CHAPTER

Introduction
1.1 GENERAL INTRODUCTION

Glaucoma is a leading cause of irreversible blindness worldwide. It is characterized by the progressive death of retinal ganglion cells (RGCs), which leads to characteristic structural retinal changes and a distinct pattern of visual field loss. Elevated intraocular pressure (IOP) is recognized as a major risk factor for developing glaucoma, but the exact pathophysiology of glaucoma is yet to be determined.

While the clinical management of glaucoma remains focused on assessing glaucomatous changes at the level of the eye, magnetic resonance imaging (MRI) studies of glaucoma patients have shown overwhelming evidence of glaucomatous degeneration affecting the visual pathway beyond the retina. This has led to a great deal of interest in the potential role of the central visual system in the pathophysiology of glaucoma, and whether glaucomatous retinal degeneration is a primary process or a secondary manifestation of a degeneration occurring downstream in the visual pathway. Furthermore, some studies have reported the presence of degenerative brain changes outside the visual system in glaucoma patients, bringing into question the involvement of the entire brain in the glaucomatous disease process.

Although the presence of glaucomatous visual pathway degeneration beyond the retina is evident, the underlying pathophysiology of this degeneration remains unclear. Given the unique division of the visual pathway into two major white matter (WM) tracts, investigating glaucomatous WM changes could potentially hold the key to understanding the underlying mechanisms of visual pathway degeneration in glaucoma. Indeed, WM changes in glaucoma patients have been extensively investigated using diffusion tensor imaging (DTI). DTI is the most commonly utilized approach for analyzing WM using diffusion-weighted MRI (DWI). By modelling the diffusion of water molecules in nerve axons, DTI produces proxy measures of WM structural integrity. However, these measures lack direct biological interpretability, which has caused our understanding of the structural nature of glaucomatous WM changes to remain ambiguous. Furthermore, prior to the work presented in this thesis, all DTI studies of glaucoma have been cross-sectional in nature, which limited our understanding of the source and mechanism of spread glaucomatous WM changes.

The main aim of my thesis was to unravel the underpinnings of glaucomatous visual pathway degeneration. To this end, I utilized novel DWI techniques for analyzing WM to advance our understanding of the structural nature of glaucomatous visual pathway WM changes. Furthermore, to identify the source and mechanism of spread of these WM changes, I performed the first longitudinal study of visual pathway WM changes in glaucoma patients. Additionally, I explored the potential clinical application of novel DWI techniques for assessing glaucoma, particularly in scenarios where current clinical tools fail to perform adequately.
1.2 THESIS OUTLINE

• **Chapter 1** provides a general introduction to the research topic and the main aims of the research presented in this thesis. A more detailed explanation of the background of the research topic and the methodology used to undertake the research are also included.

• **Chapter 2** presents the first application of fixel-based analysis (FBA) to investigate glaucomatous visual pathway WM degeneration. FBA is a novel framework for analyzing DWI which manages to investigate differently oriented WM fiber populations within a single voxel. By doing so, FBA produces biologically interpretable measures of WM degeneration, which the conventional DTI approach is incapable of.

• **Chapter 3** explores the utility of FBA to directly assess degenerative changes of the optic nerves in glaucoma patients, and compares the performance of FBA to do so with the conventional DTI approach. The aim of this chapter is to demonstrate the potential of FBA as an alternative clinical tool for assessing glaucoma in patients where current eye exams of glaucoma fail.

• **Chapter 4** presents the first longitudinal study of the progression of glaucomatous visual pathway WM degeneration, aiming to determine the primary source and pattern of spread of this degeneration. Participants from previous cross-sectional DWI studies of glaucoma were re-invited to participate in this longitudinal study to investigate the visual pathway WM changes exhibited since the initial cross-sectional studies were conducted.

• **Chapter 5** presents the first application of neurite orientation dispersion and density imaging (NODDI) to study visual pathway WM degeneration in glaucoma. NODDI is another novel approach for analyzing DWI which aims to overcome some of the limitations of DTI. By estimating neurite density within a voxel and assessing the angular variation of neurite orientation, NODDI provides new insights into WM microstructural alterations.

• **Chapter 6** includes a general discussion of the findings presented in this thesis within the broader context of our current knowledge of the research topic, along with possible clinical implications of the findings and suggestions for future research directions.

• **Appendix 1** presents an exploratory whole-brain FBA of glaucoma patients.

