

**EXAMINING *IN VIVO* CHANGES IN THE LAMINA CRIBROSA
IN EXPERIMENTAL GLAUCOMA**

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of the Requirements for the Degree

Doctor of Philosophy

By

Nripun Sredar

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EXAMINING *IN VIVO* CHANGES IN THE LAMINA CRIBROSA IN EXPERIMENTAL GLAUCOMA

Nripun Sredar

APPROVED:

Dr. George Zouridakis
Department of Computer Science,
Department of Electrical and Computer Engineering, and
Department of Engineering Technology

Dr. Jason Porter
College of Optometry, and
Department of Biomedical Engineering

Dr. Ricardo Vilalta
Department of Computer Science

Dr. Nikolaos V. Tsekos
Department of Computer Science

Dr. Jaspal Subhlok
Department of Computer Science
University of Houston

Dean, College of Natural Sciences and Mathematics

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Abstract

Glaucoma is a disease that results in the degeneration of retinal ganglion cell axons and the death of retinal ganglion cells (RGCs). It is one of the leading causes of permanent blindness worldwide. Clinical examinations currently in practice are limited in their ability to detect glaucoma prior to loss of RGC axons. The main goal of this work is to characterize early changes in the optic nerve head of monkeys with experimental glaucoma (EG) using *in vivo* and non-invasive methods to better understand the mechanisms behind glaucoma. *In vivo* images of the lamina cribrosa were acquired using a spectral domain optical coherence tomography and an adaptive optics scanning laser ophthalmoscope (AOSLO). We transformed 2D AOSLO images onto a 3D anterior lamina cribrosa surface (ALCS) and computed the 3D morphometry of the ALCS. Using principal component analysis (PCA), we estimated the predominant local ALCS beam orientation directly from raw grayscale *in vivo* images without the need for binary segmentation. Subsequently, we developed an automated method to segment the lamina cribrosa pores using level sets.

Our 3D transformation method provides a better representation of the ALCS from *in vivo* images. Following 3D transformation, mean pore area increased by $5.1 \pm 2.0\%$ in 11 normal eyes and $16.2 \pm 5.9\%$ in 4 glaucomatous eyes due to the increased curvatures. Our PCA technique yielded small errors in local orientation ($0.2 \pm 0.2^\circ$) when tested on synthetic data, accurately determined local beam orientation and was repeatable in control eyes over time. In addition, automated segmentation of pore boundaries using level sets method was comparable to manual segmentation (sensitivity = 83%, specificity = 95%) and yielded repeatable values over time. In conclusion, the PCA beam orientation and level sets segmentation methods can be used to accurately and objectively detect and track *in vivo* changes in lamina cribrosa microarchitecture during the progression of EG.

Contents

1	Introduction	1
1.1	Motivation	1
1.2	Objectives	3
1.2.1	Three-dimensional modeling to characterize lamina cribrosa surface and pore geometries using <i>in vivo</i> images in normal and glaucomatous eyes	3
1.2.2	Automatically characterize predominant lamina cribrosa beam orientation in non-human primates with experimental glaucoma	4
1.2.3	Automated segmentation of lamina cribrosa pores in non-human primates with experimental glaucoma	4
1.3	Dissertation outline	5
2	Background	6
2.1	Basic components of the eye	6
2.2	Structure of the optic nerve head and lamina cribrosa	8
2.3	Glaucoma	10
2.3.1	Risk factors in developing glaucoma	10
2.3.2	Potential mechanisms	11
2.3.3	Role of lamina cribrosa in glaucoma	12
3	3D modeling to characterize lamina cribrosa surface and pore geometries using <i>in vivo</i> images from normal and glaucomatous eyes	22
3.1	Introduction	23
3.2	Methods	24
3.2.1	Animal preparation	24
3.2.2	<i>In vivo</i> imaging	25
3.2.3	Three-dimensional anterior lamina cribrosa surface modeling and characterization	27
3.3	Results	31

3.3.1	Validation of thin plate splines	31
3.3.2	Three-dimensional transformation of the lamina cribrosa in normal and glaucomatous eyes	33
3.4	Discussion	35
3.5	Conclusions	38
4	Automated characterization of predominant lamina cribrosa orientation in non-human primates using AOSLO	43
4.1	Introduction	44
4.2	Methods	46
4.2.1	Biometric measurements and image scaling	47
4.2.2	<i>In vivo</i> imaging using spectral domain optical coherence tomography (SD-OCT)	47
4.2.3	<i>In vivo</i> imaging using adaptive optics scanning laser ophthalmoscope	49
4.2.4	Image pre-processing to improve the quality of AOSLO montage	49
4.2.5	Estimating local dominant orientation	52
4.2.6	Statistical analysis	61
4.3	Results	62
4.3.1	Validation of principal component analysis	62
4.3.2	Assessment of repeatability in measuring lamina cribrosa beam orientation in normal monkeys	65
4.3.3	Longitudinal assessment of changes in beam orientation in monkeys with unilateral experimental glaucoma	67
4.4	Discussion	69
4.5	Conclusions	73
5	Automated segmentation of lamina cribrosa pores in non-human primates using AOSLO	74
5.1	Introduction	75
5.2	Methods	76
5.2.1	Biometric measurements and image scaling	77
5.2.2	<i>In vivo</i> imaging using spectral domain optical coherence tomography (SD-OCT)	78
5.2.3	<i>In vivo</i> imaging using adaptive optics scanning laser ophthalmoscope	79
5.2.4	Estimating lamina cribrosa pore boundary	79
5.2.5	Wellner's method to identify lamina cribrosa pores	80
5.2.6	Distance regularized level sets for identifying pore boundaries	80
5.2.7	Statistical analysis	83
5.3	Results	83
5.3.1	Validation	83

5.3.2	Assessing repeatability of automatic pore segmentation	85
5.3.3	Longitudinal assessment of structural changes in monkeys with unilateral experimental glaucoma	87
5.4	Discussion and Conclusion	87
6	General Conclusions	89
6.1	General Conclusions	89
6.1.1	Chapter 3: 3D modeling to characterize lamina cribrosa surface and pore geometries using <i>in vivo</i> images from normal and glau- comatous eyes	89
6.1.2	Chapter 4: Automated characterization of predominant lamina cribrosa orientation in non-human primates using AOSLO	90
6.1.3	Chapter 5: Automated characterization of predominant lamina cribrosa orientation in non-human primates using AOSLO	91
	Bibliography	92
	Appendices	99
A	Publications	100

List of Figures

2.1	Anatomy of eye. Reprinted with permission [77]	6
2.2	Cross-sectional illustration of the retinal layers illustrating the connection between photoreceptors (cones and rods) and RGCs. RGC axons exit the eye through the optic nerve head and travel onto the lateral geniculate nucleus and ultimately to the visual cortex of the brain. Reprinted with permission [1]	8
2.3	A simplified cross-sectional illustration of the ONH and lamina cribrosa. RGC axon bundles (white tubes) pass through the porous lamina cribrosa (red and blue columns) and exits the eye. Reprinted with permission [13]	9
2.4	Schematic of AO system. Reprinted with permission [8, 22]	14
2.5	Schematic of OCT system. Reprinted with permission [19]	16
2.6	An illustration of progression of glaucoma. A) Normal LC B) Elevated IOP strains and deforms the lamina resulting in bowing and initial thickening of LC C) Advanced stage glaucoma is represented by thinning and degeneration of LC. Reprinted with permission [13]	19
2.7	An illustration of progression of glaucoma. A) Normal LC. B) Stress normal to the ONH surface strains the lamina resulting in posterior bowing of LC. C) Stress radially from the center of the ONH surface expands the scleral canal wall resulting in the deformation of LC towards the anterior surface. Reprinted with permission [13]	20
3.1	(a) <i>En face</i> SLO image showing locations of SDOCT radial B-scans acquired in a normal eye. Scale bar = 150 μm . (b) Manual delineation of the innermost terminations of the RPE/BM complex (blue points) and ALCS (red points) within the optic nerve head in a single SDOCT radial B-scan [denoted by the green bold line in (a)]. ALCS points that were located underneath a blood vessel shadow were not marked. (c) AOSLO montage of laminar pores (dark holes) on the temporal side of the anterior laminar surface scaled, registered and overlaid on the corresponding SLO image. Scale bar = 150 μm	26

3.2	(a) Model of laminar surface constructed by fitting a thin plate spline to the marked ALCS point cloud (denoted by black points that reside above and below the fit surface). (b) Mean ALCSD was calculated as the mean normal distance between a plane fit to the BMO point cloud (shaded gray) and the 3D anterior laminar surface (red arrows) for points located within the BMO ellipse (blue ellipse). (c,d) Graphical representation for determining the radius of curvature of a 2-dimensional curve (blue) at a point, P0. (c) An osculating circle (dashed, red circle with radius, r) is drawn through point P0 and two points on the 2-dimensional curve that are infinitesimally close to P0 (i.e., P1 and P2). (d) Magnified view of the region enclosed by the green dashed rectangle in (c). The radius of curvature at point P0 is calculated as the ratio of the arc length between P1 and P2 (green arc, labeled ds) to the change in the angle of a line tangent to the curve (solid orange lines) at points P1 and P2 (i.e., $ d\theta = \theta_1 - \theta_2 $).	29
3.3	(a) Registered AOSLO montage overlaid on the SLO image and projected onto the thin plate spline representing the ALCS. Laminar pores are marked and filled in white. (b) Magnified image of a laminar pore from (a) illustrating the tessellation of its surface into a collection of triangles.	30
3.4	(a) Zernike spherical aberration was created as a known surface for fitting validation. Surfaces were created by fitting a (b) thin plate spline and (c) bi-cubic spline to the same regularly sampled subset of points (45% of the total points) from the known surface. The RMS errors calculated from the non-fitted points were 3.2% and 2.6% of the total peak-to-valley height of the known surface ($3.35\ \mu m$) for the thin plate spline and bi-cubic spline, respectively.	31
3.5	(a) Hemisphere of known surface area prior to tessellation. (b) Hemisphere surface after tessellation. The area of the post-tessellated hemisphere was 0.6% smaller than the known surface area.	32
3.6	(a-d, i-l) 2D AOSLO montages of the anterior laminar surface registered and overlaid on the SLO images acquired in 8 of 11 normal monkey eyes. Laminar pores are marked and filled in white. Scale bar = $150\ \mu m$. (e-h, m-p) Each 2D AOSLO montage was registered and projected onto the thin plate spline fit to the ALCS point cloud to generate a 3D transformed image of anterior laminar pores. The RMS errors of the thin plate splines fit to the manually marked ALCS points are reported underneath each 3D transformed AOSLO image. The mean RMS fit error across all 11 normal eyes was $9.8 \pm 1.2\ \mu m$	40

3.7	(a,b) 2D AOSLO montages of the anterior laminar surface registered and overlaid on the SLO images acquired in 2 of 4 eyes with unilateral experimental glaucoma. Laminar pores are marked and filled in white. Scale bar = 150 μm . (c,d) 3D transformed AOSLO images. While the anterior laminar surfaces in the glaucoma eyes were more steeply curved, the RMS fit errors for the thin plate splines were similar to those in normal eyes. The increased surface curvatures yielded a greater change in 3D pore area compared to that calculated in normal eyes.	41
3.8	(a,b) Thin plate spline models of the anterior lamina cribrosa surface (colored) in 2 monkey eyes (Subjects OHT-63 and OHT-64) relative to the BMO plane (gray rectangles) prior to the induction of experimental glaucoma. Both eyes had mean ALCS values comparable to normal eyes (191 μm and 175 μm , respectively) with relatively high mean radii of curvature (RoC) of 2.9 mm and 3.4 mm, respectively. (c,d) Thin plate spline models of the anterior lamina cribrosa surface (colored) in the same 2 eyes from (a,b) relative to the BMO plane (gray rectangles) after induction of experimental glaucoma. Mean ALCS increased (405 μm and 429 μm , respectively) while the mean radii of curvature decreased in each eye (1.3 mm and 1.4 mm, respectively), indicating that the laminar surface posteriorly deformed and became more steeply curved.	42
4.1	ONH features marked on the B-scan. (a) Single b-scan from a normal monkey eye corresponding to the white scan line in the inset SLO image (b) Manual delineation of the innermost terminations of the RPE/BM complex (red points) and ALCS (yellow points). The minimum distance from RPE/BM to ILM (green line with dots) is denoted by the white lines. Scale bar = 300 μm	48
4.2	Flowchart of the steps in preprocessing the AOSLO image	52
4.3	A. Original AOSLO image B. Homomorphic filtered image to remove non-uniform illumination C. Contrast improved image using CLAHE. Scale bar = 150 μm	53
4.4	Mean intercept length. (a) Synthetic image of known orientation. (b) Probe lines (red) overlaid on the image (c) Probe lines intercepts at angle 126° (d) Probe lines intercepts at angle 53°	54
4.5	Predominant orientation is the direction of the major axis of the ellipse fitted to MIL in polar coordinates	55
4.6	Radon transform. (a) Synthetic image of known orientation. (b) Radon transform probe lines (red) overlaid on the image	56

4.7	Radon transform. (a) Radon transformation of the synthetic image (Fig. 4.6(a)). (b) Predominant orientation is orthogonal to the angle of maximum summed intensity variance	57
4.8	Steerable filters (a) Basis filter in horizontal direction (b) Basis filter in vertical direction (c) Response R_1^0 of horizontal basis filter to synthetic image (d) Response R_1^{90} of vertical basis filter to synthetic image Fig. 4.4(a)	58
4.9	Synthetic images of known orientation (a) Original clean synthetic image (b) Synthetic image with noise ($\sigma^2 = 0.6$) (c) Synthetic image with blur ($\sigma^2 = 10$)	63
4.10	Synthetic concentric ellipses (a) Original clean synthetic concentric ellipses (b) Synthetic concentric ellipses with noise ($\sigma^2 = 0.6$) (c) Synthetic concentric ellipses with blur ($\sigma^2 = 10$)	63
4.11	(a) Change in orientation of concentric ellipses with noise (b) Change in orientation of concentric ellipses with blur	64
4.12	AOSLO images of the ALCS and the corresponding Homomorphic filtered images from 4 normal monkey eyes (a-b) OHT-63 (c-d) OHT-64 (e-f) OHT-66 (g-h) OHT-69. Scale bar = 150 μm	66
4.13	Local predominant orientation vectors of the ALCS of monkey OHT-63 acquired at three different time points. The vectors colored red correspond to baseline measurements(a) Baseline (b) Day 13 (c) Day 35. Scale bar = 150 μm	67
4.14	Local predominant orientation vectors of the ALCS of monkey OHT-69 acquired at three different time points. The vectors colored red correspond to baseline measurements(a) Baseline (b) Day 84 (c) Day 182. Scale bar = 150 μm	68
4.15	First significant change in beam orientation coincided with the first significant change in manual measure of ALCS pore geometry for OHT-64. Scale bar = 150 μm	69
4.16	First significant change in beam orientation (red line) preceded or coincided with the first significant change in ONH parameters (yellow dot) for OHT-64	70
4.17	First significant change in beam orientation coincided with the first significant change in manual measure of ALCS pore geometry for OHT-68. Scale bar = 150 μm	71
4.18	First significant change in beam orientation (red line) coincided with the first significant change in ONH parameters (yellow dot) for OHT-68. Scale bar = 150 μm	72

5.1	Pore localization. (a) Original gray scale lamina cribrosa montage. (b) The red contours represent the pores identified using Wellner's method. Scale bar = 150 μm	81
5.2	Validation of automatic segmentation (a) Original AOSLO montage (b) Masked montage (c) Color codes of raters (d) Pores marked by 3 raters, where the overlap of two or more raters in pink is the ground truth (e) Overlap of ground truth and automatic segmentation (f) Overlap regions of automated and ground truth. Scale bar = 150 μm	84
5.3	Repeatability of automatic segmentation assessed by examining the differences in area at 2 different time-points (a) Overlap of automatic segmentation of normal monkey eye OHT64 (b) Overlap of automatic segmentation of normal monkey eye OHT-66. Scale bar = 150 μm	86

List of Tables

3.1	Comparison of lamina cribrosa surface geometry and pore geometry after 3D transformation in living eyes	34
4.1	Mean error in dominant orientation of 180 synthetic test images for MIL, Radon transform, Steerable filter, and PCA	65
4.2	Summary of first structural change(s) to occur in each EG eye, where (X) indicates the first ONH parameter(s) to change	73
5.1	Summary of first structural change(s) to occur in each EG eye, where (X) indicates the first ONH parameter(s) to change	87

Chapter 1

Introduction

1.1 Motivation

Glaucoma is the leading cause of irreversible blindness worldwide. A recent study has estimated that there will be 79.6 million people with open angle glaucoma by the year 2020 [50]. Glaucoma is generally a bilateral disease with asymmetric severity, meaning that the individual may not perceive a loss of vision immediately thereby causing delay in availing treatment. Moreover, glaucoma progresses slowly over the years making it difficult to diagnose and detect during the early stages. There are several risk factors associated with glaucoma [21, 23]. Mean age of onset of glaucoma is typically after 60 years, and the frequency increases with age. High prevalence of glaucoma in people of African and Hispanic ethnicities was reported compared to Asian and European descent. Genetic influences also play a role in glaucoma where first-degree relatives of people with

glaucoma are at a higher risk. Another main risk factor for developing glaucoma is elevated intraocular pressure (IOP). Intraocular pressure greater than 21mmHg is the clinical definition of elevated IOP. However, individuals with normal levels of IOP ($< 21\text{mmHg}$) can develop glaucoma and individuals with a sustained level of elevated IOP may never develop glaucoma. The most common treatment is to lower the IOP in patients with primary angle glaucoma. Laser treatment of the trabecular mesh-work is a surgical procedure that is performed to lower IOP [50]. Some of these procedures and treatments have side effects. Hence, early detection of the disease using non-invasive methods are desirable to start treatment early and prevent progression of the disease.

