Background

People have always been fascinated by the human eye. As the organ that provides us with the sense of sight, it allows us to recognize and learn from our environment from a safe distance. Our eyes are used for almost every activity we perform, like reading this thesis, and blindness is one of the most feared disabilities. It is therefore not surprising that the earliest known reports on the value of the human eye and the treatment of ocular pathology were written several thousands of years ago in ancient Babylon and Egypt [1]. In these times the practice of medicine was mixed with superstition and magic, and up to today people still believe the eye holds spiritual properties. As an example, a famous proverb says that “the eyes are the mirror of the soul” which means as much as that the eyes express the true intent and feelings of a person. From a biological point-of-view this is partly true, since the eye and its movements reflect activity of the brain and can for instance tell us where you are looking on this page. However, before you (and your brain) are aware of the text you are reading, your eyes process an image of this page and convert it into electrical signals that are transmitted to the brain. The work presented in this thesis focuses on the visualization and functional assessment of the structures at the back of the living human eye that are responsible for this signal conversion. This was achieved by the construction of specialized microscopes for the eye based on optical coherence tomography (OCT) imaging techniques.
1.1 Introducing the human eye

The clinical discipline that deals with the anatomy, physiology and diseases of the eye is called ophthalmology. However, in order to comprehend the complex structure and function of the eye often knowledge from other disciplines is also used. A good example is the use of physics for the calculation of appropriate spectacles to correct someone’s vision. Vision science is the scientific field that brings together all scientific disciplines that perform research on vision and the eye, and encompasses ophthalmology and parts of the fields of neuroscience, physics and many others. It is thanks to the effort of the people in this field that so much is (already) known about the eye.

1.1.1 Eye anatomy

The human eye is an approximately spherical organ with a diameter of around 24 mm in its mature state [2]. Several (semi-) transparent tissue structures guide incoming light towards the back of the eye, which are shown schematically in Fig. 1-1. The front part of the eye is called the cornea, a spherical tissue structure that is responsible for two-thirds of the light refraction. Light refracted by the cornea travels through the anterior eye chamber, which is filled with aqueous fluid, and
passes the iris. The aperture of the iris is called the pupil and has an adaptable diameter to control the amount of light that is sent through. Consecutively, the (crystalline) lens focuses the light at the back of the eye. The lens is responsible for a third of the refractive power of the eye and can change in curvature, and therefore in refractive power, through a process called accommodation. This allows us to see objects sharply at different distances. Together, the aforementioned components are called the anterior eye segment.

After passing the lens the light enters the posterior eye segment through a gel body called the vitreous before it arrives at the back of the eye. The inside surface of the back of the eye consists of the retina, the tissue layer that holds the photosensitive rods and cones that convert light into electrical signals. The choroid is a dense layer of vasculature adjacent to the outside of the retina. The posterior eye is enclosed by the sclera, an opaque tissue layer that merges with the transparent cornea to provide together an protective enclosure to the eye. Our central vision is registered by the center part of the retina, called the macula, which is also known as the yellow spot. In the center of the macula the tissue structures form a small depression at the inner retinal surface with a high concentration of cones. This retinal location is called the fovea and creates our sharp central color vision which is used to observe details.

The retinal tissues are supplied with nutrients and oxygen by two distinct vascular networks. The choroid supplies the outer retinal tissues, while the retinal circulation provides the inner retinal tissues. The retinal circulation consists of several arteries and veins that enter the eye via the optic nerve head (ONH), and branches out into an elaborate network of smaller vessels and capillaries. This network is characterized by an avascular zone in the fovea which prevents distortions and light loss in our central vision. The ONH is placed ~5 mm to the nasal side from the foveal center and consists of a depression that interrupts the retina and acts as a passageway to the outside of the eye. At this location the axons (nerve fibers) from the retina bundle together into the optic nerve which leaves at the back of the eye to conduct electrical signals to the brain. The ONH is also known as the blind spot, since the local absence of rods and cones causes a break in the visual field.

