Chapter 1

General introduction
Glaucoma has become the leading cause of global irreversible blindness (Tham et al., 2014; Bourne et al., 2016). It has been estimated that 64 million people aged 40–80 years were affected by glaucoma worldwide in 2013, and this number is predicted to increase to 76 million in 2020 and to 112 million in 2040 (Bourne et al., 2016). Glaucoma is a heterogeneous group of diseases characterized by progressive loss of retinal ganglion cells (RGCs), thinning of the retinal nerve fiber layer (RNFL) and loss of visual field function. Primary open angle glaucoma (POAG) is one of major types of glaucoma, which comprises the majority of glaucoma cases around the world. It is well known that the progression of POAG can be limited with effective treatment (Leske et al., 2003). As POAG is usually painless and symptoms occur late, early detection is important.

1. Diagnosing Glaucoma

Currently, there is not yet a single discriminatory test to diagnose glaucoma accurately. The diagnosis of glaucoma relies mainly on the assessment of structural damages of the optic nerve and corresponding functional damages (such as visual field defects). Evaluation of both the structural and functional damage of the optic nerve is important in glaucoma diagnosis. Glaucomatous structural damage of the optic nerve head includes retinal nerve fibre layer thinning, neuroretinal rim tissue loss (presented as an enlargement and deepening of the optic disc cup), and optic disc hemorrhages. These structural changes can be evaluated by ophthalmoscopy in clinical practice or by imaging modalities. However, considerable anatomical variation of the measurements of the optic nerve has been widely reported, which may confound the detection of glaucoma (Ghadiali et al., 2008; Wang et al., 2013; Cheung et al., 2011; Mwanza et al., 2011).

2. Myopia and glaucoma

Myopia has been emerging as a global public health issue because of its growing prevalence during the past few decades, especially in China. Xu et al. reported in
the Beijing Eye Study that the prevalence of myopia for definitions of spherical equivalent (SE) of $<-0.50 \text{ D}$, and $<-6.0 \text{ D}$ were 22.9% and 2.6% in people aged 40 to 90 years, respectively (Xu et al., 2005). In another rural Chinese adult (aged 30 years and older) population study, the prevalence of myopia, defined as a spherical equivalent (SE) in the right eye of more than -0.5 diopter (D), was reported to be 26.7% (Liang et al., 2009).

The association between myopia and glaucoma has been well described previously (Marcus et al., 2011). Although the underlying mechanism between myopia and glaucoma is not fully understood, several population-based studies demonstrated that the prevalence of glaucoma increased with increasing myopia (Marcus et al., 2011). As myopia is a worldwide common condition and a major risk factor for glaucoma (Marcus et al., 2011; Morgan et al., 2012), it is clinically important to be able to diagnose glaucoma in myopic subjects. However, myopia has been reported to be associated with anatomical changes of the optic disc, increased intraocular pressure, and visual field defects. These factors make the diagnosis of glaucoma in myopic subjects challenging.

2.1. Challenges of functional assessment in diagnosing glaucoma in myopic eyes

It is well known that myopic eyes are associated with a variety of visual field defects (Chang et al., 2013; Shoeibi et al., 2017; Lee et al., 2018). Optic disc tilt, disc torsion, and peripapillary atrophy (PPA) have been reported to be associated with myopia and visual field abnormalities (Tay et al., 2005; Shimada et al., 2007; Lee et al., 2014; Sung et al., 2016). This hampers the use of perimetry for excluding glaucoma in cases in which the optic disc is difficult to assess.

2.2. Challenges of structural assessment in diagnosing glaucoma in myopic eyes
Myopia is characterized by axial elongation of the eyeball. Besides, asymmetrical elongation could exist in myopic eyes. Different globe shape, including spheroidal, ellipsoidal, conical, nasally distorted, temporally distorted, and barrel shapes have been reported in myopia especially high myopia (Guo et al., 2017). Due to the globe elongation, myopia is associated with optic disc abnormalities and ocular complications. It has been reported that myopic eyes are more likely to have tilted, rotated, large disc (Takasaki et al., 2013; Sung et al., 2016). Moreover, myopic eyes are associated with chorioretinal abnormalities such as chorioretinal atrophy, posterior staphyloma, and myopic maculopathy (Chang et al., 2013; Ohno-Matsui et al., 2016). As a result, anatomical structures of myopic eyes easily fall outside the normal limits based on emmetropic eyes, and if the normal limits would be widened to include the myopic eyes as well, early glaucoma could easily be overlooked.