• **Appendix 2** presents a framework which enables the use of FBA to assess glaucomatous degeneration of the optic nerves on an individual basis.
1.3 BACKGROUND

1.3.1 PRIMARY OPEN-ANGLE GLAUCOMA

Glaucoma is a leading cause of progressive, irreversible blindness worldwide, and its prevalence is expected to increase in the future as the world’s population ages. The term glaucoma refers to a group of optic neuropathies characterized by the death of RGCs. While the pathophysiology of glaucoma remains unclear, elevated intraocular pressure (IOP) is undoubtedly associated with RGC death, and remains the only treatable risk factor of glaucoma. IOP is dependent on the volume of aqueous humor in the anterior chamber of the eye, which is regulated through a balance between the production of aqueous humor by the ciliary body and its drainage through the trabecular meshwork.

Primary open-angle glaucoma (POAG) refers to a specific subtype of glaucoma where aqueous humor drainage is not physically obstructed, yet there is increased resistance to aqueous outflow at the trabecular meshwork, often leading to an increase in IOP. POAG is the most common subtype of glaucoma, accounting for up to 85% of glaucoma cases worldwide. POAG is also the main subtype of glaucoma investigated in this thesis.

The increased IOP in POAG is thought to exert mechanical stress on the lamina cribrosa and optic nerve (ON) fibers at the posterior pole of the eye. This leads to the deformation and remodeling of the lamina cribrosa and the interruption of the delivery of trophic factors to the RGCs from the lateral geniculate nucleus (LGN), which eventually causes the death of RGCs. These processes lead to distinct optic disc morphological changes and characteristic patterns of visual field loss. Glaucomatous optic disc morphological changes include progressive and asymmetric optic disc cupping, a large cup-to-disc ratio, a thin neuroretinal rim, and optic disc hemorrhage. Glaucomatous visual field loss usually starts as localized areas of visual field defects in the periphery, which may go unnoticed by the patients at early disease stages. If left untreated, POAG could eventually lead to complete irreversible blindness.

The latest European Glaucoma Society guidelines recommend lowering IOP as the main treatment strategy of glaucoma. This could be done through several approaches, including medication, laser surgery (such as trabeculoplasty), and incisional surgery (such as trabeculectomy). However, lowering the IOP does not seem to halt disease progression in all POAG patients. Furthermore, some POAG patients present with normal levels of IOP, a condition known as normal tension glaucoma. Therefore, while increased IOP is a major risk factor for developing POAG, other IOP-independent mechanisms likely play a role in the pathophysiology POAG. Recent evidence of visual pathway degeneration beyond the retina in POAG patients has brought into question the role of the entire visual system in the pathophysiology of POAG.
1.3.2 VISUAL PATHWAY DEGENERATION IN POAG

The visual pathway starts at the retina, where the photoreceptor cells convert light into electrical signals. These signals are then transmitted to the bipolar cells of the retina, which, in turn, transmit the signals to the RGCs. The axons of the RGCs converge at the optic disc to escape the posterior pole of the eye through the lamina cribrosa, forming the ON. The ONs of the right and left eyes meet to form the optic chiasm, where the axons arising from the nasal retinas cross to form part of the contralateral optic tracts, while those arising from the temporal retinas continue without crossing to form part of the ipsilateral optic tracts (Figure 1). The optic tracts terminate at the LGN, the neurons of which project directly to the primary visual cortex through the optic radiations. The visual pathway can be divided in relation to the LGN into pre-geniculate (or anterior) visual pathway, and post-geniculate (or posterior) visual pathway.

Postmortem and neuroimaging studies of POAG have found evidence of degenerative changes in both the pre- and post-geniculate visual pathways of POAG patients. The primary source and underlying pathophysiology of this degeneration have yet to be determined. This has led to some speculation as to whether the retinal degeneration exhibited by POAG patients is a primary process or if it is a secondary manifestation of a degenerative process occurring downstream in the post-geniculate pathway. Furthermore, some neuroimaging studies of POAG patients have reported evidence of degenerative changes outside the visual system, bringing into question the role of the brain in POAG pathophysiology.
The mechanism of spread of degenerative changes throughout the visual pathway in POAG patients can potentially be explained based on the primary source of this degeneration, which is yet to be definitively determined. Assuming that the death of RGCs in POAG is a primary degenerative process, its spread downstream to the post-geniculate pathway can be explained through a process known as anterograde trans-synaptic degeneration (Figure 2). However, if the death of RGCs in POAG is a secondary manifestation of post-geniculate visual pathway degeneration, the upstream spread of this degeneration would be caused by retrograde trans-synaptic degeneration (Figure 2). Whether antero- or retrograde trans-synaptic degeneration is responsible for the widespread degenerative changes exhibited by the entire visual pathway of POAG patients remains unclear. This lack of clarity can be attributed to the fact that, prior to the work presented in this thesis, all neuroimaging studies of POAG patients have been cross-sectional in nature, which hindered our ability to determine the primary source and pattern of spread of visual pathway degeneration in POAG. In Chapter 4, I address this issue by presenting the first longitudinal study of visual pathway degeneration in POAG.