1.1.2 Retinal anatomy

The healthy retina has a thickness roughly between 150 µm and 300 µm in the macula [3] and is organized into multiple tissue layers with different functions and cell components [2,4]. In Fig. 1-2 a schematic drawing of the macula including the fovea is given together with a magnified view of the layered structure. Light that falls onto the retina travels through these layers for which the order and a description are given below.
The inner limiting membrane (ILM) forms the boundary between the retina and the vitreous, and consists of a basal lamina.

The retinal nerve fiber layer (RNFL) contains the axons of the retinal Ganglion cells and forms the top layer at the inner retinal surface.

The Ganglion cell layer (GCL) holds displaced amacrine cells and the Ganglion cell bodies that generate the retinal action potentials.

The inner plexiform layer (IPL) holds the synapses that connect the GCL with the lower located bipolar cells.

The inner nuclear layer (INL) contains the cell bodies of the bipolar cells, amacrine cells and horizontal cells, which carry out the initial processing of the visual signals.

The outer plexiform layer (OPL) consists of the synapses that connect the bipolar cells with the ends of the rods and cones. In the macula, this layer is also called Henle’s fiber layer.

The outer nuclear layer (ONL) contains the cell bodies of the rods and the cones.

The photoreceptor layer (PRL) includes the external limiting membrane, and the photoreceptive segments of the rods and the cones that are responsible for the light detection.
• The retinal pigment epithelium (RPE) is the most outer layer of the retina and consists of a single layer of cuboidal cells which contain the pigment melanin.
• The choriocapillaris (Cc) is a mesh-like capillary layer of the choroid directly below the retina from which nutrients diffuse into the retina.
• The choroid (C) is the layer of vasculature that supplies the choriocapillaris.

It is noteworthy here that the rods and the cones are not located at the inner retinal surface as one might expect, but deeper inside the retina. Consequently the signal transduction pathway that forms the retinal action potentials follows the reverse path of the light and occurs from the PRL to the RNFL.

In the fovea the inner nuclear and ganglion cells are displaced from the foveal center towards its periphery [4]. This creates the characteristic foveal depression that supports the high concentration of cones. Consequently, the RNFL, GCL, IPL and INL are (nearly) absent in the center of the fovea.

1.1.3 Ocular pathology in this thesis

Pathology affecting the posterior eye often leads to irreversible damage to the retina and the ONH. If left untreated, the damage is likely to progress over time and causes a (partial) loss of vision and eventually blindness. In the western world two leading causes of blindness are age-related macular degeneration (AMD) and glaucoma [5]. Patients suffering from either of these diseases are presented in this thesis to show the clinical applicability of the developed OCT imaging techniques.

In AMD the retinal tissues of the macula deteriorate and break down (degenerate) as an effect of the body’s natural aging process [6,7]. AMD affects therefore mainly older adults and causes (partial) loss of a person’s central vision. Two distinct forms of AMD are clinically classified: dry (classic) AMD and wet (neovascular) AMD. In dry AMD proteins and lipids accumulate between the retina and choroid in so-called drusen, which are assumed to deprive the RPE and photoreceptor layer of oxygen and nutrients. The development of small sized drusen with advancing age occurs without vision loss and is considered normal, but can become pathological when their size and number increases. In wet AMD abnormal blood vessels are formed that grow from the choroid (through the RPE) into the retina. This process is called choroidal neovascularization and often creates blood vessels that leak fluid or blood. Especially this leakage and the corresponding damage to the retina causes vision losses. It is not uncommon that patients are simultaneously affected by both AMD types. Currently no solution exists to prevent AMD and treatment focuses on slowing down the disease progression via the administration of vitamin supplements, lifestyle and dietary modifications, and drug and laser therapies that suppress and regress neovascularization.
Glaucoma describes a group of ocular disorders that all suffer from intraocular pressure-associated damage to the optic nerve (fibers) [8]. In glaucoma patients the fluid pressure in the anterior eye chamber is usually chronically elevated, which pushes the lens back against the vitreous. The subsequent pressure increase in the posterior eye chamber causes mechanical stress onto the ONH and the retinal vasculature [9]. This results in damage to the optic nerve and an insufficient blood supply to the inner retinal layers and, if left untreated, leads to progressive loss of the Ganglion cell axons. The corresponding thinning of the RNFL is among the first signs of glaucoma manifestation, and the RNFL thickness is therefore an important parameter in the detection of this disease. Vision loss typically starts in the periphery of the visual field and leads to a progressively worsening tunnel vision. Axonal loss due to glaucoma is permanent and, in analogy to AMD, treatment is focused on slowing down the disease progression. This is achieved by medication or surgery that restores the intraocular pressure to normal values.