3. Assessing structure in glaucoma

3.1. Assessment of the optic disc

Glaucomatous ONH changes are characterized by enlargement of the cup-disc ratio, progressive neuroretinal rim thinning, disc hemorrhages, and definite disc cupping in severe cases (Spaeth et al., 2006; Kim et al., 2017). Before the development of imaging devices (see 3.2.), assessment of the ONH was usually based on fundus biomicroscopy or photography (Figure 1). Jonas et al. first introduced the ISNT rule for glaucoma diagnosis (Jonas et al., 1988; Jonas et al., 1998). This rule describes that, in healthy eyes, the thickness of the rim follows the pattern inferior > superior > nasal > temporal.
It has been reported that optic discs in myopic eyes can be abnormally small or abnormally large (Hawker et al., 2006). It is also well known that myopic eyes are associated with disc tilt, disc torsion, and PPA (Figure 2) (Tay et al., 2005; Marsh-Tootle et al., 2017). These disc anomalies make it difficult to determine the disc margin in myopic eyes, hampering the determination of the cup-disc ratio and the application of the ISNT rule.
3.2. Imaging techniques for assessment of structure in glaucoma

Traditionally, assessment of the optic nerve is based on fundus biomicroscopy or photography. However, these two methods, especially biomicroscopy, rely on the ability and experience of the observer which leads to considerable variability amongst observers (Abrams et al., 1994). Recently, several non-invasive imaging techniques including confocal scanning laser ophthalmoscopy (HRT) and optical coherence tomography (OCT) have been developed for objective assessment of glaucomatous structural damage (Huang et al., 1991; Woon et al., 1992; Schuman et al., 1995; Fallon et al., 2017). OCT is the primary technique used in this thesis. HRT has been used in some of the studies as well.

The confocal scanning laser ophthalmoscope of the HRT uses a diode laser (670 nm) to scan the retinal surface at multiple focal planes axially along the optic nerve head. Subsequently, a three-dimensional image is constructed. By setting a reference plane placed on the retinal surface, relative topographic measurements (disc area, rim area, cup to disc ratio, etc) can be calculated. Previous studies have shown that HRT is useful in the diagnosis and progression monitoring of glaucoma (Leung et al., 2010; Lucenteforte et al., 2015).

Optical coherence tomography (OCT) is a high-resolution imaging technique that allows for in vivo cross-sectional imaging of the ONH and retina. OCT technology is based on low-coherence interferometry. It measures the reflectivity for near infrared radiation of retinal tissue, with a high axial resolution (1 to 5 μm). A measurement at a single location is called an A-scan (reflectivity along a line perpendicular to the retina). A series of A-scans provide a B-scan, and a series of B-scans gives a 3D image of the retina. Scan speeds of more than 50000 A-scans per second have been reported (Kiernan et al., 2010). With OCT, the peripapillary retinal nerve fiber layer as well as individual macular inner retinal layers can be measured (Figure 3). It has been shown that measurements of the macular area and the optic nerve head (ONH) are useful in glaucoma diagnosis and disease monitoring (Lucenteforte et al., 2015; Dong et al., 2016; Oddone et al., 2016).
Figure 3. The optic disc scan (A) and macula scan (B) made with a commercial OCT instrument. A. Left: The 6×6 mm² optic disc scan area with a 3.4 mm diameter measurement circle (green circle) centered at the optic disc; Right upper: The reconstructed cross sectional image (B scan image) of the peripapillary retina at the measurement circle, with segmented retinal nerve fiber layer (RNFL); Right lower: The peripapillary RNFL thickness profile at the measurement circle as derived from the cross sectional image. B. Left: The 6×6 mm² macular scan area (green square) centered at the macula; Right: The cross sectional image (B scan image) of the macula with segmentation of individual retinal layers.

