular transmission in double-pass as given in Fig. 2-8, and $\chi$ is the fraction of the detected input photons for a single tissue depth and depends on tissue scattering and the collection efficiency of the optical system. In the SNR calculation $\chi$ was assumed wavelength independent and set at $10^{-6}$ [51], $\tau_{\text{A-scan}}$ was set at 10 µs corresponding to a 100 kHz A-scan repetition rate [24], and the detector quantum efficiency $q(\lambda)$ for each wavelength range was calculated from the responsivity of widely available photodetectors [52]. The parameters for $\text{MPE}(\lambda)$, $T_{\text{ocular}}(\lambda)$ and $q(\lambda)$ are given in Table 2-1 together with the calculated SNR. It can be seen from Table 2-1 that for the 1050 nm OCT the increase in the $\text{MPE}(\lambda)$ and $q(\lambda)$ with respect to 850 nm OCT compensates for the decrease in $T_{\text{ocular}}(\lambda)$ and achieves an equivalent SNR. This is not the case for 1310 nm OCT where the decrease in $T_{\text{ocular}}(\lambda)$ is not compensated by the higher $\text{MPE}(\lambda)$ and results in a
>23 dB lower SNR compared to the other OCT wavelength ranges. In the human retina the OCT signal intensity of the different tissue layers varies over a 20 dB to 30 dB range [53]. Under this condition the >40 dB SNR provided by 850 nm and 1050 nm OCT allows for the visualization of all retinal tissue tissues, while the ~20 dB SNR of 1310 nm OCT is not sufficient for this purpose. OCT in the 1310 nm range is therefore never used for in vivo imaging of the posterior eye.

In the scientific work presented in this thesis SS-OCT was used in the 1050 nm wavelength range to benefit from its increased penetration depth in tissue for improved visualization of the choroid.

<table>
<thead>
<tr>
<th>Wavelength range</th>
<th>MPE(λ)</th>
<th>Tocular(λ)</th>
<th>q(λ)</th>
<th>SNR</th>
</tr>
</thead>
<tbody>
<tr>
<td>850 ± 25 nm</td>
<td>0.78 mW</td>
<td>0.82</td>
<td>0.77</td>
<td>43.3 dB</td>
</tr>
<tr>
<td>1040 ± 50 nm</td>
<td>1.93 mW</td>
<td>0.30</td>
<td>0.84</td>
<td>44.1 dB</td>
</tr>
<tr>
<td>1310 ± 50 nm</td>
<td>15.6 mW</td>
<td>1.15E-4</td>
<td>0.88</td>
<td>20.2 dB</td>
</tr>
</tbody>
</table>

**Table 2-1.** Calculation of the SNR for in vivo OCT measurements of the posterior eye with three different wavelength ranges. The calculations were done according to Eqs. (2.13) and (2.31) and used the tabulated values for MPE(λ), Tocular(λ) and q(λ) which were the average values for the wavelength ranges.

**References**


52. Thorlabs, “Manual for Balanced Amplified Photodetectors PDB400 Series”, v1.0 (08/12/2010).