1.2 Posterior eye imaging with ophthalmoscopes

In 1851 a new era began for ophthalmology with the introduction of the ophthalmoscope by Hermann von Helmholtz [10]. He constructed and popularized a fairly simple device to solve the mystery of how to look through the black pupil and view the in vivo fundus (the back of the eye). The ophthalmoscope, or Augenspiegel as Helmholtz called it, consisted of three basic components: an illumination source, a reflecting surface to direct light in to the subject’s eye, and a concave lens to correct the out-of-focus fundus image [11]. The ophthalmoscope was operated by holding it manually in front of the subject’s eye and the operator looked through it to observe the fundus. The introduction of new stable light sources and improved optical designs developed the ophthalmoscope into the modern day fundus camera. A fundus camera acquires a two-dimensional photo from the back of the eye with visible light for which an example from a healthy subject is shown in Fig. 1-3(A). The fundus photo gives a flattened view of the structures of the retina from which the larger retinal vessels, the ONH and the fovea can easily be identified. The visualization of vasculature can be improved by the administration of the fluorescent contrast dyes fluorescein or indocyanin green to the subject [12]. So-called fluorescein angiography and indocyanin green angiography are routinely used in the clinic to study retinal pathology that involves vascular changes, such as choroidal neovascularizations and fluid leakage.

The development of laser light sources and optical raster scanning methods gave rise to a new class of ophthalmoscopes: the scanning laser ophthalmoscope (SLO). An SLO projects a focused laser spot onto the retina from which the reflected light is detected while the spot is rapidly scanned over an area [13]. This
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Technique can be performed at high scanning speeds to create real-time images of the retina and requires substantially less light than conventional ophthalmoscopes and fundus cameras. In addition SLO can be complemented with confocal detection to reject out-of-focus light for improved contrast [14], which turns SLO into an in vivo confocal microscope for the retina. An example of a confocal SLO (cSLO) image is given in Fig. 1-3(B) which shows the retina of the same healthy subject as earlier visualized with a fundus camera. cSLO has developed over the years into a versatile retinal imaging technology including tailored implementations for the detection of RPE autofluorescence [15] and RNFL polarization properties [16]. In the work presented in this thesis cSLO and its aforementioned tailed implementations were used as auxiliary measurement devices for the guidance, comparison, and improvement of the performed OCT measurements.

The tremendous amount of knowledge that can be obtained via ex vivo microscopic histology made researchers realize that visualization of all three dimensions (including depth) is essential for a better understanding of in vivo tissue morphology and pathology [17]. In the late 1980s and early 1990s a novel optical imaging technology was introduced for this exact purpose called optical coherence tomography (OCT). In OCT the echo of reflected light is measured to determine the distance to a tissue structure with micrometer resolution, and used to obtain a cross-sectional (depth) profile of the tissue. In order to capture the very short echo delay times of light, OCT uses low-coherence laser interferometry for which the methodology is described in chapter 2 of this thesis. Also called the optical analogue of ultrasound imaging, OCT was originally used to measure the anatomical distances between the