Glaucoma is a multi-factorial disease of the optic nerve head (ONH) that results in the degeneration of retinal ganglion cell (RGC) axons and the death of retinal ganglion cells [54]. The RGCs receive visual information from the photoreceptors and transmit the information via the connecting RGC axons that exit the eye through the optic nerve head (ONH) and travel to the brain. *Ex vivo* and post mortem studies support the idea that the initial damage to these axons occurs at the lamina cribrosa, a 3-dimensional (3D) porous mesh-work consisting primarily of flexible, collagenous beams located within the ONH that support and nourish the RGC axon bundles passing from the retina to the brain [38, 52]. Stress due to increased IOP can strain and deform the lamina cribrosa, resulting in permanent structural alterations that could potentially crimp RGC axon bundles passing through the laminar pores [53]. Hence, it is important to detect and characterize changes in the lamina cribrosa using *in vivo* and non-invasive methods to better understand the mechanisms behind glaucoma.

1.2 Objectives

The overall goal of this project is to develop computational tools to analyze images of the lamina cribrosa acquired using an adaptive optics scanning laser ophthalmoscope (AOSLO) and spectral domain optical coherence tomography (SDOCT) to better understand early changes in lamina cribrosa microarchitecture in an experimental model of glaucoma.

1.2.1 Three-dimensional modeling to characterize lamina cribrosa surface and pore geometries using *in vivo* images in normal and glaucomatous eyes

En face adaptive optics scanning laser ophthalmoscope images of the anterior lamina cribrosa surface represent a 2D projected view of a 3D surface. As a result, measurements of laminar surface and pore geometries from 2D AOSLO images do not incorporate differences in curvature between normal and glaucomatous eyes. The inherent limitation of this method is addressed by modeling the anterior lamina surface in 3D using SD-OCT images and projecting 2D AOSLO images onto the 3D surface to achieve a more accurate representation of the surface morphology. This study validates the methodology and demonstrates the difference in lamina surface and pore geometries before and after transformation.

1.2.2 Automatically characterize predominant lamina cribrosa beam orientation in non-human primates with experimental glaucoma

The lamina cribrosa is implicated as the initial site of damage to retinal ganglion cell axons in glaucoma. We sought to characterize predominant lamina cribrosa beam orientation *in vivo* in normal eyes and changes in predominant beam orientation in non-human primate eyes during experimental glaucoma (EG). We assessed the repeatability of principal component analysis in quantifying the lamina cribrosa beam orientation *in vivo* in non-human primate eyes from 2D AOSLO images acquired at different time points. This method can be extended to track longitudinal changes in laminar microarchitecture in monkeys with unilateral experimental glaucoma and in human glaucomatous patients.

1.2.3 Automated segmentation of lamina cribrosa pores in non-human primates with experimental glaucoma

Ex vivo studies have established that there is posterior bowing of anterior lamina cribrosa surface during glaucoma. Structural changes in lamina cribrosa surface may change geometry of laminar pores and potentially damage the axons that pass through the pores. Hence, there is a need for objective and accurate *in vivo* analysis and examination of laminar pore microarchitecture to have a better insight into structural changes during glaucoma. The purpose of this study is to develop an automated method to segment lamina cribrosa using a boundary evolution method that can accurately and objectively track changes in laminar pore geometry during glaucoma.

1.3 Dissertation outline

In Chapter 1, we discuss the motivation of this work and present the overall objectives and specific objectives targeted in this work.

In Chapter 2, we present background related to the research presented in this work. Here we introduce the pathology of glaucoma and previous work done in this field.

In Chapter 3, we discuss the methods and results of the work related to **Specific aim 1** which is high-resolution 3D modeling of anterior lamina cribrosa.

In Chapter 4, we discuss the methods and results of the work related to **Specific aim 2** which is automated characterization of lamina cribrosa beam orientation in normal eyes and longitudinal study of changes in beam orientation in non-human primate eyes with EG.

In Chapter 5, we discuss the methods and results of the work related to **Specific aim 3** which is automated segmentation of lamina cribrosa pores and the use of this technique to objectively characterize changes in laminar pores in a longitudinal study of EG.

In Chapter 6, we layout in brief the general conclusions of our study.

Chapter 2

Background

2.1 Basic components of the eye

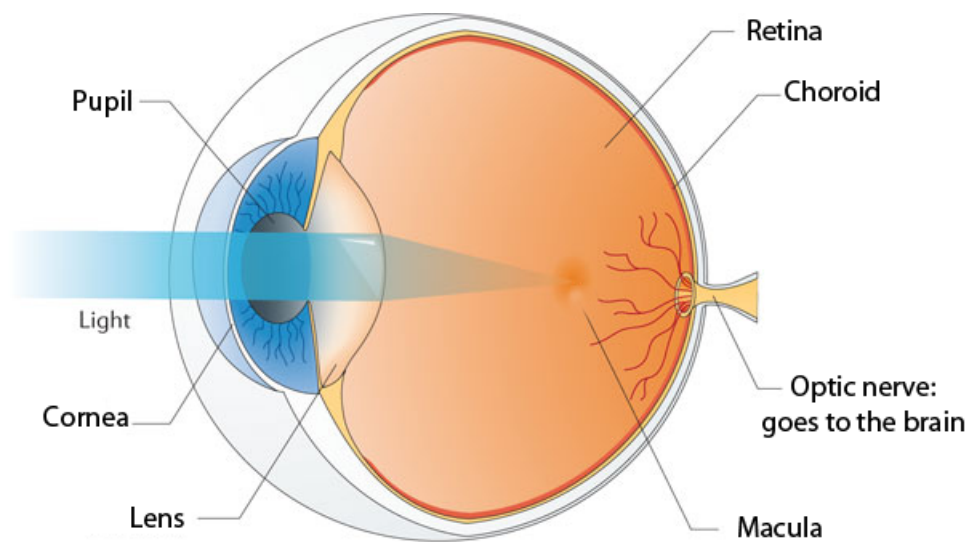


Figure 2.1: Anatomy of eye. Reprinted with permission [77]

The visual system and ocular anatomy of the non-human primate is similar to those of

humans. The non-human primate eye is an asymmetrical globe with an average axial length of 14 *mm*. Different parts of the eye (as shown in Fig. 2.1) include:

- Iris: The colored, pigmented circular muscle whose innermost boundary defines the eye's pupil. The iris dilates and constricts to help regulate the amount of light that enters the eye.
- Pupil: The aperture in the center of the iris through which light travels to reach the retina.
- Crystalline lens: A transparent biconvex lens in the eye that can change its shape (in younger aged individuals) to focus on objects at various distances. The lens becomes more opaque with increasing age and gradually loses its ability to change focus.
- Cornea: The transparent, outermost surface that covers the pupil and the iris. The cornea acts as a lens and, together with the crystalline lens, focuses light ideally on the retina.
- Sclera: The white part of the eye, which is fibrous and helps maintain the shape of the globe.
- Retina: The light incident on the eye is refracted by the cornea and the crystalline lens, then propagates to the retinal surface which is the sensory part of the eye. The photoreceptors in the retina receive light energy and which is then converted to electrical impulses that are transmitted through the retinal ganglion cells (RGCs) and its axons out of the ONH and to the brain.

2.2 Structure of the optic nerve head and lamina cribrosa

The optic nerve head (ONH) is also termed the "blind-spot" because of its lack of light sensitive photoreceptor cells. The optic nerve head is the location through which approximately 1 million RGC axons exit the eye and travel to the brain (Fig. 2.2) [48]. In

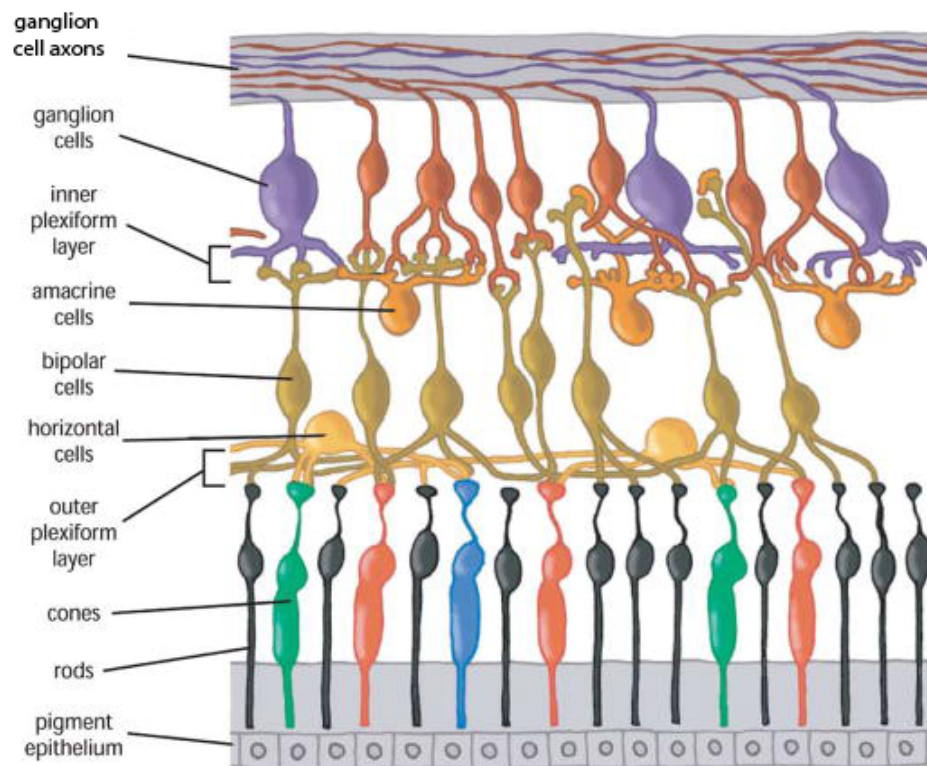


Figure 2.2: Cross-sectional illustration of the retinal layers illustrating the connection between photoreceptors (cones and rods) and RGCs. RGC axons exit the eye through the optic nerve head and travel onto the lateral geniculate nucleus and ultimately to the visual cortex of the brain. Reprinted with permission [1]

non-human primates, there is a large variability in the size of the ONH. The ONH tends to be elliptical, where typical lengths of vertical and the horizontal axes are 1.4 mm and 1.1

mm, respectively [75].

The ONH primarily consists of RGC axons, vasculature and glial tissue [69]. The sclera

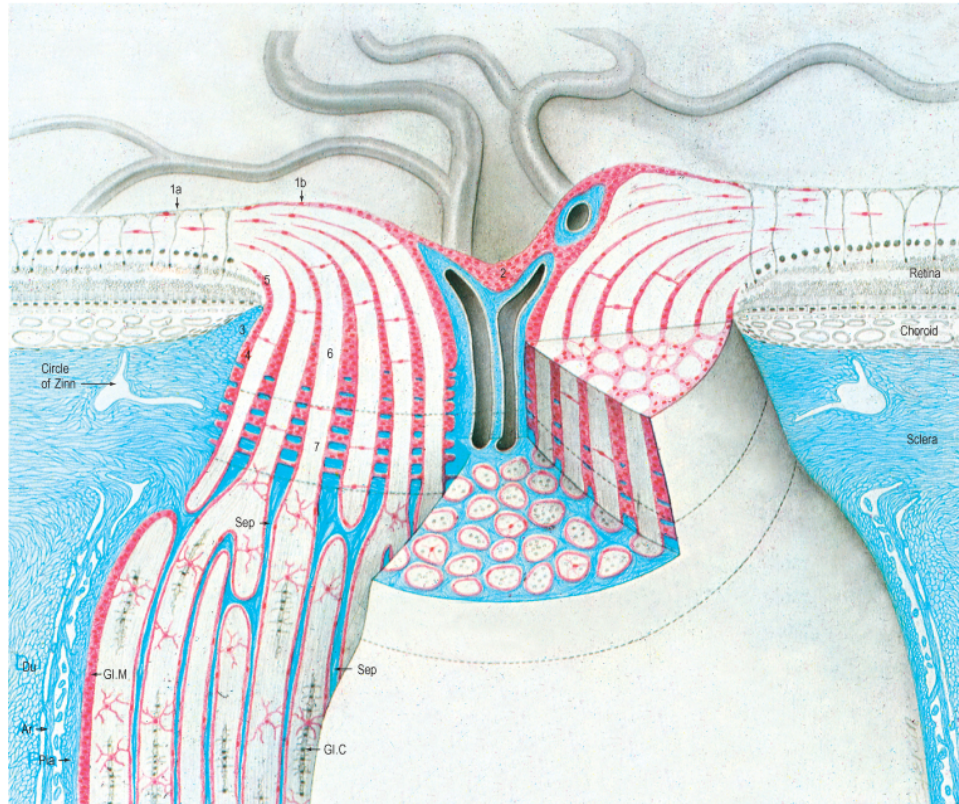


Figure 2.3: A simplified cross-sectional illustration of the ONH and lamina cribrosa. RGC axon bundles (white tubes) pass through the porous lamina cribrosa (red and blue columns) and exits the eye. Reprinted with permission [13]

extends through the ONH and forms a complex 3-dimensional porous meshwork called the lamina cribrosa (Fig. 2.3). Therefore, the ONH is typically stratified into pre-laminar, laminar and post-laminar regions. In normal monkeys, the thickness of the pre-laminar region is approximately $160\mu m$ and that of the lamina cribrosa is approximately $100\mu m$ [75].

The lamina cribrosa (LC) provides functional and structural support to the RGC axon bundles that pass through the ONH. The lamina cribrosa consists of hyper-reflective beams and pores through which the RGC axons pass into the brain. The lamina cribrosa beams are primarily composed of collagen fibers, capillaries, and elastin, and are lined with astrocytes. Elastin fibers are responsible for the flexibility and resilience of the tissue to fluctuations in IOP. Early studies found the elastin fibers to become thicker with age [26]. Additionally, there was marked disappearance of elastin in primary open angle glaucoma. The astrocytes insulate neurons and provide metabolic and physical support.

2.3 Glaucoma

Glaucoma describes a group of common ocular neuropathies which result in the death of RGCs and a progressive loss of vision if left untreated. Clinical characteristics of the neuropathy are clinically visible changes in the ONH, including excavation and enlargement of the optic cup, and thinning of the neuroretinal rim width. This irreversible form of blindness affects millions of people worldwide and 90% of affected people go undiagnosed [54], thus making glaucoma the leading cause of irreversible blindness worldwide.

2.3.1 Risk factors in developing glaucoma

The main risk factors are elevated IOP, family history, age, and ethnicity [21]. IOP is measured in millimeters of mercury (*mmHg*). Normal IOP in humans range from 10 – 21*mmHg*, while IOP greater than 21*mmHg* is classified as high IOP. There is no

elevated level of IOP that will definitely lead to glaucoma and conversely there is no lower level that will eliminate the risk of glaucoma. Therefore, individuals with normal IOP can develop glaucoma while individuals with elevated IOP may not develop glaucoma. These variations make it difficult to detect and diagnose the early onset of glaucoma. Treatments currently employed include the use of IOP lowering eye drops, trabeculoplasty, and trabeculectomy to prevent and minimize damage to the optic nerve head. Some of these procedures may have undesirable side effects which may deter patients from undergoing these invasive treatments. Another risk factor for developing glaucoma is having a family history of glaucoma. One's chance of acquiring glaucoma increases 10-fold if an immediate family member is diagnosed with the disease [50]. Increased age is also a major risk factor for developing glaucoma. The risk of developing glaucoma in humans increase with age with a mean age of onset greater than 60 years [32]. Finally, individuals of African and Hispanic ethnicity have a higher chance of developing glaucoma compared with those of Asian and European descent [56].

2.3.2 Potential mechanisms

Irrespective of the type, glaucoma in all its forms can lead to progressive vision loss if left untreated. Early detection of glaucoma is vital to prevent permanent losses of vision. Biomarkers that provide early detection and diagnosis of glaucoma are not known yet. Additionally, the mechanisms of glaucoma that lead to the death of RGCs and degeneration of RGC axons are not fully understood. Three primary theories that have been proposed to explain the mechanisms by which glaucoma occurs are the mechanical, vascular, and glial hypotheses.

- Mechanical theories suggests that the optic nerve head is vulnerable to changes in IOP. Stress due to elevated IOP can strain and deform the ONH, resulting in degradation and permanent deformation of load bearing lamina beams, changes in the diameter of the scleral canal opening, and potential compression and shearing of RGC axons that traverse through the LC [7, 13, 4, 59]. Changes in the ONH manifest as changes in lamina cribrosa cupping, thickness, and geometry (eg: the shape and size of the pores, and change in orientation of the lamina beams).
- Vascular theories suggest that elevated IOP affects the ocular blood flow resulting in inconsistent perfusion pressure within the eye and compromising the diffusion of nutrients thus leading to axonal damage [26].
- Glial theories suggest that elevated IOP results in the reactivation of astrocytes that induce an enzyme that maybe toxic to the RGC axons. This mechanism is counter-intuitive as the reactivation of astrocytes is typically a response to isolate healthy neural tissues to prevent further damage [25].

The theories discussed above may occur as mutually exclusive events or may occur simultaneously making it challenging to isolate the mechanisms that lead to developing glaucoma. However, the general consensus is that the initial site of axonal damage during glaucoma is at the level of lamina cribrosa.