NFL: nerve fiber layer; GCL: ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; IS/OS: photoreceptor; RPE: retinal pigment epithelium.

3.3. Assessment of the peripapillary RNFL

Evaluation of peripapillary RNFL is useful for the assessment of glaucomatous damage. The RNFL can be assessed through ophthalmoscopy and by using imaging techniques such as red-free fundus photography and OCT imaging. Evaluation of the RNFL in red-free fundus photos is much more difficult in
myopic eyes, and especially in high myopic eyes, due to atrophy and thinning of choroid and retina (decreased visibility of the RNFL).

Although in vivo measurement of RNFL thickness with OCT is emerging as an important diagnostic technology for glaucoma (Figure 4), considerable anatomical variation of the RNFL thickness profile has been reported, which confounds the assessment of glaucoma (Ghadiali et al., 2008; Wang et al., 2013; Cheung et al., 2011). Previous studies have investigated the peripapillary RNFL thickness profile in myopic eyes (Kim et al., 2010; Hong et al., 2010; Wang et al., 2010; Leung et al., 2012; Yamashita et al., 2017). It has been shown that myopic eyes have a thicker temporal RNFL (Kim et al., 2010; Wang et al., 2010). Leung et al. (2012) studied the radial axes of the superotemporal and inferotemporal RNFL bundles as determined in the RNFL thickness map in 189 myopic eyes. They reported that the superotemporal and inferotemporal RNFL bundles converge temporally with increasing myopia (Leung et al., 2012).

A high proportion of abnormal (that is, false-positive, Figure 5) diagnostic classification for OCT RNFL thickness measurements was reported in healthy subjects, especially in myopic subjects (Qiu et al., 2011; Aref et al., 2014; Kim et al., 2015). This could hamper the diagnostic performance of RNFL parameters in myopic eyes. Indeed, a worse diagnostic performance of RNFL parameters was reported in myopic eyes compared to nonmyopic eyes (Choi et al., 2013; Akashi et al., 2015). It has been reported that the diagnostic performance of RNFL measurements for the detection of glaucoma in myopia significantly improved after application of a myopic normative database (Biswas et al., 2016; Seol et al., 2017).
Figure 4. A peripapillary RNFL thickness printout from a glaucoma patient made with a commercial OCT instrument (Cirrus HD OCT). The patient has glaucoma in his left eye. He has a normal visual field in his right eye and a superior visual field defect in his left eye. The OCT printout shows thinning of the RNFL in the inferior region (in agreement with a superior visual field defect) in the left eye and a normal RNFL thickness in the right eye, based on the built-in normative database.
Figure 5. A peripapillary RNFL thickness printout from a healthy myopic subject made with the same commercial OCT instrument as Figure 4. The subject has normal visual fields in both eyes. The OCT printout shows thinning of the RNFL (false-positive results) in both eyes, based on the built-in normative database.

3.4. Assessment of macular thickness and macular inner retinal layers

The macular region is important for the assessment of glaucomatous damage, as this area has the highest density of retinal ganglion cells (RGCs) (Curcio et al.,
With the development of OCT technology, in vivo measurement of the thickness of various macular layers has become feasible. This includes the macular RNFL (mRNFL), the ganglion cell-inner plexiform layer (GCIPL), and the ganglion cell complex (GCC; a combined measurement of mRNFL and GCIPL). A significant thinning of the thickness of the macular inner retinal layers has been reported in glaucomatous eyes, compared to nonglaucomatous eyes (Figure 6). Previous studies have shown that thickness measurements of macular inner retinal layers have a similar glaucoma discriminating performance when compared to thickness measurements of the peripapillary retinal nerve fiber layer (pRNFL) (see Springelkamp et al., 2014 for an overview).