![Fig. 1-3. Photos from the back of the left eye of a healthy volunteer taken with (A) a fundus camera and (B) a confocal scanning laser ophthalmoscope (cSLO). Both give a flattened view on the structures of the retina from which the larger retinal blood vessels, the optic nerve head and the fovea can easily be identified. The bright spot in the center of the cSLO image is a lens reflection from within the instrument.](image)
different ocular components [18,19]. Following cSLO, OCT was complemented with optical raster scanning to visualize the microscopic tissue structures at the back of the eye in three dimensions [20,21]. The strength of OCT is best visualized in an image, as given in Fig. 1-4, that displays an in vivo cross-section of the back of a healthy eye. In this image the structures of the fovea and ONH are immediately recognized as well as several different tissue layers within the retina. Depending on the optical properties of each tissue the amount of reflected light changes, which forms the contrast (the grayscale) in the OCT image. The real-time non-invasive cross-sectional visualization of in vivo tissue microstructures with OCT approaches the quality of ex vivo histology and forms an entirely new way to study and diagnose pathology. After its initial application in ophthalmology, OCT rapidly spread to other medical fields including dermatology [22], cardiology [23], pulmonology [24], and gastroenterology [25].

![Fig. 1-4. Cross-sectional image of the back of the eye as obtained in vivo with OCT from a healthy volunteer. The structures of the fovea and ONH are easily recognized as well as several different tissue layers within the retina. The image size is 1.5 mm in depth and 9.0 mm in width. Abbreviations: see caption of Fig. 1-2. The image is based on data from chapter 6 of this thesis.](image)

### 1.3 Thesis aim and outline

Nowadays OCT is an established technique in ophthalmology for structural imaging and routinely used for diagnosing retinal pathology. Structure is, however, only one aspect of living tissue. Functional processes within the tissue, like blood flow, can give additional information on tissue health. The extraction of functional information from OCT signals has led to several novel OCT implementations including Doppler OCT for the visualization of blood flow (angiography) and polarization-sensitive OCT for optical polarization analysis of fiber structures (polarimetry). The aim of the work presented in this thesis was to further develop these two functional OCT methods, to combine them with the state-of-the-art OCT technology, and to apply them for non-invasive angiography and polarimetry of the in vivo
posterior human eye.

In order to extract functional information from OCT signals one has to exactly understand its origin and the corresponding physical modeling. For this purpose, chapter 2 of this thesis discusses the principles of OCT (low coherence laser interferometry) and gives an introduction on Doppler OCT and polarization-sensitive OCT. The fundamental knowledge of this chapter will form the basis for the scientific work presented in chapters 3 to 6.

Chapter 3 describes the construction and calibration of an experimental swept-source OCT instrument operating in the 1050 nm wavelength range for the improved visualization of the choroid. Doppler OCT was implemented on this instrument to detect blood flow in the retinal and choroidal vasculature in a healthy individual. The clinical applicability of the instrument is demonstrated by Doppler OCT measurements in an AMD patient to visualize the process of revascularization after transplantation surgery on the RPE and choroid.

In chapter 4 a novel Doppler OCT method is presented using a backstitched raster scanning pattern that was designed to improve the blood flow detection sensitivity. The scanning pattern was optimized to visualize blood flow down to the capillary level and allowed angiographic imaging of the complete retinal and choroidal circulation in a healthy individual. The clinical feasibility of the system is shown by the detection of neovascular blood vessels in an AMD patient.

The correction of eye motion artifacts in Doppler OCT angiography using real-time eye tracking is described in chapter 5. A separate high-speed cSLO system was coupled together with the OCT instrument and provided real-time feedback to correct the OCT raster scanning for motion of the eye. This allowed the acquisition of undistorted Doppler OCT angiograms and the accurate compounding of multiple datasets for noise reduction. High quality artifact-free angiograms are presented of the retina, the choroid, and the choriocapillaris from the fovea of a healthy subject.

Chapter 6 describes the construction of a polarization-sensitive swept-source OCT instrument based on passive components in the 1050 nm wavelength range. A novel Jones matrix analysis method is presented in which the retinal tissues are used as a calibration object to compensate for polarization distortions induced by hardware components. The instrument was used to visualize the polarization properties of fibrous structures in the retina, in particular the RNFL and Henle’s fiber layer (outer plexiform layer), of a healthy subject and a glaucoma patient. In the glaucoma case significant loss of the RNFL was observed which demonstrated the clinical applicability of polarimetry with PS-OCT using the novel Jones matrix analysis method.

The scientific content of this thesis ends with chapter 7 in which an overall discussion on the obtained results and an outlook on future research is given.
References

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