2.3.3 Role of lamina cribrosa in glaucoma

The lamina cribrosa has been implicated as the initial site of damage to RGC axons in glaucoma. Early studies showed blockade of axonal transport as a result of IOP elevation

in monkey eyes [3, 53, 38]. *Ex vivo* and *in vivo* studies were carried out in both human and monkey eyes. Scanning electron microscopy images of lamina cribrosa in *ex vivo* human eye with glaucoma showed 0.7mm posterior displacement of the lamina surface and a lateral extension of the lamina cribrosa as a result of scleral expansion [52]. Previous studies in *ex vivo* eyes showed that lamina cribrosa connective tissue were significantly larger in central nasal-temporal region compared to peripheral superior-inferior region in 16 normal human subjects [11]. They hypothesized that RGC axons in the superior and inferior regions are susceptible to damage earlier potentially due to decreased connective tissue support in these regions. The findings of *ex vivo* studies were validated by *in vivo* studies using different imaging modalities like optic disc photography, confocal scanning laser ophthalmoscopy, confocal scanning laser tomography, spectral-domain optical coherence tomography, adaptive optics SLO, and adaptive optics OCT.

- **Optic disc photography** : The optic disc is photographed using a clinical ophthalmic instrument and the photographs are examined for optic disc shape, optic disc hemorrhages, and cup-to-disc ratio.
- **Confocal scanning laser tomography (CSLT)** : CSLT is a technique that measures the surface topography of the posterior pole structures of the eye. CSLT provides clinicians diagnostic tools to discriminate between normal and glaucomatous optic discs.
- **Confocal scanning laser ophthalmoscope (cSLO)** : cSLO scans the object point by point and captures the reflected light through a pinhole. Using cSLO one can acquire *en face* images of the optic nerve head.

- **Adaptive optics scanning laser ophthalmoscope (AOSLO) :** Acquiring images of a living eye was a challenging task due to low reflectance of the retina and the presence of high-order aberrations in the optical system. Adaptive optics correction of wave aberration originated in astronomy, as a solution to imaging distant stars by correcting the distortion caused by atmospheric turbulence. Adaptive optics made its way into vision science to improve retinal images in 1989. Miller et al. devel-

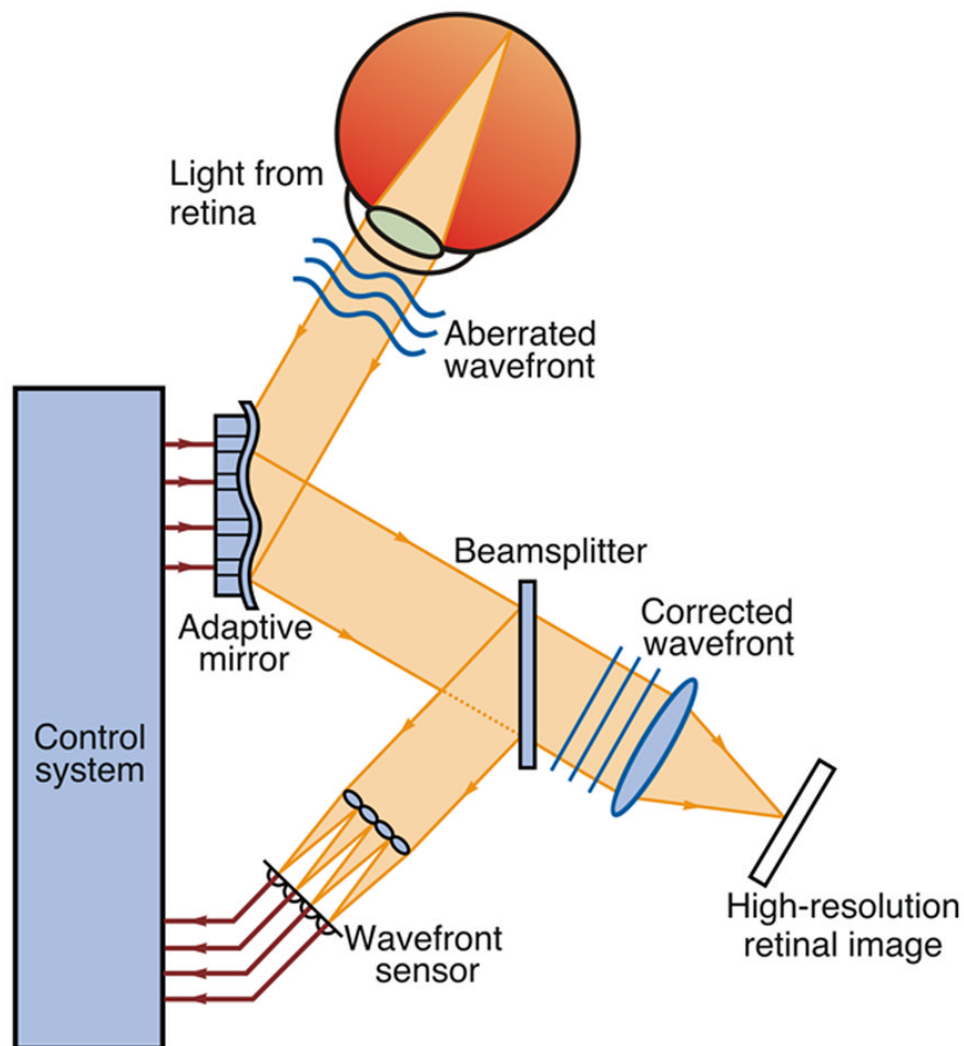


Figure 2.4: Schematic of AO system. Reprinted with permission [8, 22]

oped a high-resolution wavefront sensor to better characterize the wave aberrations present in human eyes [37]. Fig. 2.4 illustrates the three principal components in a traditional adaptive optics system: a wavefront sensor, a wavefront corrector, and a control system.

- Wavefront sensor: A wavefront sensor is used to measure the aberration of the wavefront. The most commonly used wavefront sensor is the Shack-Hartmann wavefront sensor. It consists of an array of lenslets in x and y direction with uniform diameter and focal length.
- Wavefront corrector: The wavefront corrector is used to compensate for the wavefront aberrations in the eye. By applying voltages to a series of actuators on the wavefront corrector which push and pull the deformable surface, we can achieve a surface that will compensate for the low order and high order wavefront aberrations.
- Control system: The control system measures the wavefront aberrations by computing the slope of the wavefront displacement, computes the deformable mirror actuator voltages and corrects the wavefront aberrations in an iterative loop.

The remaining components of an AOSLO system are light source, horizontal scanner, vertical scanner, and a detector. A scanning laser ophthalmoscope raster scans a focused beam of light on the retina and the scattered light is captured using a photo-multiplier tube (PMT). The confocal nature of the system blocks light not originating from the object plane using a pinhole, thereby enabling axial sectioning

of the retina.

- **Spectral domain optical coherence tomography (SDOCT)** : Optical coherence tomography (OCT) is an optical imaging modality that is pervasive in the field of biomedical optics and clinical ophthalmology. The first retinal imaging using OCT was performed in 1989 by James Fujimoto, David Huang and Joel Schuman [66]. The original prototype was time domain optical coherence tomography (TDOCT) which through the use of Fourier transformation lead to the development of SDOCT. Fig. 2.5 illustrates the principle behind TDOCT and SDOCT. A single axial scan im-

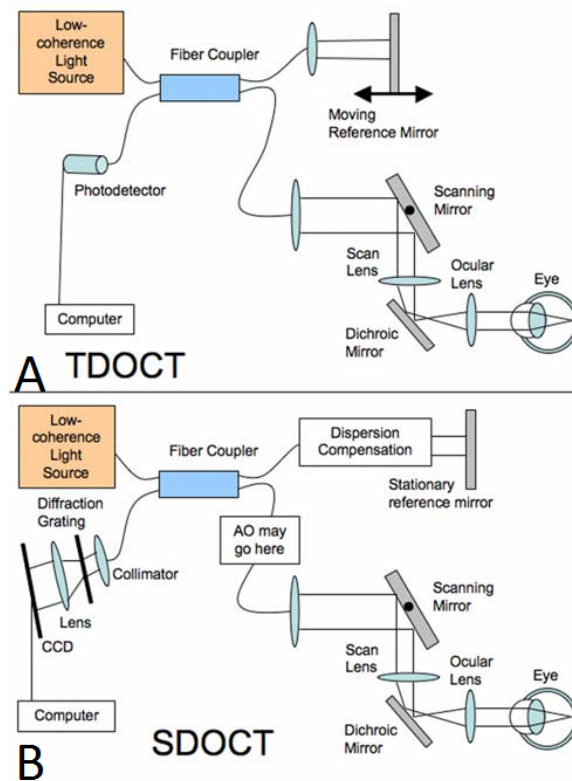


Figure 2.5: Schematic of OCT system. Reprinted with permission [19]

age known as the A-scan was acquired using optical reflections from various depths

of the tissue along a point, and multiple axial scans linearly across the tissue generated a cross-sectional scan, also known as the B-scan. A three-dimensional volumetric data set can be generated by combining these sequential B-scans. The general principle of reflective OCT imaging is analogous to that of an ultrasound where imaging is performed by measuring the echo time delay and the intensity of the backscattered light. Direct measurement of time-delay of light is impossible because it requires a time resolution in the order of femtoseconds. TDOCT uses low coherence interferometry to indirectly measure the echo time delay and magnitude of light. The light source travels to a beam splitter where one part goes to a mirror known as the reference arm and the rest goes to the sample arm where the light reflects and scatters off the tissue. Light from the two arms which recombines to form an interference pattern, combine constructively only if the tissue and the reference arm are relatively at the same distance. The position of the reference arm is moved back and forth to image the tissue at known depths. The mechanical movement of the reference arm makes TDOCT acquisition slower. The principle of SDOCT differs from TDOCT in the fact the depth of imaging is not known in advance. In SDOCT the reference arm is stationary and the interference pattern is detected using a spectrometer that can gather all points on the A-scan simultaneously thereby increasing the speed of acquisition.

In vivo analysis of human eyes with glaucoma using optic disc photography showed that pore area became smaller with time [67]. Comparison of the number of pores and other pore parameters like pore area using color disc photograph, SLO, and AOSLO in humans

found the pore area to be larger during disease [2]. High-resolution AOSLO images acquired *in vivo* of the lamina cribrosa surface showed excellent reproducibility [28]. Recent studies measured the repeatability of analyzing lamina cribrosa in normal and glaucomatous human eyes using swept-source OCT and adaptive optics OCT [40, 71]. However, the slow progression of glaucoma in humans pose a challenge to learn different stages of the disease in human patients. An experimental model of glaucoma in animals offers researchers valuable opportunity to track and study longitudinal changes in ONH during glaucoma.

A monkey model of experimental glaucoma was used to better understand changes in lamina cribrosa during disease progression. An argon laser treatment is performed unilaterally to damage the trabecular meshwork thereby elevating the IOP, while the fellow eye is used as a normal/control. The hypothesis that LC deforms due to elevated IOP was tested by increasing the IOP from 5 to 50 mm Hg for 24 hrs which produced a posterior deformation in human donor eyes [5]. 3D reconstruction of the optic nerve connective tissue in *ex vivo* monkey eyes with EG revealed posterior deformation of LC, thickening of LC and expansion of neural canal opening [6]. 3D histomorphometric reconstruction of histological data showed significant increase in thickness of lamina cribrosa during early glaucoma as illustrated in Fig. 2.6. Assessment of the location of lamina cribrosa *in vivo* in 54 human glaucoma subjects using SDOCT showed the lamina cribrosa to be more posterior in glaucomatous than in normal human eyes [20]. Additionally, confocal scanning laser tomography imaging of *in vivo* experimental glaucoma eyes in monkeys revealed expansion of scleral canal [13].

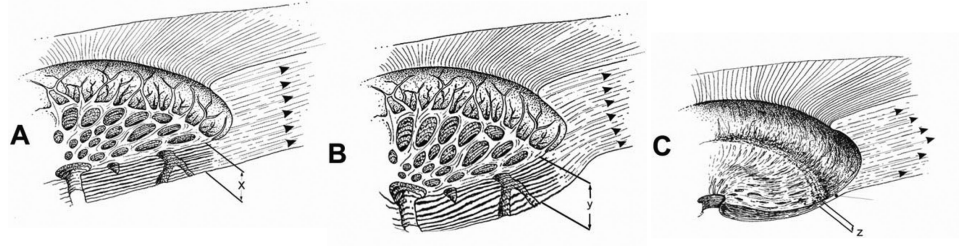


Figure 2.6: An illustration of progression of glaucoma. A) Normal LC B) Elevated IOP strains and deforms the lamina resulting in bowing and initial thickening of LC C) Advanced stage glaucoma is represented by thinning and degeneration of LC. Reprinted with permission [13]

- Biomechanical modeling of lamina cribrosa using *ex vivo* EG data:** From a biomechanical perspective, the ONH is a weak-spot compared to the corneo-scleral shell. Several attempts have been made to model the biomechanical behavior of the lamina cribrosa. Finite element models of varying complexity were used to present the biomechanical behavior of lamina cribrosa [13, 59]. These studies found that significant levels of strain are produced within the lamina cribrosa due to elevated IOP. Additionally, the scleral compliance affects the lamina strain more than lamina compliance and the variations in scleral properties may be a potential influencing factor in glaucoma [59]. A micro-scale finite element models of the lamina cribrosa microarchitecture in normal rhesus monkeys was developed to capture the biomechanical behavior of the laminar meshwork. The 3-dimension lamina cribrosa was segmented based on an expectation-maximization anisotropic Markov random field algorithm which incorporated prior information about the geometry of the structure [14]. The material properties of the lamina tissue for the model was defined

using the connective tissue volume fraction and the predominant laminar beam orientation. The stress acting normal to the ONH may bow the lamina cribrosa posteriorly and the force acting radially may expand the sclera resulting in anterior deformation of LC as shown in Fig. 2.7.

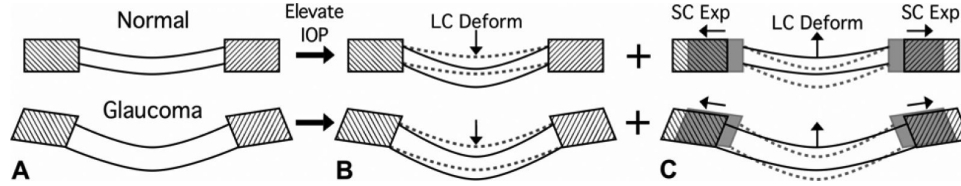


Figure 2.7: An illustration of progression of glaucoma. A) Normal LC. B) Stress normal to the ONH surface strains the lamina resulting in posterior bowing of LC. C) Stress radially from the center of the ONH surface expands the scleral canal wall resulting in the deformation of LC towards the anterior surface. Reprinted with permission [13]

In vivo studies using SDOCT with high axial resolution detected *in vivo* longitudinal changes like anterior lamina cribrosa depth increase, neuroretinal rim reduction, and eventual RNFL thinning in rhesus monkeys with experimental glaucoma [64]. These *in vivo* studies validated the results seen in *ex vivo* studies. However, the low lateral resolution limits its capability to resolve and monitor changes in laminar pore geometry.

These studies shed light on changes that occur at the level of lamina cribrosa in human and monkey eyes. However, it is imperative to detect early changes during glaucoma and to subsequently track these changes longitudinally to better understand the time-course of structural changes and remodeling during disease progression. We acquire *in vivo* images using high lateral resolution AOSLO and high axial resolution SDOCT to detect and track microarchitectural changes in the anterior lamina cribrosa surface which may help us better understand the mechanisms behind glaucoma.

The goal of this project is to develop computational tools to objectively and accurately characterize the lamina cribrosa microarchitecture of non-human primate eyes with experimental glaucoma. Additionally, the project aims to detect and track early longitudinal changes to better understand the biomechanical behaviors responsible for glaucoma.

Chapter 3

**3D modeling to characterize lamina
cribrosa surface and pore geometries
using *in vivo* images from normal and
glaucomatous eyes**

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3.1 Introduction

Glaucoma is the leading cause of irreversible blindness worldwide [57, 49] and results in the degeneration of retinal ganglion cell axons and the death of retinal ganglion cells. Several studies support the idea that the initial damage to these axons occurs at the lamina cribrosa [52, 39], a 3-dimensional (3D) porous meshwork consisting primarily of flexible, collagenous beams located within the optic nerve head (ONH) [3, 53, 63]. Increases in intraocular pressure (IOP) in a monkey model of experimental glaucoma have been shown to result in a posterior bowing and thickening of the lamina cribrosa early in the disease process, potentially stretching the load-bearing laminar beams and altering laminar pore shape and geometry [13, 5]. Changes in laminar pores could damage the encompassed retinal ganglion cell axons that pass through the laminar pores from the retina to the brain, potentially resulting in axonal degeneration and vision loss.

The lamina cribrosa has been studied in vivo in normal and glaucomatous eyes. Most reports have used spectral domain optical coherence tomography (SDOCT), a technique that provides cross-sectional views of the lamina with high axial resolution, to characterize changes in laminar surface height with disease [63, 30]. However, the poor lateral resolution inherent in commercial SDOCT systems ($15\ \mu m$) currently limits the suitability of this technique to monitor and quantify changes in laminar pore geometry. *En face* images of laminar pores have been acquired and analyzed in living eyes using fundus photography and scanning laser ophthalmoscopy [65, 67, 16]. While these studies improved understanding of laminar pore geometry in normal and diseased eyes, the size and total number of pores that could be resolved and the number of eyes in which laminar pores

could be successfully imaged were limited due to the reduced lateral resolution imposed by uncorrected ocular aberrations. Adaptive optics has recently been used to correct for the eyes optical imperfections and yield high-resolution ($2 - 3 \mu m$) in vivo images of laminar pores [68, 70, 28, 2]. Studies using adaptive optics scanning laser ophthalmoscope (AOSLO) imaging have dramatically improved the ability to quantify anterior laminar pore geometry, but have not accounted for the curvature of the anterior lamina cribrosa surface (ALCS), i.e., the fact that *en face* images acquired using an AOSLO represent a 2D projection of a 3D curved surface [70, 28, 2].