While macular measurements with OCT are useful for glaucoma detection, significant variation of macular structures in healthy individuals and especially myopic subjects has confounded the detection of glaucoma (Figure 7) (Lam et al., 2007; Mwanza et al., 2011; Koh et al., 2012; Takeyama et al., 2014; Zhao et al., 2013; Kim et al., 2015; Leal-Fonseca et al., 2014; Aref et al., 2014; Akashi et al., 2015; Zhao et al., 2016;). As such, the macula does not offer a simple solution for the confounding effects of myopia in the assessment of the peripapillary RNFL.
Figure 6. Two macular imaging printouts from a normal eye (A) and a glaucomatous eye (B) made with a commercial OCT instrument (Topcon 3D OCT 2000). A: The printout shows that the thicknesses of the RNFL (retinal nerve fiber layer), GCL+ (ganglion cell layer and inner plexiform layer), and GCL++ (combination of RNFL and GCL+) are within normal range. B: The printout shows significant thinning (mainly in inferior region) of the RNFL, GCL+, and GCL++. 
Figure 7. A macular imaging printout from a healthy myopic eye made with the same commercial OCT instrument as Figure 6. The printout shows significant thinning of the RNFL (retinal nerve fiber layer), GCL+ (ganglion cell layer and inner plexiform layer), and GCL++ (combination of RNFL and GCL+), based on the built-in normative database.

3.5. Assessment of the retinal nerve fiber bundle (RNFB) trajectories and structure-function map

As there is no single discriminatory test to diagnose glaucoma accurately, a detailed anatomical knowledge of the retinal nerve fiber bundle (RNFB) trajectories is helpful to integrate structure and functional visual field data to produce the structure-function map, which could improve the detection of glaucoma. Based on fundus photographs, the Garway-Heath model for describing nerve fiber bundle trajectories was reported in 2000 and has been shown to be
useful in clinical practice. Later, several other models describing the RNFB trajectories have been reported (Wigelius, 2001; Ferreras et al., 2008; Turpin et al., 2009; Jansonius et al., 2009). Moreover, a considerable variability of the RNFB trajectories was reported and the influence of refraction/axial length has been studied (Denniss et al., 2012; Jansonius et al., 2012; Lamparter et al., 2013). The exact role of myopia in these maps still has to be defined.

4. Aim and outline of this thesis

Diagnosing glaucoma in myopic eyes is challenging. The aim of the current thesis is to uncover the determinants and characteristics of the anatomical structures relevant to glaucoma in myopic eyes. For this purpose, we studied:
(1). The determinants and characteristics of the RNFB trajectories in myopic eyes;
(2). The determinants and characteristics of the pRNFL thickness profile in myopic eyes;
(3). The determinants of macular inner retinal layer thicknesses in myopic eyes;
(4). The glaucoma diagnostic classification based on thickness measurements of the pRNFL and the macular inner retinal layers in myopic eyes, as provided by commercially available OCT devices.

Chapter 2 addresses the question whether retinal vessel topography affects the RNFB trajectories in the Caucasian human retina. In Chapter 3, the characteristics and determinants of the RNFB trajectories in Chinese myopic eyes are determined and compared to that of Caucasians.

Chapter 4 evaluates the ISNT rule in myopic eyes. In this chapter, application of the ISNT rules to both the pRNFL thickness and the neuroretinal rim area in healthy myopic eyes is investigated and discussed.

In Chapter 5, I assess the influence of various ocular factors on the pRNFL thickness profile in healthy myopic eyes. Of all the included ocular factors, retinal vessel topography was uncovered as the most prominent predictor of the RNFL
thickness profile, for both the superior and the inferior hemiretina. **Chapter 6** describes the characteristic patterns of RNFL defects relative to the major retinal vessels in myopic eyes with and without glaucoma. This is a follow-up of the study described in Chapter 5, which confirms the important influence of the retinal vessel topography on the RNFL thickness profile. In this study, a simple and valuable approach for differentiating between false-positive and glaucoma in myopic eyes is described and discussed.

**Chapter 7** and **Chapter 8** evaluate the effect of the disc-fovea distance on the overall macular thickness and the individual retinal layers in healthy eyes. In these two studies, I found that a greater disc-fovea distance was independently associated with thinner inner retinal layers and overall retina in the macular region. **Chapter 9** is a follow-up study on Chapters 7 and 8. In this study, the influence of the optic disc-fovea distance on the glaucoma diagnostic classification based on thickness measurements of macular inner retinal layers with OCT is studied in healthy subjects.
References