Figure 2. Potential mechanisms of spread of glaucomatous degeneration along the visual pathway. (A) Schematic representation of the organization of the pre- and post-geniculate visual pathways. (B) Anterograde trans-synaptic degeneration describes the downstream spread of retinal ganglion cell (RGC) degeneration (black) to the post-geniculate pathway. (C) Retrograde trans-synaptic degeneration describes the upstream spread of post-geniculate pathway degeneration (black) to the pre-geniculate pathway, causing the death of the RGCs. Figure adapted from Davis, B. et al. (2016)
1.4 METHODS

1.4.1. DIFFUSION-WEIGHTED IMAGING
DWI refers to a specific type of MRI which derives tissue contrast from the random motion of water molecules within a voxel. In biological tissues, the diffusion of water molecules can be characterized as free, hindered or restricted (Figure 3). Free diffusion occurs when water molecules are free to move without encountering any boundaries or obstacles; for example, water molecules in the cerebrospinal fluid (CSF) experience free diffusion. Hindered diffusion, on the other hand, occurs when water molecules are slowed down due to encountering obstacles along their trajectory, but are not completely confined into a space by impermeable boundaries. Water molecules occupying the extracellular space experience hindered diffusion. Finally, restricted diffusion describes the constrained diffusion of water molecules by an impermeable boundary. Intracellular water molecules experience restricted diffusion, the properties of which are highly dependent on the shape of the cell in which the water molecules are confined. Due to the unique linear architecture of neuronal axons, intra-axonal restricted diffusion occurs more readily along the axis of the axons and less so across them, a phenomenon known as anisotropic diffusion (Figure 3B). This characteristic anisotropic diffusion is exploited to probe the state of WM axonal bundles, where a loss of anisotropy implies deterioration of the linear architecture of axons (Figure 3C).

1.4.2 DIFFUSION TENSOR IMAGING AND ITS LIMITATIONS
DTI is one of the most commonly used techniques to derive quantitative measures of WM structural integrity from DWI data. DTI uses a tensor composed of three eigenvectors and three eigenvalues to model the diffusion of water molecules within each voxel. This tensor can be represented geometrically by an ellipsoid, where the longitudinal axis of the ellipsoid indicates the principal direction of water molecules in each voxel (Figure 4A). The more anisotropic the diffusion of water molecules in a WM voxel is, the more elongated the ellipsoid is, while the more isotropic the diffusion is, the more spherical the ellipsoid is (Figure 4). The degree of elongation of the tensor model, and hence the anisotropy of diffusion, can be quantified using a measure called fractional anisotropy (FA), where FA is directly proportional to the degree of elongation of the tensor. As a loss of the characteristic anisotropic diffusion in WM implies a degree of deterioration of the linear architecture of the axons, a decrease in FA is generally associated with a loss of “structural integrity” of WM. Mean diffusivity (MD) is another parameter which can be derived from the tensor model. MD describes the overall diffusivity of water molecules in all directions in a voxel.
Figure 3. Diffusion trajectory of water molecules in different environments. (A) Illustration of hindered diffusion (green) in the extracellular compartment and restricted diffusion (blue) in the intracellular compartment, separated by cell boundaries (black). (B) Illustration of anisotropic restricted diffusion (blue) within the linear boundaries of axons (black lines). Note how diffusion is less restricted along the axons and more restricted across them, which leads to anisotropic diffusion. (C) Illustration of a decrease in diffusion anisotropy (blue) due to a deterioration of the linear architecture of axons (dotted black lines). Note how diffusion becomes less restricted along the axons due to architectural deterioration, which leads to a relative decrease in diffusion anisotropy.