To obtain a more accurate representation of the native pore geometry in living eyes, we transformed 2D AOSLO images onto a 3D ALCS that was modeled using surface points marked on SDOCT images acquired through the optic nerve head (ONH). We computed the mean curvature of the 3D surface for each eye and compared pore parameters calculated from 2D and 3D transformed images taken in normal and glaucomatous eyes to assess the impact of 3D transformation on pore geometry.

3.2 Methods

3.2.1 Animal preparation

All animal care experimentation procedures were approved by the University of Houston's Institutional Animal Care and Use Committee and adhered to the Association for Research in Vision and Ophthalmology (ARVO) statement for the Use of Animals in Ophthalmic and Vision Research. The anterior lamina cribrosa was examined in 11 eyes from six

normal rhesus monkeys (*Macaca mulatta*) between the ages of 1.6 to 7 years. The anterior lamina cribrosa was also examined in 4 monkeys following the induction of unilateral experimental glaucoma (i.e., one glaucomatous eye with the fellow eye as a control). Monkeys were anesthetized with 20 to 25 mg/kg ketamine, 0.8 to 0.9 mg/kg xylazine, and 0.04 mg/kg atropine sulfate to minimize eye movements during in vivo imaging [24]. Monkey pupils were dilated using 2.5% phenylephrine and 1% tropicamide and a contact lens was placed on the eye to prevent corneal dehydration during imaging.

3.2.2 *In vivo* imaging

Wide field (20°) scanning laser ophthalmoscope (SLO) images of the ONH were acquired in all eyes using a Spectralis HRA+OCT (Heidelberg Engineering, Germany). *En face* SLO images were taken such that the anterior lamina cribrosa was at best focus. The same instrument was used to acquire 24-48 SDOCT cross sectional radial B-scans (20° in extent) centered on the ONH [Fig. 3.1(a)]. The termination of the retinal pigment epithelium (RPE) / Bruchs membrane (BM) interface (defining Bruchs Membrane Opening [BMO]) and ALCS were manually marked on SDOCT B-scans [Fig. 3.1(b)] using custom MATLAB code (The MathWorks, Inc., Natick, MA) to generate a point cloud in 3-dimensional space.

En face AOSLO reflectance images (840 nm) of the anterior lamina cribrosa were captured over a 1.5° field at a rate of 25 Hz during closed-loop adaptive optics correction [28, 18]. The power of the 840 nm superluminescent diode (Superlum, Ireland) at the corneal plane was 300 μ W) (which is more than 10 times below the maximum permissible exposure

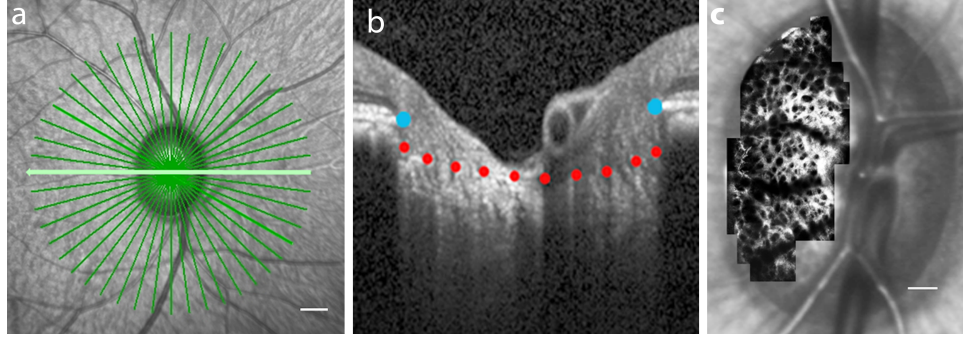


Figure 3.1: (a) *En face* SLO image showing locations of SDOCT radial B-scans acquired in a normal eye. Scale bar = $150\ \mu\text{m}$. (b) Manual delineation of the innermost terminations of the RPE/BM complex (blue points) and ALCS (red points) within the optic nerve head in a single SDOCT radial B-scan [denoted by the green bold line in (a)]. ALCS points that were located underneath a blood vessel shadow were not marked. (c) AOSLO montage of laminar pores (dark holes) on the temporal side of the anterior laminar surface scaled, registered and overlaid on the corresponding SLO image. Scale bar = $150\ \mu\text{m}$.

dictated by the ANSI standards for a 1.5° field size, 1.5 hour exposure, and wavelength of 840 nm) [33]. Individual frames from the AOSLO videos were registered offline using a normalized cross-correlation technique to remove distortions caused by eye movements. Registered frames were averaged to produce images with high signal-to-noise ratios. A montage of the anterior laminar surface was created from registered AOSLO images [Fig. 3.1(c)]. Laminar pore boundaries were manually marked using Photoshop (Adobe Systems, San Jose, CA) [28]. Pore area and elongation (ratio of the major to minor axis of an ellipse best-fit to the boundary of a given pore) were calculated in the *en face* montages.

Because SLO, SDOCT, and AOSLO images were taken over fixed angular field sizes, the lateral distance covered by each scan on the ONH could vary depending on the eye's ocular biometry. We measured each eye's axial length, anterior chamber depth, and anterior corneal curvature using an ocular biometer (IOLMaster, Carl Zeiss Meditec, Dublin, CA)

and incorporated these measurements into a model eye to convert the lateral field size in our images from angular (i.e., degrees) to physical (i.e., micrometers) units [28]. All SLO, SDOCT, and AOSLO images from a given eye were scaled to the same resolution (microns/pixel) and registered in each eye.

3.2.3 Three-dimensional anterior lamina cribrosa surface modeling and characterization

A thin plate spline was fit to the marked ALCS point cloud to model the surface in 3 dimensions using the MATLAB curve fitting toolbox (The MathWorks, Inc., Natick, MA) [Fig. 3.2(a)]. The kernel used for the thin plate spline was:

$$f(r) = r^2 \log(r^2) \quad (3.1)$$

where $f(r)$ is depth of the surface at a given radius, r , from the center of the kernel.

The thin plate spline is an interpolator formed as the linear combination of multiple kernels, which minimizes the bending energy, $E(f)$, defined as:

$$E(f) = \iint_{R^2} \left(\left(\frac{\partial^2 f}{\partial x^2} \right)^2 + \left(\frac{\partial^2 f}{\partial xy} \right)^2 + \left(\frac{\partial^2 f}{\partial y^2} \right)^2 \right) dx dy \quad (3.2)$$

To estimate a smooth surface, a smoothing parameter (p) was added to the energy function for regularization. The thin plate smoothing spline was estimated by minimizing the energy function, $E_s(f)$, defined as:

$$E_s(f) = p \sum_{i=1}^n f(x_i, y_i) - z_i + (1 - p) \iint_{R^2} \left(\left(\frac{\partial^2 f}{\partial x^2} \right)^2 + \left(\frac{\partial^2 f}{\partial xy} \right)^2 + \left(\frac{\partial^2 f}{\partial y^2} \right)^2 \right) dx dy \quad (3.3)$$

According to the Matlab manual, the smoothing parameter, p , was estimated a priori. The smoothing parameter, p , was computed from the coefficient matrix of the linear equations obtained using a radial basis function applied to the (x, y) coordinates of the marked points:

$$p = \frac{1}{\text{mean}(\text{diagonal}(\text{Coefficient Matrix}))} \quad (3.4)$$

In our case, we found a consistently low value for the smoothing parameter (mean value of $p = 1.6 \times 10^{-6} \pm 6.1 \times 10^{-7}$) across all eyes. The standard deviation about the mean value was very small, indicating a very similar smoothing parameter across eyes.

Following this surface estimation, the mean anterior lamina cribrosa surface depth (ALCSD) was calculated as the mean perpendicular distance from a plane best-fit to the BMO point cloud to the thin plate spline surface (fit to the marked ALCS points) for locations within the BMO ellipse [Fig. 3.2(b)] [63]. The 3D anterior lamina cribrosa surface was also characterized by computing the mean radius of curvature of the surface for points located within the projection of the BMO ellipse onto the ALCS. In two dimensions, the radius of curvature (ρ) at a given point on the curve may be calculated as the ratio of the change in arc length (ds) to the change in the angle of a line tangent to the curve ($d\theta$) between 2 points adjacent to the given point [Fig. 3.2(c,d)].

$$\rho = \left| \frac{ds}{d\theta} \right| \quad (3.5)$$

The curvature (κ) at the given point is the reciprocal of the radius of curvature:

$$\kappa = \frac{1}{\rho} \quad (3.6)$$

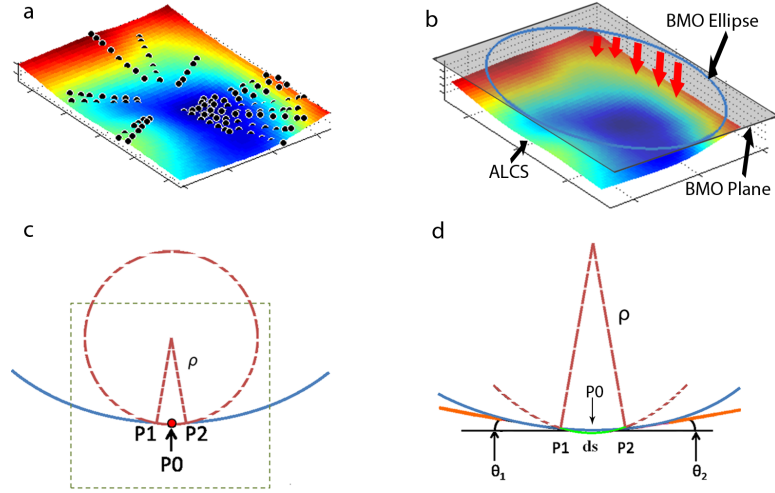


Figure 3.2: (a) Model of laminar surface constructed by fitting a thin plate spline to the marked ALCS point cloud (denoted by black points that reside above and below the fit surface). (b) Mean ALCSD was calculated as the mean normal distance between a plane fit to the BMO point cloud (shaded gray) and the 3D anterior laminar surface (red arrows) for points located within the BMO ellipse (blue ellipse). (c,d) Graphical representation for determining the radius of curvature of a 2-dimensional curve (blue) at a point, P0. (c) An osculating circle (dashed, red circle with radius, ρ) is drawn through point P0 and two points on the 2-dimensional curve that are infinitesimally close to P0 (i.e., P1 and P2). (d) Magnified view of the region enclosed by the green dashed rectangle in (c). The radius of curvature at point P0 is calculated as the ratio of the arc length between P1 and P2 (green arc, labeled ds) to the change in the angle of a line tangent to the curve (solid orange lines) at points P1 and P2 (i.e., $|d\theta| = |\theta_1 - \theta_2|$).

The mean curvature of the 3D laminar surface was calculated as the average of the values of the mean curvature calculated at points on the fitted surface (1 m separation) located within the projection of the BMO ellipse. The mean curvature at a given point on a 3D surface, H , can be calculated as the mean of the principal curvatures at that point. The principal curvatures (κ_1, κ_2) are the maximum and minimum curvatures at the given point:

$$H = \frac{(\kappa_1 + \kappa_2)}{2} \quad (3.7)$$

The mean radius of curvature for the 3D anterior laminar surface was calculated as the

reciprocal of the mean 3D curvature.

The AOSLO montage was then registered with the SLO and SDOCT images and projected onto the thin plate spline representing the ALCS [Fig. 3.3(a)]. The 3D surface was tessellated into a mesh consisting of triangular elements with regularly sampled vertices in the $x - y$ (lateral) plane spaced between $1.5 - 2 \mu m$ [Fig. 3.3(b)]. The 3D area of a given pore was calculated by integrating the area of the tessellated triangles located within the pore boundary. If a triangle was not completely located inside the pore boundary, its contributing area was computed as the product between the ratio of the number of triangle vertices contained within the pore boundary and the triangle area. To compute the 3D elongation of a given pore, an ellipse was first fit in 3 dimensions to points located on the pore boundary of the 3D surface. The ratio of the major to minor axis of this best-fit ellipse was computed as the 3D elongation of the pore.

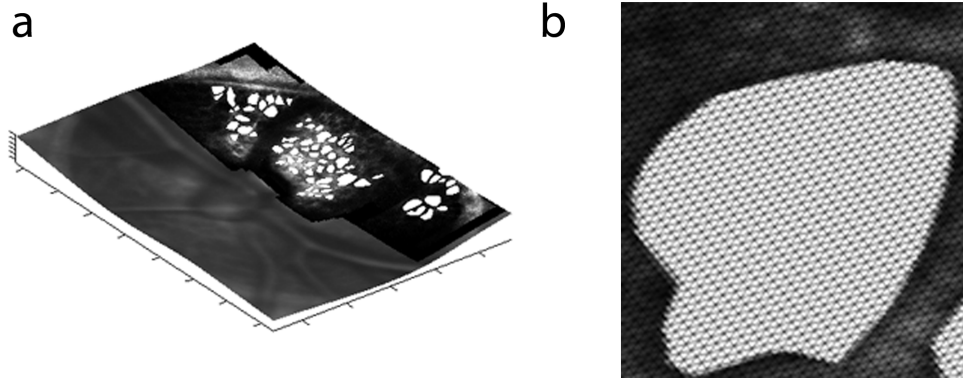


Figure 3.3: (a) Registered AOSLO montage overlaid on the SLO image and projected onto the thin plate spline representing the ALCS. Laminar pores are marked and filled in white. (b) Magnified image of a laminar pore from (a) illustrating the tessellation of its surface into a collection of triangles.

3.3 Results

3.3.1 Validation of thin plate splines

Arbitrary known surfaces were created and used to validate and compare the goodness-of-fit of the thin plate spline with other fitting functions (e.g., bi-cubic spline). As shown in Fig. 3.4, thin plate and bi-cubic splines were fit to the same regularly sampled subset of points (25-100% of the total points) used to generate a known surface. The residual root-mean-square (RMS) errors of the thin plate and bi-cubic spline fits with respect to the known surfaces were calculated from the non-fitted points (or from all points if 100% of the points were used for the fit). For example, Zernike spherical aberration was constructed as one of the known surfaces (Fig. 3.4(a)). Thin plate and bi-cubic splines were fit to 45%

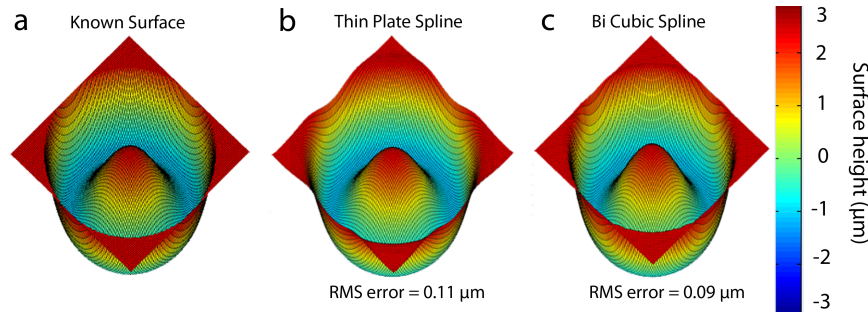


Figure 3.4: (a) Zernike spherical aberration was created as a known surface for fitting validation. Surfaces were created by fitting a (b) thin plate spline and (c) bi-cubic spline to the same regularly sampled subset of points (45% of the total points) from the known surface. The RMS errors calculated from the non-fitted points were 3.2% and 2.6% of the total peak-to-valley height of the known surface ($3.35 \mu m$) for the thin plate spline and bi-cubic spline, respectively.

of the total known surface points that were regularly sampled. The RMS errors of the thin plate and bi-cubic splines (calculated from the residual 55% of the total known surface

points) were 3.2% and 2.6% of the original peak-to-valley surface height of the known surface ($3.35 \mu\text{m}$), respectively (Fig. 3.4(b,c)). Similarly, we found the RMS errors for the thin plate and bi-cubic spline fits to be comparable when the data were regularly sampled for other known surfaces. However, because the ALCS points that were manually marked from the SDOCT images were not regularly sampled (which is a requirement for a bi-cubic spline fit), we chose to use a thin plate spline as our fitting function (as further elaborated in the Discussion).

To validate the area measurements calculated using our tessellation method, the areas of surfaces with known parameters were compared with the areas generated after tessellating the known surface into a mesh of triangular planes. Fig. 3.5 shows the area validation results obtained when using a hemisphere as a known surface. The difference between

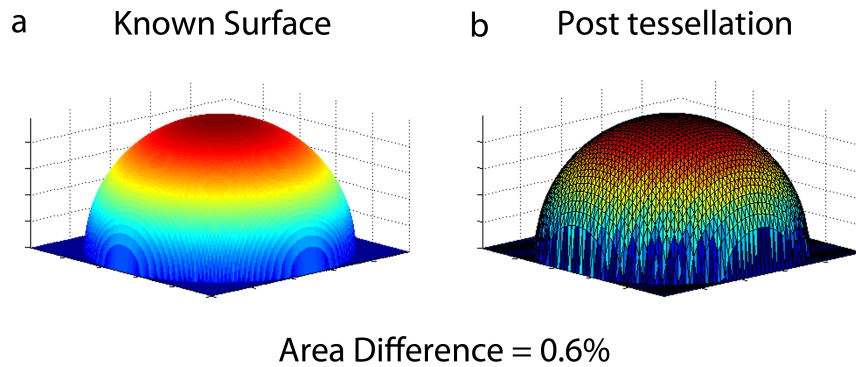


Figure 3.5: (a) Hemisphere of known surface area prior to tessellation. (b) Hemisphere surface after tessellation. The area of the post-tessellated hemisphere was 0.6% smaller than the known surface area.

the theoretical area of the hemisphere and the area after tessellation was 0.6%. Similarly, the differences between the theoretical areas and the post-tessellated areas were less than 1% in all surfaces tested. These small differences were due to the accumulation of errors

contributed by areas of the triangles not completely contained within the border (or edge) of the surface.

3.3.2 Three-dimensional transformation of the lamina cribrosa in normal and glaucomatous eyes

Thin plate splines accurately fit the manually marked ALCS points (Fig. 3.6). The mean RMS fit error of the thin plate spline across the 11 normal eyes was ($9.8 \pm 1.2 \mu m$), a value that is only 2.5 times larger than the SDOCT instrument's axial resolution (corresponding to a 23 pixel error). In addition, the anterior laminar surface was relatively flat in normal eyes as exhibited by the large mean radius of curvature of the surface (mean = $3.0 \pm 0.5 mm$ across eyes) and the small mean ALCS D (190 $\pm 13 \mu m$), as seen in Fig. 3.6 and Table 1.

There were small changes in laminar pore parameters following the 3D transformation of the AOSLO images in normal eyes. The mean 2D pore area and elongation across all normal eyes were $1092 \pm 195 \mu m^2$ and 1.66 ± 0.44 , respectively (Table 1). Following 3D transformation, mean pore area increased by $5.1 \pm 2.0\%$ ($52.2 \pm 35.5 \mu m^2$) while the mean change in pore elongation was $0.0 \pm 0.2\%$ (0.0 ± 0.004) due to the relatively flat laminar surfaces. The mean 3D transformed pore area and elongation were $1137 \pm 260 \mu m^2$ and 1.67 ± 0.45 , respectively (Table 1).

Despite having a more steeply curved ALCS (Fig. 3.7(c,d)), the mean RMS error of the thin plate spline fit across the 4 glaucomatous eyes was $10.8 \pm 1.3 \mu m$, a value comparable to that measured in normal eyes. However, the mean radius of curvature of the laminar

surface in the glaucomatous eyes was $1.3 \pm 0.1 \text{ mm}$, demonstrating a more steeply curved (or cupped) anterior laminar surface. This observation was also reflected in the larger mean ALCSD value ($392 \pm 31.4 \mu\text{m}$) compared to normal eyes. The mean 2D pore area and elongation across all experimental glaucoma eyes were $1049 \pm 389 \mu\text{m}^2$ and 1.83 ± 0.64 , respectively [Table 3.1].

Table 3.1: Comparison of lamina cribrosa surface geometry and pore geometry after 3D transformation in living eyes

	Mean laminar surface and pore parameters	
	11 Normal Eyes	4 Glaucoma Eyes
Mean ALCS Depth (μm)	190 ± 13	392 ± 31.4
Mean Radius of Curvature (mm)	3.0 ± 0.5	1.3 ± 0.1
Mean 2D Pore Area (m^2)	1092 ± 195	1049 ± 389
Mean 3D Pore Area(m^2)	1137 ± 260	1209 ± 433
Mean % Difference in Area	5.1 ± 2.0	16.2 ± 5.9
Mean 2D Pore Elongation	1.66 ± 0.44	1.83 ± 0.64
Mean 3D Pore Elongation	1.67 ± 0.45	1.84 ± 0.63
Mean % Difference in Elongation	0.0 ± 0.2	-0.2 ± 2.0

Following 3D transformation of the AOSLO images onto the thin plate spline surfaces, the mean pore area increased across the 4 glaucoma eyes by $16.2 \pm 5.9\%$ ($160 \pm 121 \mu\text{m}^2$) due to the increased curvatures, while the mean change in pore elongation was $-0.2 \pm 2.0\%$ (-0.005 ± 0.04). The mean 3D transformed pore area and elongation were $1209 \pm 433 \mu\text{m}^2$ and 1.84 ± 0.63 , respectively [Table 3.1].

3.4 Discussion

It is important to have a faithful representation of the anterior lamina cribrosa surface from the *in vivo* images as the ALCS serves as the basis for characterizing laminar surface and pore parameters in 3 dimensions. We considered parametric and non-parametric approaches to estimate the ALCS based on our manually marked data points. Parametric approaches, such as polynomial surfaces, were not suitable for estimating the ALCS because the shape of the marked data points did not conform to a single polynomial surface. For example, a lower order polynomial could provide a smooth surface fit to the marked data points in 3-dimensional space with a potential consequence of yielding a high RMS fit error. Using a higher degree polynomial would yield a lower RMS fit error, but could result in undesirable ringing effects in the estimated surface (Runge's phenomenon). Hence, a non-parametric approach was found to be more suitable to describe the ALCS.

Multiple non-parametric approaches were considered for modeling the ALCS, including locally weighted regression, bi-cubic splines and thin plate splines. Locally weighted regression surface estimation is a popular approach to estimate a smooth surface and model data points. However, this smoothing surface approach requires a set of dense data points for regression within a local (or span) window that typically form a point cloud of data points which lie above/below and to the left/right of the actual surface. This type of point cloud is not generated in our data, as our data points are manually marked just along the top of the ALCS in each SDOCT B-scan. Therefore, a locally weighted regression approach was not suitable for our data.

We used a thin plate spline to model the anterior lamina cribrosa surface in 3D and quantified the goodness-of-fit of the thin plate spline method by calculating the residual RMS fitting error. This RMS error was found to be comparable to the residual RMS fitting error of a bi-cubic spline. Despite the fact that a bi-cubic spline tended to yield surfaces with slightly lower RMS fitting errors, we chose to fit all of our ALCS point clouds using thin plate splines because fitting with a bi-cubic spline requires that the data be regularly sampled, whereas the thin plate spline does not. The latter was more suitable for fitting a manually marked lamina cribrosa surface, as the generated point cloud was not regularly sampled [Fig. 3.2(a)]. For example, it is often difficult to mark laminar surface points that reside underneath overlying blood vessels [Fig. 3.1(b)]. Moreover, the residual RMS error was very low for the thin plate spline [Fig. 3.4]. In addition, thin plate splines have been used previously to accurately represent the anterior and posterior laminar surfaces of histomorphometric reconstructions of the monkey lamina cribrosa [74] and have been shown to be an effective method for mapping and tracking non-rigid surface deformations longitudinally in the heart.

An accurate representation and characterization of the ALCS is necessary to better understand and track structural changes in the ALCS during disease progression. Several studies have shown an increase in mean ALCS D during the progression of glaucoma [3, 63, 13]. However, the use of this single parameter provides only a partial characterization of the structural changes in the laminar surface. While an increase in the mean ALCS D indicates an overall posterior shift in the anterior laminar surface (relative to the BMO reference plane), this value provides no characterization of the shape or curvature of the actual surface. For example, it is possible to have two differently curved anterior laminar surfaces

with the same mean ALCSD; for instance, one surface could be uniformly depressed in all locations (i.e., a large radius of curvature) whereas the second surface could have a large degree of bowing or cupping (i.e., a small radius of curvature) about the same mean depth. Likewise, two anterior laminar surfaces with the same mean curvature could give rise to different mean ALCSD values if, for example, one surface is more posterior to the BMO reference plane than the other. Therefore, we believe that specifying the mean ALCSD and mean radius of curvature provides increased information on the structural changes that occur in the ALCS during disease progression and will facilitate better tracking of the laminar surface over time [Fig. 3.8]. An increase in mean ALCSD accompanied by a decrease in the mean radius of curvature would indicate a more steeply curved laminar surface that is more posteriorly located with respect to the BMO reference plane, whereas an increased mean ALCSD accompanied by no change in the mean radius of curvature would indicate a more uniform posterior deformation of the entire surface.

The 3D transformed pore parameters better represent the native state of lamina cribrosa surface pore geometry compared with values calculated from 2D AOSLO images. Table 1 shows that larger increases in the 3D transformed pore area were calculated in the experimental glaucoma eyes. These increases were due to the fact that the mean radius of curvature was lower (i.e., the curvature was greater) in glaucoma eyes relative to normal eyes (Table 1). The small mean increases in laminar pore parameters calculated after 3D transformation in normal eyes were due to the fact that the anterior laminar surface was relatively flat with a high radius of curvature [Fig. 3.6]. However, the change in mean elongation of the laminar pores following 3D transformation was nearly identical between normal eyes and eyes with experimental glaucoma. The change in the elongation of a pore

after 3D transformation depends on the spatial location and orientation of the pore. The 3D elongation of a transformed pore would increase relative to the 2D elongation of the same pore if the slope along the major axis of the ellipse best-fit to the transformed pore is greater than the slope along the minor axis of the same ellipse. Alternatively, the 3D elongation of a transformed pore would decrease from its 2D elongation value if the slope is greater along the minor axis of the ellipse best-fit to the pore. The fact that the mean changes in elongation following 3D transformation in normal and glaucoma eyes were $0.0 \pm 0.2\%$ and $-0.2 \pm 2.0\%$, respectively, of the original 2D pore elongation likely indicates the absence of a predominant orientation of the anterior laminar surface pores that were imaged.

3.5 Conclusions

As evidenced by the low RMS fitting errors, thin plate splines can accurately fit laminar surfaces with different slopes in normal and glaucomatous eyes. Changes in mean ALCSD and the mean radius of curvature of the anterior laminar surface can better describe structural changes that occur during disease. The laminar surfaces in normal eyes are fairly flat (i.e., exhibit large radii of curvature), yielding small changes in 3D pore geometry. As the curvature of the anterior laminar surface increases, which typically occurs during disease progression, larger changes can be noted in the 3D transformed pore geometry. The proposed 3D transformation and tessellation method can be employed with *en face* and axial imaging techniques, such as AOSLO and SDOCT imaging, to better understand 3D laminar geometry. This approach could be used *in vivo* to further examine global and

regional differences in the 3D laminar geometry of normal eyes, 3D changes in laminar geometry that occur at different stages of the disease in experimental models of glaucoma and in human glaucoma patients, and 3D differences in laminar geometry between normal and glaucomatous eyes.

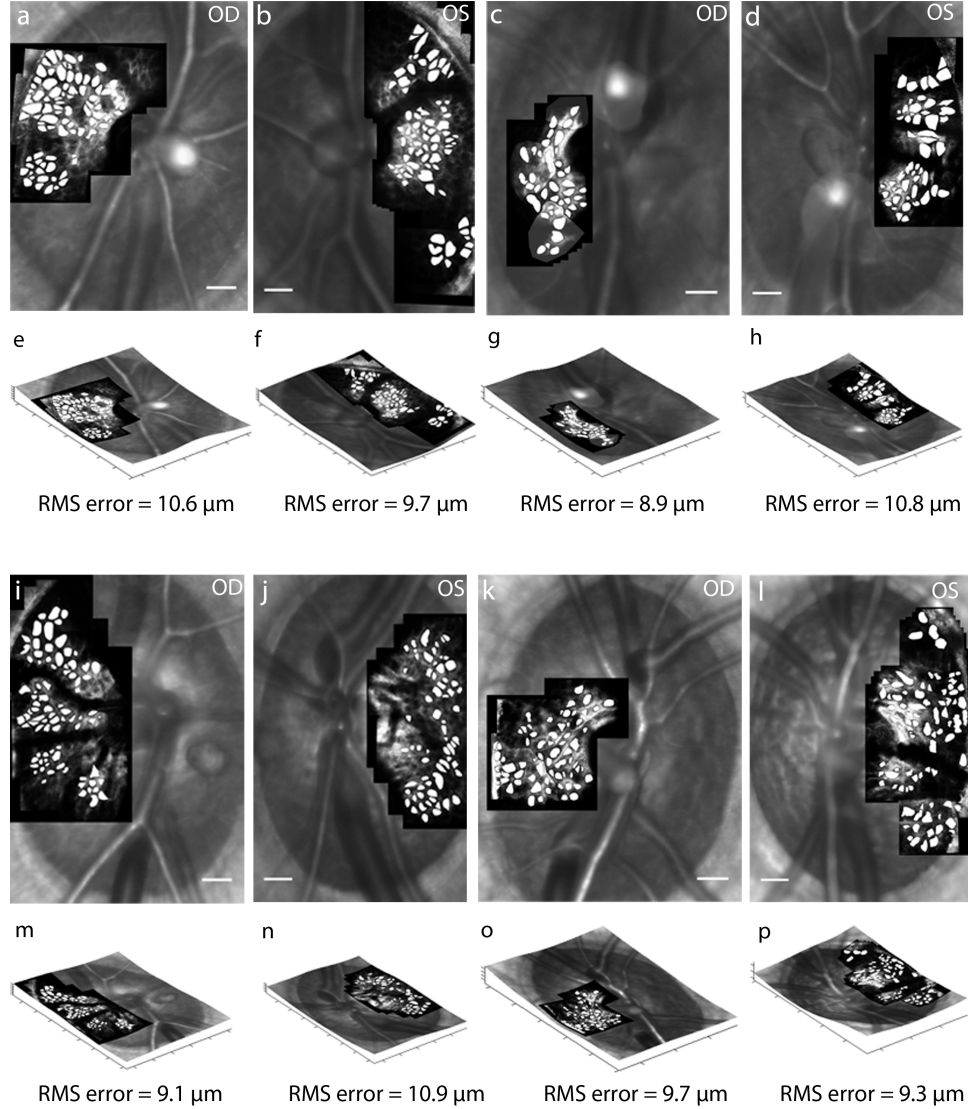


Figure 3.6: (a-d, i-l) 2D AOSLO montages of the anterior laminar surface registered and overlaid on the SLO images acquired in 8 of 11 normal monkey eyes. Laminar pores are marked and filled in white. Scale bar = $150 \mu m$. (e-h, m-p) Each 2D AOSLO montage was registered and projected onto the thin plate spline fit to the ALCS point cloud to generate a 3D transformed image of anterior laminar pores. The RMS errors of the thin plate splines fit to the manually marked ALCS points are reported underneath each 3D transformed AOSLO image. The mean RMS fit error across all 11 normal eyes was $9.8 \pm 1.2 \mu m$.

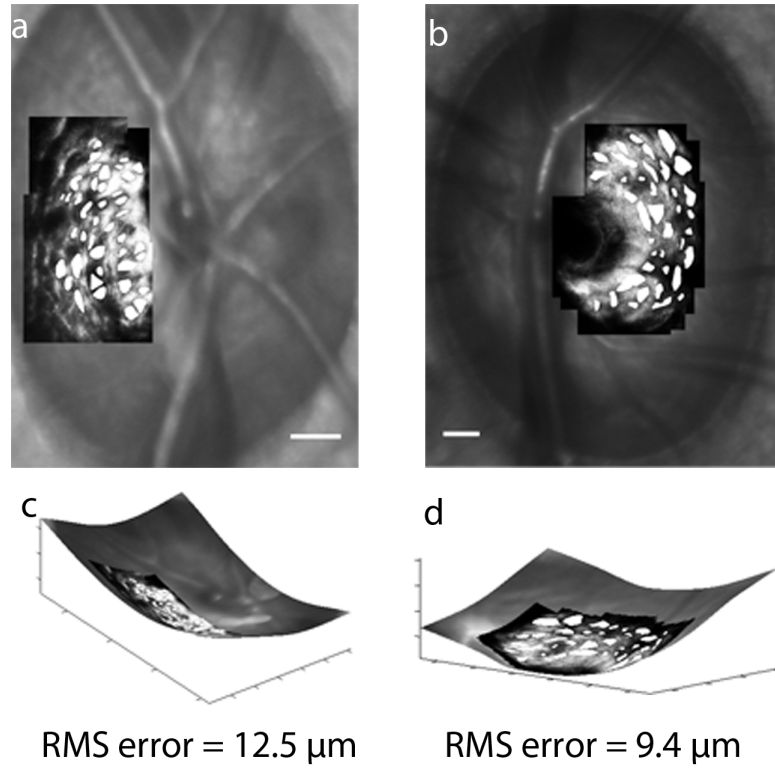


Figure 3.7: (a,b) 2D AOSLO montages of the anterior lamellar surface registered and overlaid on the SLO images acquired in 2 of 4 eyes with unilateral experimental glaucoma. Lamellar pores are marked and filled in white. Scale bar = 150 μm . (c,d) 3D transformed AOSLO images. While the anterior lamellar surfaces in the glaucoma eyes were more steeply curved, the RMS fit errors for the thin plate splines were similar to those in normal eyes. The increased surface curvatures yielded a greater change in 3D pore area compared to that calculated in normal eyes.