Figure 4. The diffusion tensor. (A) Geometric representation of the tensor model (ellipsoid) and its three eigenvectors ($\lambda_1$, $\lambda_2$ and $\lambda_3$). (B) Example of relatively high fractional anisotropy (FA; denoted by the elongated tensor model) due to the maintained linear architecture of the axons (green). (C) Example of relatively low FA due to the deterioration of the linear architecture of the axons (green). Figure adapted from Roberts, R. et al. (2013)\textsuperscript{13}
A major limitation of DTI is that the tensor model assumes the presence of a single WM fiber population of a single orientation within each voxel, which is an obvious oversimplification of the complex nature of WM. While this limitation is often referred to as the “crossing fibers problem,” it is encountered in voxels containing WM fibers which are curving, diverging, fanning, or crossing. The inability to model such fibers results in unreliable DTI measures of WM structural integrity. This limitation is further compounded by the fact that around 90% of WM voxels were found to contain multiple fiber populations. Another major limitation of DTI is the lack of direct biological interpretability of its measures. A change in FA could be an indication of a myriad of WM changes, including changes in myelination, morphology, axonal packing density, axonal diameter and membrane permeability. To address these limitations, multiple higher-order diffusion models have been recently introduced.

1.4.3 FIXEL-BASED ANALYSIS
Fixel-based analysis (FBA) is a novel framework for analyzing DWI in a fiber tract specific manner, where a “fixel” refers to a specific WM fiber population within a voxel. FBA uses constrained spherical deconvolution to disentangle and independently assess differently oriented WM fiber populations (or simply “fixels”) within the same voxel. By doing so, FBA manages to overcome the crossing fibers problem inherent to the use of DTI. FBA produces biologically meaningful measures of WM structural changes (Figure 5), namely fiber density (FD), fiber bundle cross-section (FC), and fiber density and cross-section (FDC). FD is an estimate of the axonal density of a specific WM fiber population within a voxel, and is therefore a measure of microstructural WM changes occurring at an intra-voxel level. FC, on the other hand, is a measure of morphological changes in fiber bundle cross-section perpendicular to the fixel direction, and is therefore a measure of macrostructural WM changes. Finally, FDC is a combined measure of FD and FC, and is therefore a more representative measure of the information-carrying capacity of a WM fiber bundle. In Chapter 2, I present the first application of FBA to study visual pathway WM changes in POAG patients, while in Chapter 3 I explore the potential of FBA as an alternative clinical tool for assessing degeneration of the ONs in glaucoma patients.

1.4.3 NEURITE ORIENTATION DISPERSION AND DENSITY IMAGING
Neurite orientation dispersion and density imaging (NODDI) is another higher-order diffusion model which attempts to address some of the limitations of the DTI approach. NODDI models the diffusion of water molecules in the different biological compartments, namely restricted diffusion in the intracellular compartment, hindered diffusion in the extracellular compartment, and free diffusion in the CSF. By doing so, the NODDI approach produces biologically meaningful measures of microstructural WM changes, which the conventional DTI approach is incapable of. These measures are: neurite density index (NDI), orientation dispersion index (ODI), and fraction of isotropic diffusion (FISO). NDI is an estimate of neurite (or axonal) density within a voxel, where a loss of NDI is generally associated with
degenerative WM changes. ODI quantifies the angular variation of neurite orientation, and hence estimates the degree of dispersion of neurites. A low ODI indicates the preservation of the linear WM architecture, while a high ODI indicates the loss of coherency of WM fibers. Finally, FISO is an estimate of the volume fraction of a voxel which is occupied by CSF. In Chapter 5, I present the first application of NODDI to investigate microstructural visual pathway WM alterations in POAG patients.

Figure 5. Schematic of a white matter (WM) fiber bundle cross-section and the different WM changes assessed by fixel-based measures. Grey circles represent axons within the WM fiber bundle. A change in fiber density (FD) represents a microstructural change in axonal density; a change in fiber bundle cross-section (FC) represents a macrostructural change in the cross-sectional area of a fiber bundle; a change in fiber density and cross-section (FDC) represents a change in both FD and FC. Figure adapted from Haykal, S. et al. (2020)
1.5 REFERENCES


18. Haykal S, Jansonius NM, Cornelissen FW.
Investigating changes in axonal density and morphology of glaucomatous optic nerves using fixel-based analysis. Eur J Radiol. 2020;133(October):109356.