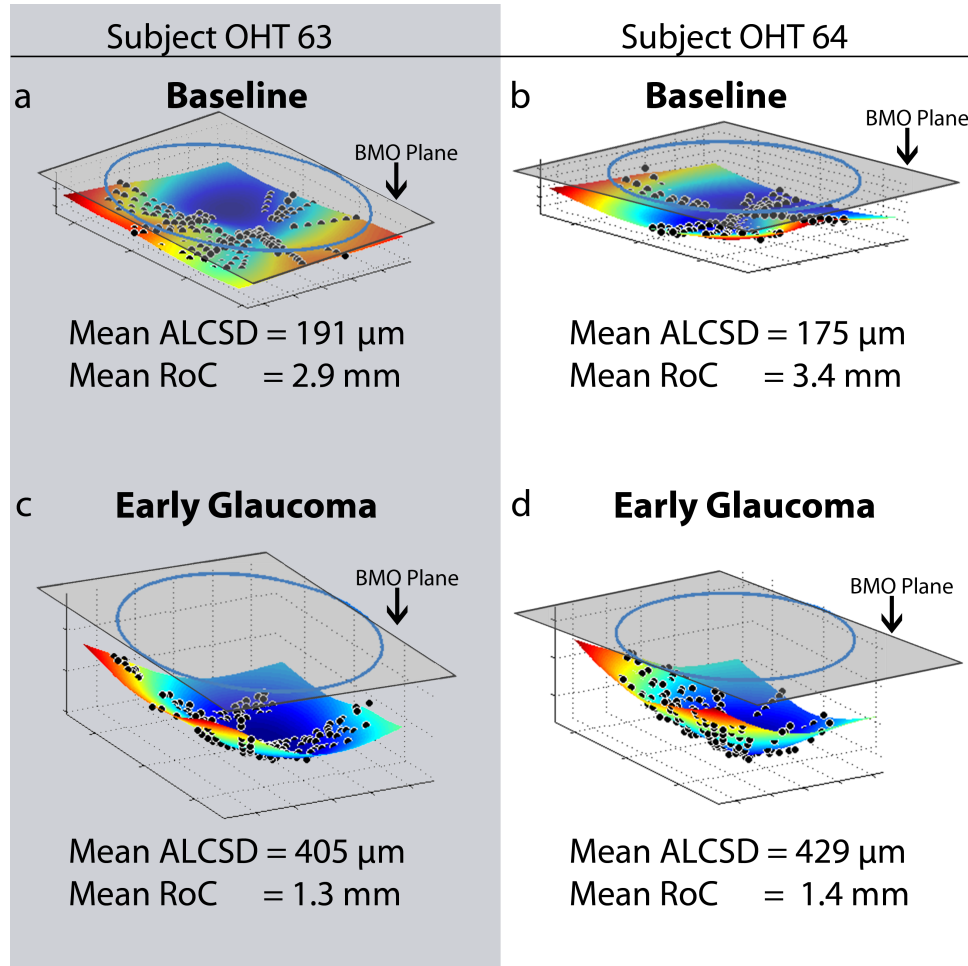


Figure 3.8: (a,b) Thin plate spline models of the anterior lamina cribrosa surface (colored) in 2 monkey eyes (Subjects OHT-63 and OHT-64) relative to the BMO plane (gray rectangles) prior to the induction of experimental glaucoma. Both eyes had mean ALCSD values comparable to normal eyes (191 μm and 175 μm , respectively) with relatively high mean radii of curvature (RoC) of 2.9 mm and 3.4 mm, respectively. (c,d) Thin plate spline models of the anterior lamina cribrosa surface (colored) in the same 2 eyes from (a,b) relative to the BMO plane (gray rectangles) after induction of experimental glaucoma. Mean ALCSD increased (405 μm and 429 μm , respectively) while the mean radii of curvature decreased in each eye (1.3 mm and 1.4 mm, respectively), indicating that the laminar surface posteriorly deformed and became more steeply curved.

Chapter 4

Automated characterization of predominant lamina cribrosa orientation in non-human primates using AOSLO

4.1 Introduction

Glaucoma is a group of eye diseases that results in the degeneration of retinal ganglion cell axons and the death of the retinal ganglion cells. A major risk factor for glaucoma is elevated intraocular pressure (IOP). Substantial evidence suggests that the initial site of axonal damage due to elevated intraocular pressure occurs at the level of the lamina cribrosa [3, 53, 55, 51]. The lamina cribrosa is a three-dimensional porous meshwork in the optic nerve head, consisting primarily of collagenous beams that provide functional and structural support to the retinal ganglion cell axons passing through it from the retina to the brain.

Previous studies have shown that stress due to elevated intraocular pressure in a monkey model of the experimental glaucoma strains the flexible lamina leading to posterior bowing and stretching of the load-bearing lamina cribrosa [5, 6, 74]. Straining the flexible lamina may potentially change the geometric and material properties of the connective tissue structure, and damage the laminar capillaries and astrocytes that may compromise axonal structure and function. Several *ex vivo* studies have compared the structure of lamina cribrosa between normal and glaucomatous eyes in non-human primates. 3D histomorphometric techniques were used to quantify ONH structures by delineating neural canal landmarks such as Bruchs membrane opening, anterior sclera canal opening etc [74]. Previous studies characterized the orientation of the lamina cribrosa beam microarchitecture in *ex vivo* eyes by estimating the mean intercept length [58]. While this method works well on high-contrast histological images, the method's utility on *in vivo* images with varying degrees of contrast, noise and blur is limited due to the fact that the mean intercept length

requires accurate binary segmentation of the raw gray scale image. While *ex vivo* have improved our understanding of glaucoma, post-mortem studies are limited by its ability to track longitudinal changes. Hence, it is necessary to validate the *ex vivo* results and examine longitudinal changes *in vivo* during the progression of glaucoma. High-resolution, *in vivo* analysis of the lamina cribrosa have been carried out in normal and glaucomatous eyes using various techniques [2, 70, 40, 71, 28]. Ivers et al. showed good repeatability in imaging and assessing manually marked lamina cribrosa pore geometry *in vivo* in normal monkeys and humans using AOSLO [28]. These studies support the need for objective and accurate *in vivo* analysis and examination of structural characteristics of lamina cribrosa.

We used principal component analysis to estimate the predominant local anterior lamina cribrosa beam orientation directly from raw gray scale *in vivo* images without the need for binary segmentation. To validate the method, we employed synthetic images of known characteristics to estimate the predominant orientation of overlapping local regions of interest and compared the resulting estimates with the known values for different levels of noise and blur. We estimated the orientation of synthetic images of known characteristics using principal component analysis and compared the results with other methods like mean intercept length, Radon transform and steerable filter. We assessed the repeatability of quantifying the predominant local anterior lamina cribrosa beam orientation *in vivo* within the individual normal eyes of six macaques imaged using an adaptive optics scanning laser ophthalmoscope at different time points. Statistical tests were performed to investigate whether significant differences were present in the local anterior lamina cribrosa beam orientations within individual normal eyes. This work provides an automatic and repeatable method for determining dominant orientations of the lamina cribrosa and can

be extended to track longitudinal changes in local beam orientation in experimental glaucoma.

4.2 Methods

All animal care experimental procedures were approved by the University of Houston's Institutional Animal Care and Use Committee, and adhered to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

The adaptive optics scanning laser ophthalmoscope (AOSLO) montages of anterior lamina cribrosa beams and optical coherence tomography scans of the optic nerve head were examined *in vivo* in the fellow eyes of six rhesus monkeys at multiple time-points before and after induction of unilateral experimental glaucoma. Argon laser treatment was performed in stages around the trabecular meshwork to elevate the intraocular pressure of each monkey's right eye, while the left eye served as controls. Monkeys were anesthetized with 20-25 mg/kg ketamine, 0.8-0.9 mg/kg xylazine, and 0.04 mg/kg atropine sulfate to minimize eye movements during *in vivo* imaging. Monkey pupils were dilated with 2.5% phenylephrine and 1% tropicamide [18]. A lid speculum was used to hold the monkey eyelid open and a contact lens was placed on the monkey eye to prevent corneal dehydration during imaging. A head mount with tip, tilt, and rotation capabilities was attached to an XYZ translation stage used to stabilize the monkey's head and align the eye's pupil with the AOSLO system for lamina imaging.

4.2.1 Biometric measurements and image scaling

We measured axial length, anterior chamber length, and anterior corneal curvature in all eyes at every time-point using an ocular biometer (IOLMaster; Carl Zeiss Meditec, AG, Jena, Germany). These biometric measurements were incorporated into a model eye to laterally scale AOSLO images using methods described by [28].

4.2.2 *In vivo* imaging using spectral domain optical coherence tomography (SD-OCT)

Wide field (20°) scanning laser ophthalmoscope (SLO) images of the ONH were acquired in all eyes using a Spectralis HRA+OCT (Heidelberg Engineering, Germany). *En face* SLO images were taken such that the anterior lamina cribrosa was at best focus. The same instrument was used to acquire 24-48 SDOCT cross sectional radial B-scans (20° in extent) centered on the ONH [Fig. 4.1]. The termination of the retinal pigment epithelium (RPE) / Bruchs membrane (BM) interface (defining Bruchs Membrane Opening [BMO]) and ALCS were manually marked on SDOCT B-scans (Fig. 4.1) using custom MATLAB code (The MathWorks, Inc., Natick, MA) to generate a point cloud in 3-dimensional space. The radius of curvature of ALCS, BMO area and the mean anterior lamina cribrosa surface depths were computed using thin plate spline [61]. The internal limiting membrane (ILM) was automatically segmented in each B-scan using the SD-OCT software and manually corrected wherever necessary. The mean minimum rim width (MRW), which is the average of the minimum distances between each RPE/BM termination point to the ILM, were calculated for all marked B-scans. The peripapillary retinal nerve fiber layer (RNFL) was

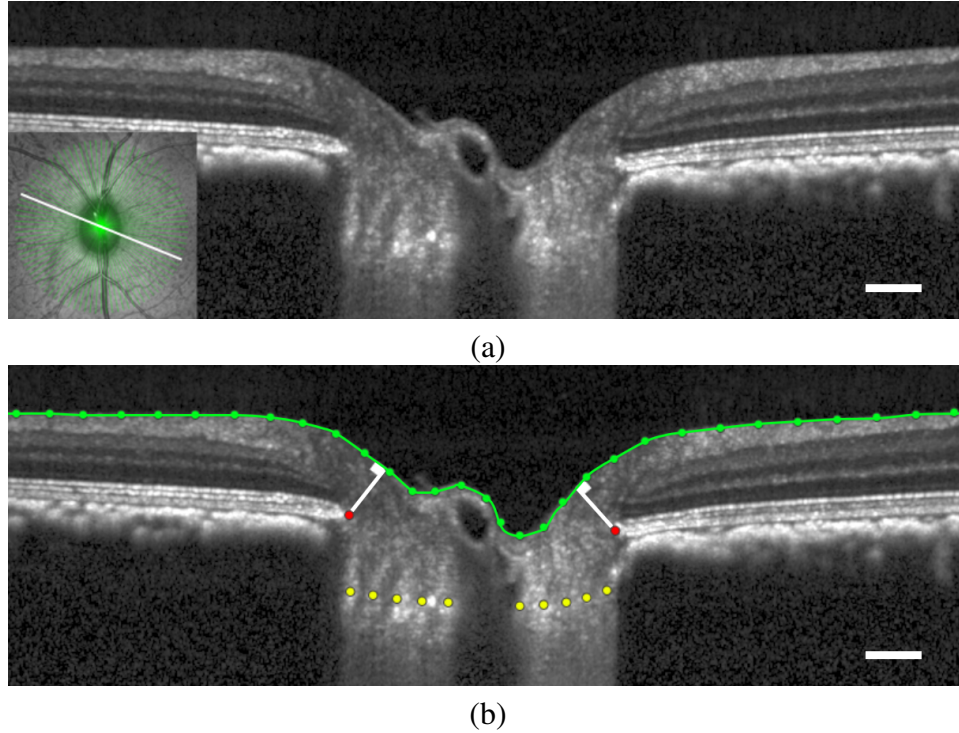


Figure 4.1: ONH features marked on the B-scan. (a) Single b-scan from a normal monkey eye corresponding to the white scan line in the inset SLO image (b) Manual delineation of the innermost terminations of the RPE/BM complex (red points) and ALCS (yellow points). The minimum distance from RPE/BM to ILM (green line with dots) is denoted by the white lines. Scale bar = 300 μm .

automatically segmented from the 12° circular scans centered on the ONH at every time-point and manually corrected wherever necessary. Subsequently, the mean RNFL thickness (RNFLT) was measured.

4.2.3 *In vivo* imaging using adaptive optics scanning laser ophthalmoscope

En face images of the anterior lamina cribrosa at best-focus was acquired using an AOSLO over a field of (1.5°) at a rate of 25Hz using an 840 nm superluminescent diode as described previously. The power of the 840 nm SLD at the corneal plane conformed to the ANSI standards and was set to $300 \mu W$) [33]. Individual frames from the AOSLO videos were registered offline using a custom cross-correlation technique and averaged to reduce the noise in the image [28]. These registered frames were then stitched manually to form a montage. The pores were manually marked using Adobe Photoshop [28]. The pore area, elongation, and nearest neighbor distance of each marked pore were quantified in each montage after 3D transformation [61]. The predominant local beam orientation of the anterior lamina cribrosa surface was estimated.

4.2.4 Image pre-processing to improve the quality of AOSLO montage

It is well known that performance of image analysis methods are affected by the presence of noise, low contrast, and irregular illumination of the object of interest. Image pre-processing filters were applied to the lamina cribrosa montages in order to ease the effects of variations in image acquisition quality. Homomorphic filtering can be used to remove the effects of non-uniform illumination. According to the illumination-reflection model, the intensity value at the pixel coordinate (x, y) of the image I is a product of the illumination of the object and the reflectance of the object in the scene.

$$I(x, y) = L(x, y) R(x, y)$$

where, L is the illumination and R is the reflectance of the object. Reflectance R depends on the property of the object and illumination L depends on the property of light source that shines on the object. When a homogeneous object is uniformly illuminated, the intensity of the image it forms on a camera will also be uniform. However, non-uniform illumination results in irregular intensities in the image. Since reflectance is dependent on the intrinsic property of the object, it may vary abruptly in space, thus making it the high spatial frequency component in the image. For example, the AOSLO *en face* image of the lamina cribrosa is composed of the hyper-reflective lamina cribrosa beam and the non-reflective dark holes known as lamina cribrosa pores. Conversely, the illumination is a low spatial frequency component because it varies gradually within an image. To correct the effects of non-uniform illumination it is necessary to separate the illumination component and the reflectance component. Homomorphic filter does exactly that by filtering the low frequency illumination component. However, a high-pass filter cannot be employed directly on the image because these two components are multiplicative and not additive. Hence, a simple mathematical trick is employed to transform the illumination-reflection model with multiplicative components to an expression with additive components by taking the natural log (base-e) of the expression.

$$\ln [I(x, y)] = \ln [L(x, y) R(x, y)]$$

$$\ln [I(x, y)] = \ln [L(x, y)] + \ln [R(x, y)]$$

Now, apply a high-pass filter in the log domain to filter the high frequency reflectance component. A simple Gaussian high-pass filter is constructed and applied to the Discrete-Fourier transform of the log transformed image. A Gaussian high-pass filter can be constructed by first creating a Gaussian low-pass filter with $\sigma = 20$ and then subtracting 1 from it. Take the inverse Fourier transform of the high-pass filtered image followed by the exponential transform. The resulting image is composed of only the reflectance component.

The next step is to enhance the contrast of the image using contrast limiting adaptive histogram equalization or CLAHE for short. It is well known that histogram equalization can enhance the global contrast of the image by non-linear transformation of the intensity values such that the resulting image has a flatter histogram. However, this does not address the local contrast enhancement requirements. Another drawback with histogram equalization is that it amplifies noise in the image. CLAHE is a method that addresses these two problems and requires two input parameters: tile size and clip limit. To enhance the local contrast the image is divided into several tiles/blocks and histogram equalization is applied to these individual tiles. The neighboring tiles are combined using a bilinear interpolation to avoid sharp edges at the tile boundaries. To overcome the noise problem, the amplification is limited by clipping the histogram at a value specified by the clip limit parameter. Appropriate values for the parameters were heuristically chosen by the user. Fig. 4.2 summarizes the flow of steps involved in pre-processing AOSLO montages of lamina cribrosa. Fig. 4.3 show the output images obtained at each stage of pre-processing.

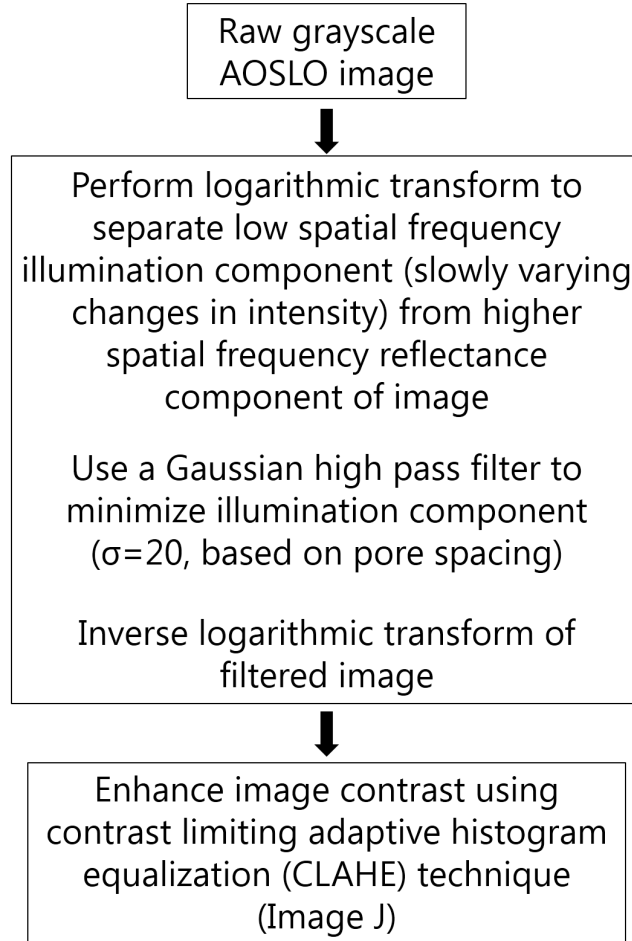


Figure 4.2: Flowchart of the steps in preprocessing the AOSLO image

4.2.5 Estimating local dominant orientation

4.2.5.1 Mean intercept length

In an earlier study [58], MIL was used to locally characterize fabric in terms of predominant orientation of the lamina cribrosa beams. In addition, MIL has been used to characterize patterns in other binary segmented biological structures [42, 10]. The basic idea of MIL is to count the number of intersections between a set of parallel probe

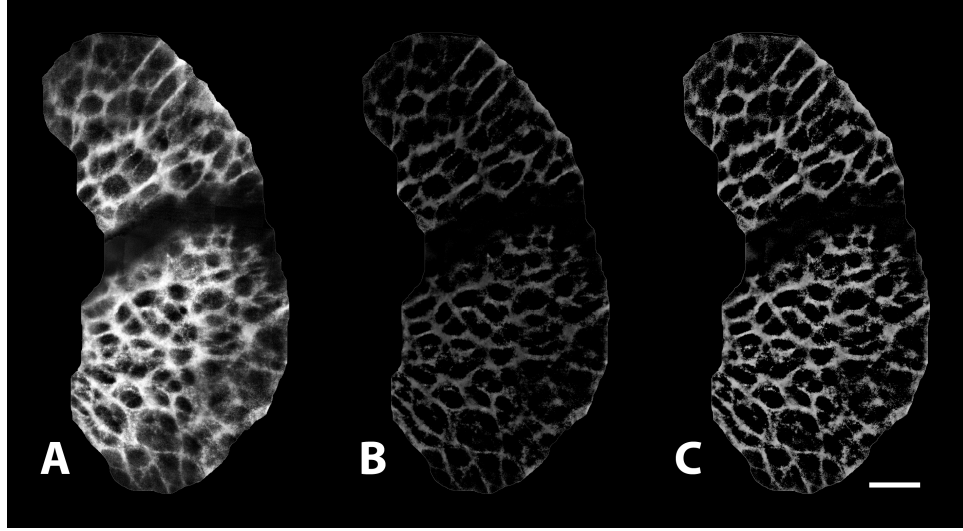


Figure 4.3: A. Original AOSLO image B. Homomorphic filtered image to remove non-uniform illumination C. Contrast improved image using CLAHE. Scale bar = 150 μm .

lines (red) and the object in the foreground (white) as a function of probe line orientation θ . Hence, the mean intercept length will be higher when the foreground pattern and the probe lines have the same orientation. The mean intercept length of a local region of a binary image (Fig. 4.4(c)) is defined as follows.

$$MIL(\theta) = \frac{\text{Total length of intersection (blue) of probe lines and white foreground objects}}{\text{Number of locations where probe beams change phase (orange)}} \quad (4.1)$$

The dominant orientation of the local region is the direction of the major axis of an ellipse fitted to the MIL measurements at all angles. For example, to estimate the dominant orientation of the synthetic image in Fig. 4.4(a) follow the steps given below.

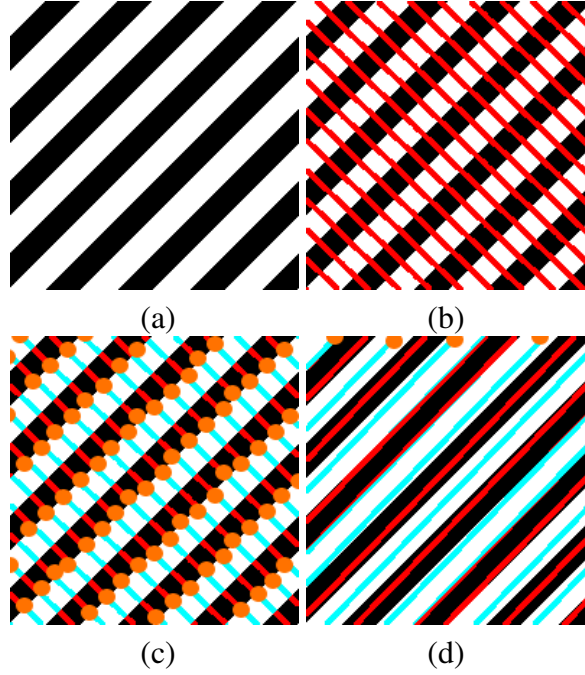


Figure 4.4: Mean intercept length. (a) Synthetic image of known orientation. (b) Probe lines (red) overlaid on the image (c) Probe lines intercepts at angle 126° (d) Probe lines intercepts at angle 53°

Algorithm *Mean Intercept Length*

1. **Step 1:** Draw the red probe lines onto the synthetic image Fig. 4.4(b) .
2. **Step 2:** Compute the number of intercepts where probe lines change phase (Fig. 4.4(c) and d) orange locations).
3. **Step 3:** Compute the number of pixels where the probe lines overlap with the foreground (cyan pixels).
4. **Step 4:** Compute the MIL for angle θ using (Eq. 4.1).
5. **Step 5:** Repeat steps 1 - 4 for different values of θ .
6. **Step 6:** Plot MIL values in polar coordinates and fit an ellipse. The angle made by the major axis with x -axis is the dominant orientation Fig. 4.5 .

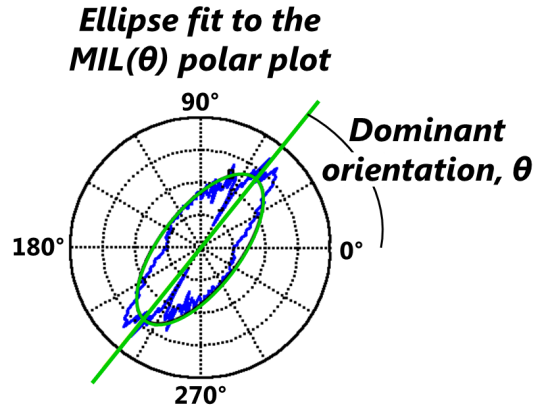


Figure 4.5: Predominant orientation is the direction of the major axis of the ellipse fitted to MIL in polar coordinates

4.2.5.2 Radon transform

The Radon transform was used to measure the trabecular bone orientation of the bird knee [12, 47]. The dominant orientation computed using Radon transform is roughly along the direction with maximum straight lines. For a set of parallel probe lines at an angle θ the sum of image intensities is calculated along each probe line (red) Fig. 4.6(b). Repeat this process for angles from 0° to 180° . Plot the Radon transform values on a 2D plane where the x -axis represents the angle of the probe lines (θ) and y -axis represents r , the perpendicular distance of the probe line from the center of the image (Fig. 4.7(a)). Compute the variance of the summed intensities for angles from 0° to 180° . The dominant orientation is the angle possessing the peak variance as shown in Fig. 4.7(b).

Radon transform of an image $I(x,y)$ is defined as:

$$R(r, \theta) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} I(x, y) \delta(x \cos \theta + y \sin \theta - r) dx dy \quad (4.2)$$

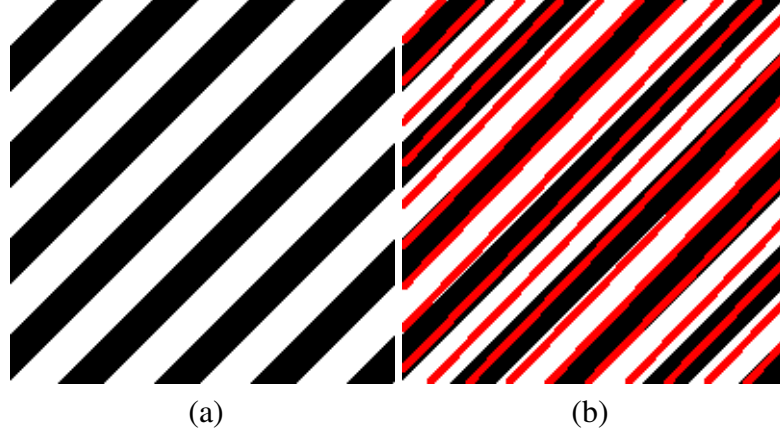


Figure 4.6: Radon transform. (a) Synthetic image of known orientation. (b) Radon transform probe lines (red) overlaid on the image

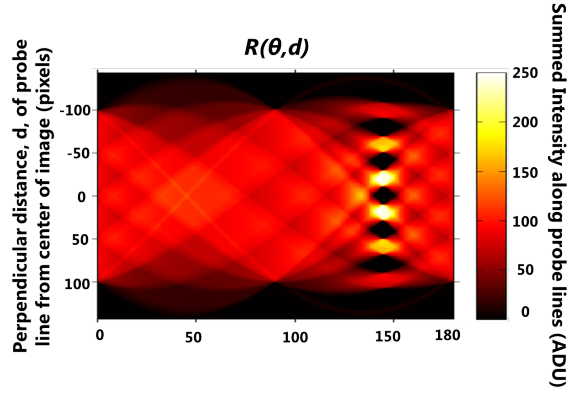
The dominant orientation is as follows:

$$\alpha = \arg \left(\min_{\theta} \frac{d^2 \sigma_{\theta}^2}{d\theta^2} \right) \quad (4.3)$$

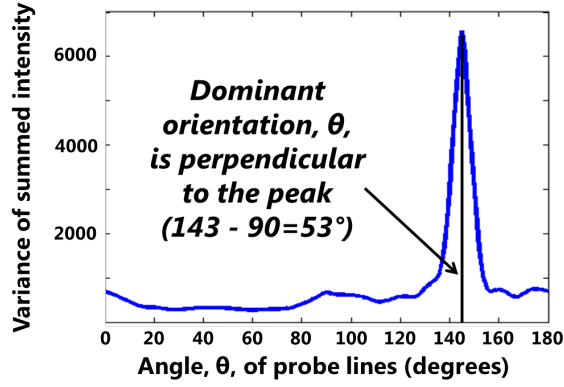
For example, the dominant orientation of the synthetic image in Fig. 4.6(a) can be estimated from its Radon transform show in Fig. 4.7

4.2.5.3 Steerable filters

Spatially oriented filters are used in the field of image processing for texture analysis, edge detection, feature detection, etc. [17, 60]. This method constructs a filter of arbitrary orientation as a linear combination of basis filters. To estimate the local orientation of an image two basis filters, one oriented horizontally and the other oriented vertically, were used. Horizontally oriented basis filter was the first derivative of a radially symmetric 2D Gaussian with respect to x and the vertically oriented basis filter was the first derivative of a radially symmetric 2D Gaussian with respect to y .



(a)



(b)

Figure 4.7: Radon transform. (a) Radon transformation of the synthetic image (Fig. 4.6(a)). (b) Predominant orientation is orthogonal to the angle of maximum summed intensity variance

Consider the following simplified 2D Gaussian.

$$G(x, y) = e^{-(x^2 + y^2)} \quad (4.4)$$

For any function f , let f_n^θ denote the n^{th} derivative of the function f rotated by an angle θ . The basis filters oriented in the horizontal direction and the vertical direction are $G_1^{0^\circ}$ (basis filter 1) [Fig. 4.8(a)] and $G_1^{90^\circ}$ (basis filter 2) [Fig. 4.8(b)] respectively. A filter of

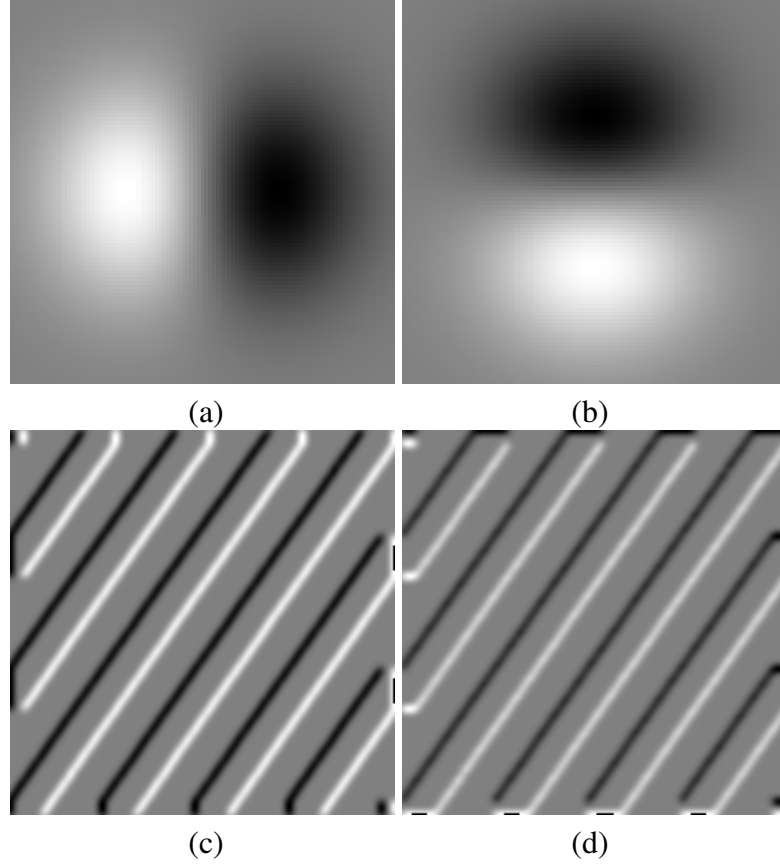


Figure 4.8: Steerable filters (a) Basis filter in horizontal direction (b) Basis filter in vertical direction (c) Response R_1^0 of horizontal basis filter to synthetic image (d) Response R_1^{90} of vertical basis filter to synthetic image Fig. 4.4(a)

arbitrary orientation can be synthesized as the linear combination of $G_1^{0^\circ}$ and $G_1^{90^\circ}$:

$$G_1^\theta = \cos(\theta) G_1^0 + \sin(\theta) G_1^{90} \quad (4.5)$$

The response R of a filter to an input image I in space is obtained by convolving the image and the filter. The response of the basis filters 1 and 2 are: $R_1^0 = G_1^0 * I$ and $R_1^{90} = G_1^{90} * I$

Since convolution is a linear operation the response of filter G_1^θ oriented at an arbitrary angle θ is the linear combination of the responses of the filters at 0° and 90° .

Hence,

$$R_1^\theta = \cos(\theta) R_1^0 + \sin(\theta) R_1^{90} \quad (4.6)$$

To estimate the dominant orientation of the image, compute the peak of the variance of the intensities of the filter response. For example, the dominant orientation of a synthetic image of is as follows.

4.2.5.4 Principal component analysis (PCA)

Principal component analysis is a mathematical procedure that is widely used in the field of image processing and image analysis. PCA also is commonly referred to as Karhunen-Love transform or Hotelling transform. This technique was first introduced by Pearson et al. to describe the best fit line or plane to a system of points in space [46]. Hotelling et al. developed PCA, where the central idea was to reduce the number of variables of a data set to an ordered uncorrelated variables while maintaining the variance of the original data [27]. This transformation gives rise to data where the first few variables preserved maximum variance. Preserving the variance of the original data is fundamental to retaining the information content. Formally, PCA is defined as a transformation of data in space to a new coordinate space wherein the new axis, also known as the principal axis, lies along the direction of maximum variance of the data. This property enables PCA to be used as a method to identify patterns in data.

We use PCA to identify the local predominant orientations of the lamina cribrosa beams acquired using an AOSLO [15, 73]. For a given image, the direction of the intensity gradient is orthogonal to the predominant orientation of the image pattern. Hence, we can

formulate the problem of finding the dominant orientation in a local region of interest or window as the task of finding a unit vector $\bar{a}$ that is orthogonal to the unit intensity gradient vectors $\bar{G}$.

$$\bar{G} = \nabla f(x_k, y_k)^T = \left[\frac{\partial f(x_k, y_k)}{\partial x}, \frac{\partial f(x_k, y_k)}{\partial y} \right]^T \quad (4.7)$$

where f is the image, k is the pixel number inside the region of interest.

When two vectors are orthogonal then the dot product is equal to zero. This corresponds to minimizing the following function.

$$\sum_{k=1}^n (\bar{a}^T \bar{G}_k)^2 \quad (4.8)$$

Since the $\bar{a}$ and $\bar{G}$ are unit vectors,

$$\bar{a}^T \bar{a} = 1 \quad (4.9)$$

$$\bar{G}_k^T \bar{G}_k = 1 \quad (4.10)$$

The function can be written as,

$$\sum_{k=1}^n (\bar{a}^T \bar{G}_k)^2 = \sum_{k=1}^n \left[(\bar{a}^T \bar{G}_k) (\bar{a}^T \bar{G}_k)^T \right] = \sum_{k=1}^n \left[\bar{a}^T (\bar{G}_k \bar{G}_k^T) \bar{a} \right] = \bar{a}^T \left(\sum_{k=1}^n \bar{G}_k \bar{G}_k^T \right) \bar{a} \quad (4.11)$$

$$\bar{a}^T \left(\sum_{k=1}^n \bar{G}_k \bar{G}_k^T \right) \bar{a} = \bar{a}^T M \bar{a} \quad (4.12)$$

where M is the covariance matrix of $\bar{G}$.

The above function can be optimized using Lagrange multiplier, subject to the condition specified in (Eq. 4.12).

$$F(\bar{a}) = \bar{a}^T M \bar{a} + \lambda (1 - \bar{a}^T \bar{a}) \quad (4.13)$$

where λ is the scalar Lagrange multiplier.

Taking the partial derivative of F with respect to $\bar{a}$,

$$2M\bar{a} - 2\lambda\bar{a} = 0 \quad (4.14)$$

$$M\bar{a} = \lambda\bar{a} \quad (4.15)$$

From Eq. 4.15 it is clear that $\bar{a}$ is the eigenvector of M corresponding to the largest eigenvalue.

The predominant beam orientation of the anterior lamina cribrosa surface montage was calculated at local regions of interest of size $150 \mu m \times 150 \mu m$. The size of the moving window ROI was empirically estimated to be three times the mean nearest neighbor distance of the lamina cribrosa pores. The step size of the moving window ROI was calculated at every $40 \mu m$.

4.2.6 Statistical analysis

The coefficients of repeatability ($1.96 \times SD \times \sqrt{2}$) were calculated for mean ALCSD, mean RoC, mean MRW, and RNFLT. The first time-point of significant change for each ONH parameter in each experimental glaucoma (EG) eye was the first time-point that fell outside the 95% confidence interval (established from the control eyes). Mean pore area,

elongation, and NND were compared globally across all time-points within each EG eye. Mann-Whitney rank sum test [35] was used to assess statistically significant differences in pore parameters with respect to baseline measurements.

The paired t-test [36] was used to assess repeatability in measuring the lamina cribrosa beam orientations across time-points within the normal eyes and to assess statistically significant difference in the lamina cribrosa beam orientations in eyes with experimental glaucoma with respect to baseline measurements. In addition, mean differences in dominant beam orientations between baseline and different time-points were computed in fellow eyes for monkeys with unilateral experimental glaucoma.

4.3 Results

4.3.1 Validation of principal component analysis

To validate the accuracy of the predominant orientation computed using principal component analysis, we estimated the orientation in images with a known orientation [Fig. 4.9(a)] and computed the error in the estimated values. Each image had a sinusoidal pattern along a single orientation. 180 images were created, each with a different orientation. Different levels of noise and blur were added to test the robustness of the method [Fig. 4.9(b)(c)].

Additionally, we compared the orientation estimated using PCA with the orientation estimated using other methods like mean intercept length (MIL), Radon transform and steerable filters. The mean error in the dominant orientation with respect to the true orientation estimated using MIL, Radon transform, steerable filter, and PCA were 1.81 ± 1.52 ,

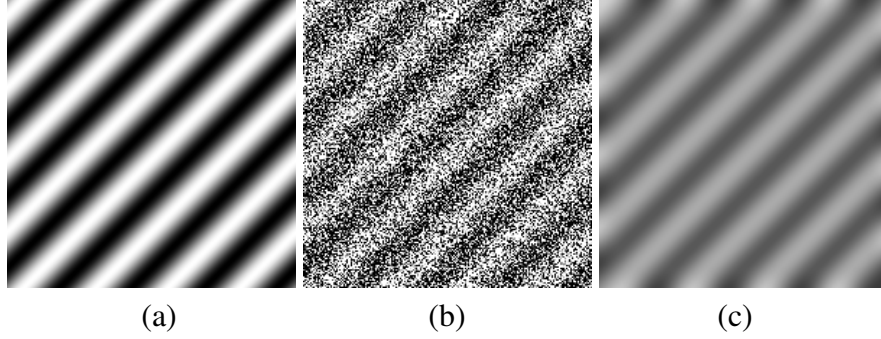


Figure 4.9: Synthetic images of known orientation (a) Original clean synthetic image (b) Synthetic image with noise ($\sigma^2 = 0.6$) (c) Synthetic image with blur ($\sigma^2 = 10$)

0.01 ± 0.10 , 1.11 ± 0.72 , and 0.2 ± 1.52 respectively (Table 4.1). To test PCA-based methods robustness to noise and blur we employed an image with concentric ellipses (Fig. 4.10) and added different degrees of Gaussian noise ($\sigma^2 = 0 - 0.6$) and Gaussian blur ($\sigma^2 = 1 - 10$).

The local orientation estimated at every location in the image for different degrees of

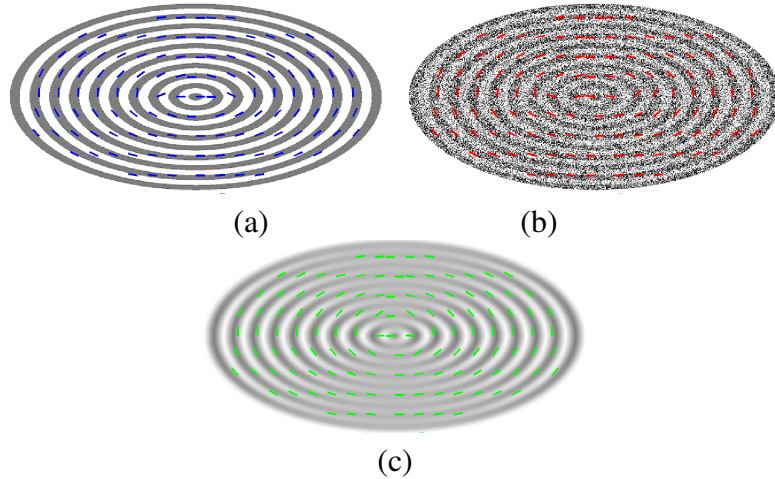
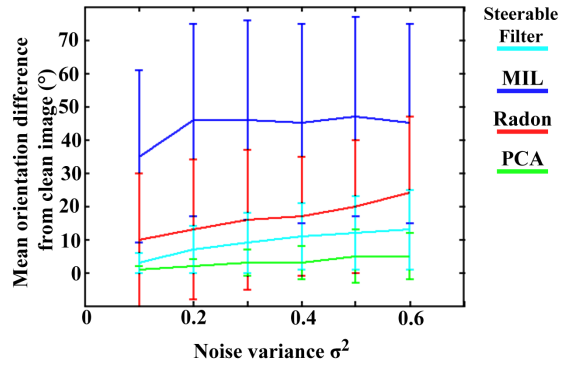


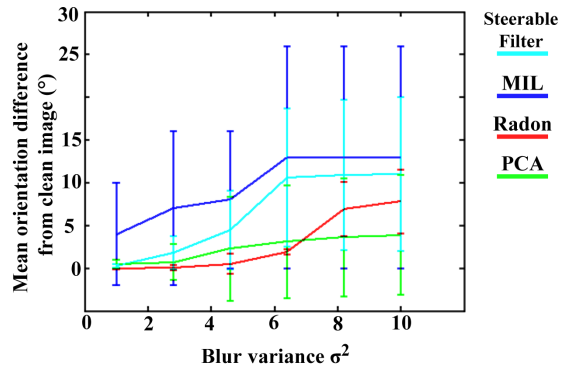
Figure 4.10: Synthetic concentric ellipses (a) Original clean synthetic concentric ellipses (b) Synthetic concentric ellipses with noise ($\sigma^2 = 0.6$) (c) Synthetic concentric ellipses with blur ($\sigma^2 = 10$)

noise and blur were compared with the orientation estimated on the original noise free and blur free image. We observed that the mean error in dominant orientation with respect to

the original image increased with rise in noise and blur. However, the PCA-based method was the most robust of the four methods (Fig. 4.11).



(a)



(b)

Figure 4.11: (a) Change in orientation of concentric ellipses with noise (b) Change in orientation of concentric ellipses with blur

Table 4.1: Mean error in dominant orientation of 180 synthetic test images for MIL, Radon transform, Steerable filter, and PCA

	Error in dominant orientation (<i>mean</i> $\pm$ <i>std</i>)
MIL	$1.81^\circ \pm 1.52^\circ$
Radon transform	$0.01^\circ \pm 0.10^\circ$
Steerable filter	$1.11^\circ \pm 0.72^\circ$
PCA	$0.2^\circ \pm 1.52^\circ$

4.3.2 Assessment of repeatability in measuring lamina cribrosa beam orientation in normal monkeys

The raw AOSLO montages of the anterior lamina cribrosa surface acquired in 4 normal monkey eyes are shown in Fig. 4.12 (a,c,e,g), along with the corresponding homomorphic filtered images (Fig. 4.12 (b,d,f,h)). Fig. 4.14 presents the montages of the ALCS of monkey OHT-69 from 3 separate imaging sessions that spanned a course of 150 days. The anterior lamina cribrosa beam orientations were computed inside a moving square region of interest of size $150\mu m \times 150\mu m$ with a step size of $40\mu m$ throughout the montage acquired at different time-points. The predominant beam orientation was compared across imaging sessions to assess the repeatability in quantifying the lamina cribrosa beam orientation of the monkey eyes imaged at different time points [62].

Fig. 4.13(a) shows the baseline image of animal OHT-63 superimposed with its dominant orientation vectors (red). Fig. 4.13(b) shows the AOSLO images of animal OHT-63 acquired on Day 13 superimposed with dominant orientation vectors (green) and the baseline orientation vectors (red) for visual comparison. Fig. 4.13(c) shows the AOSLO images of animal OHT-63 acquired on Day 35 superimposed with dominant orientation vectors (green) and the baseline orientation vectors (red) for visual comparison. Fig. 4.14(a) shows

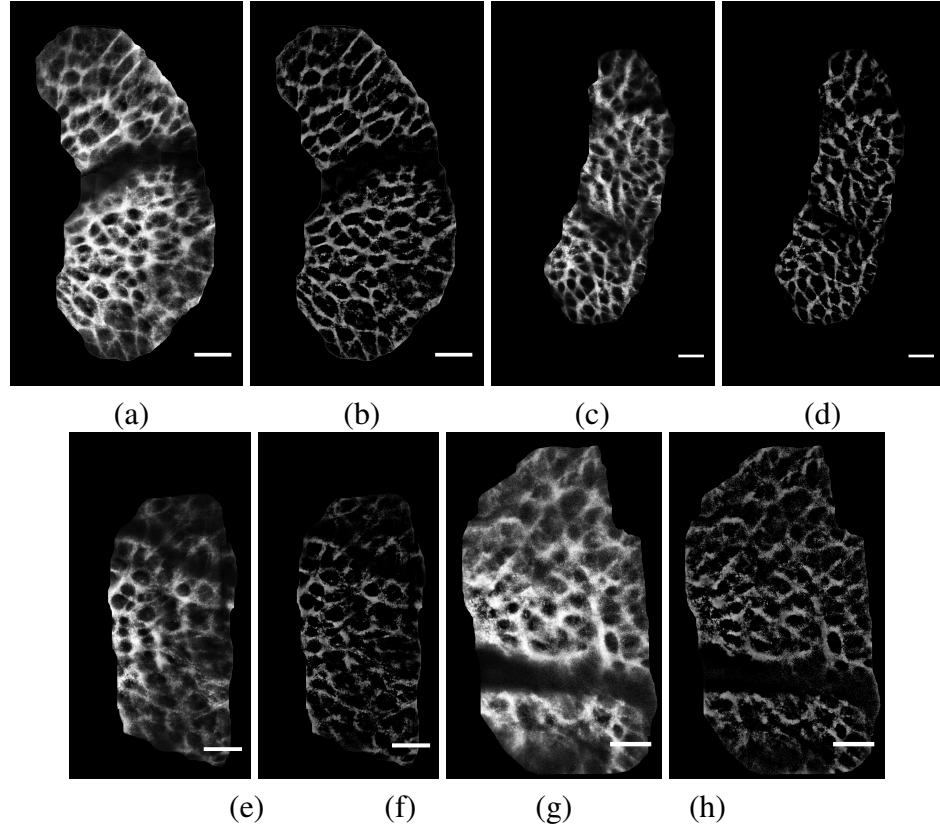


Figure 4.12: AOSLO images of the ALCS and the corresponding Homomorphic filtered images from 4 normal monkey eyes (a-b) OHT-63 (c-d) OHT-64 (e-f) OHT-66 (g-h) OHT-69. Scale bar = 150 μm .

the baseline image of animal OHT-69 superimposed with its dominant orientation vectors (red). Fig. 4.14(a-c) shows the AOSLO images of animal OHT-69 acquired at different time-points superimposed with the baseline orientation vectors (red) and their respective dominant orientation vectors (green).

The differences in dominant orientations of each time-point with respect to the baseline image were computed only in overlapping regions. The mean difference of predominant lamina orientations of 6 normal monkey eyes with respect to its baseline was $7 \pm 5^\circ$. There were no statistically significant differences in lamina cribrosa beam orientation across

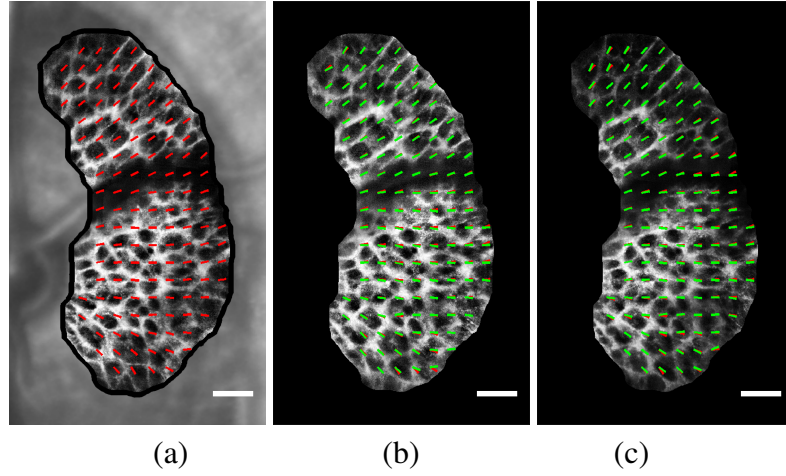


Figure 4.13: Local predominant orientation vectors of the ALCS of monkey OHT-63 acquired at three different time points. The vectors colored red correspond to baseline measurements (a) Baseline (b) Day 13 (c) Day 35. Scale bar = 150 μm .

all imaging sessions for normal macaques demonstrating good measurement repeatability ($P > 0.05$).

4.3.3 Longitudinal assessment of changes in beam orientation in monkeys with unilateral experimental glaucoma

Longitudinal changes were measured in predominant beam orientation in 6 monkeys with EG. The first time-point with statistically significant differences in beam orientation was compared with first time-points of significant changes in manually measured pore parameters, RNFLT and ONH parameters like mean ALCSD, mean MRW, and mean RoC in 6 EG eyes. The first significant change in beam orientation coincided with manually measured lamina pore changes in 5/6 EG eyes and preceded lamina pore changes in 1/6 eyes. Fig. 4.15 summarizes the lamina cribrosa microarchitecture changes for OHT 64 where

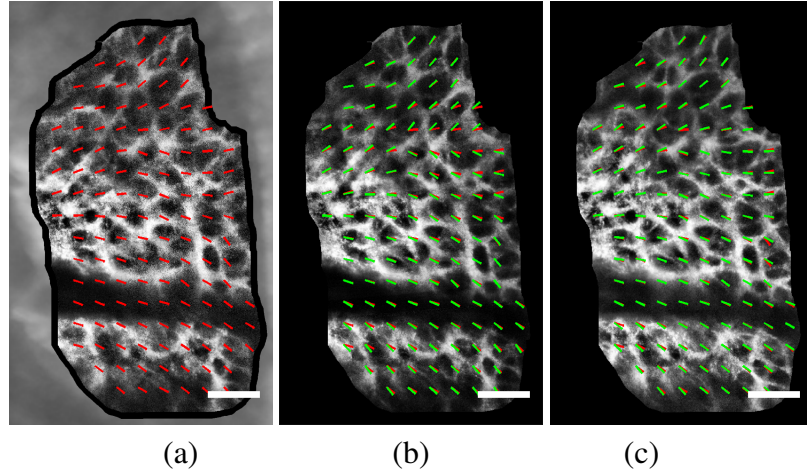


Figure 4.14: Local predominant orientation vectors of the ALCS of monkey OHT-69 acquired at three different time points. The vectors colored red correspond to baseline measurements (a) Baseline (b) Day 84 (c) Day 182. Scale bar = $150\ \mu\text{m}$.

first significant change in beam orientation coincided with first significant change in manual measures of ALCS pore geometry. Fig. 4.16 shows early changes in ONH parameters (yellow point) and the red line represents the time point where first significant change in beam orientation occurred for OHT 64. Fig. 4.17 summarizes the lamina cribrosa microarchitecture changes for OHT-68 where first significant change in beam orientation preceded first significant change in manual measures of ALCS pore geometry. Fig. 4.18 shows early changes in ONH parameters (yellow point) and the red line represents the time point where first significant change in beam orientation occurred for OHT 68. Mean ALCSD changed first in 5/6 eyes. Simultaneous change in mean MRW occurred in 4 of 6 eyes and mean ALCS beam orientation in 3 of 6 eyes. The summary of first change can be seen in Table 4.2.

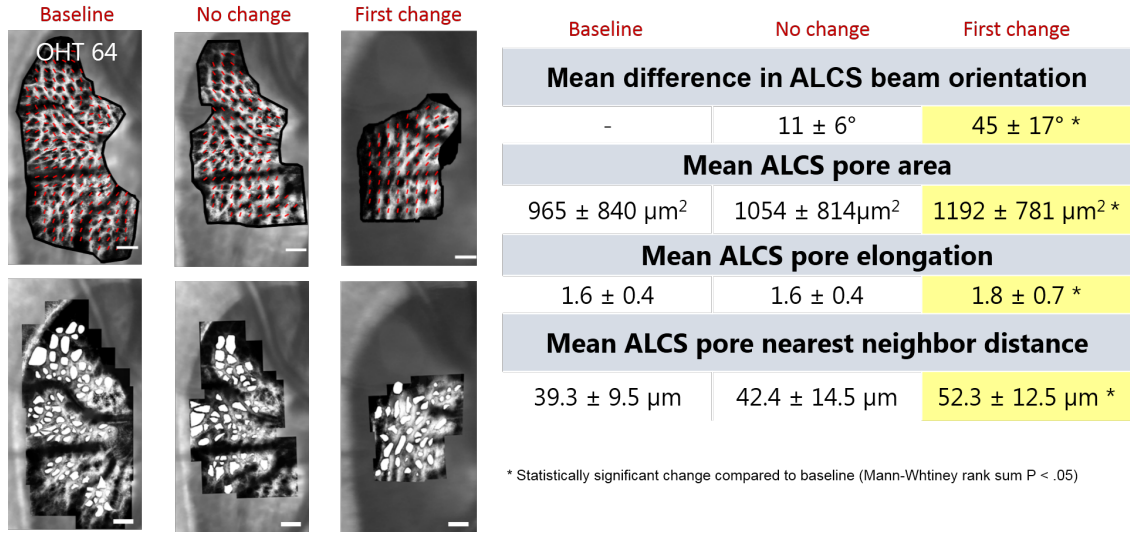


Figure 4.15: First significant change in beam orientation coincided with the first significant change in manual measure of ALCS pore geometry for OHT-64. Scale bar = $150 \mu\text{m}$.

4.4 Discussion

We investigated four methods to estimate the predominant orientation pattern present in an image. While mean intercept length works on binary images, Radon transform, steerable filters, and PCA works directly on gray scale images. All four methods yielded low errors in estimating the dominant orientation in synthetic images with a single dominant orientation. Radon transform yielded lower errors compared to other methods owing to its inherent effectiveness in identifying straight lines with a single orientation in binary images. However, in an image with noise, blur and diverse orientations the PCA yielded lower errors. Steerable filters yielded errors that are comparable to PCA-based approach. However, the convolution operation in the steerable filter method is computationally expensive than PCA-based method. Additionally, designing an appropriate filter is a complex task as it involves fine tuning several filter design parameters. We chose the PCA-based method to

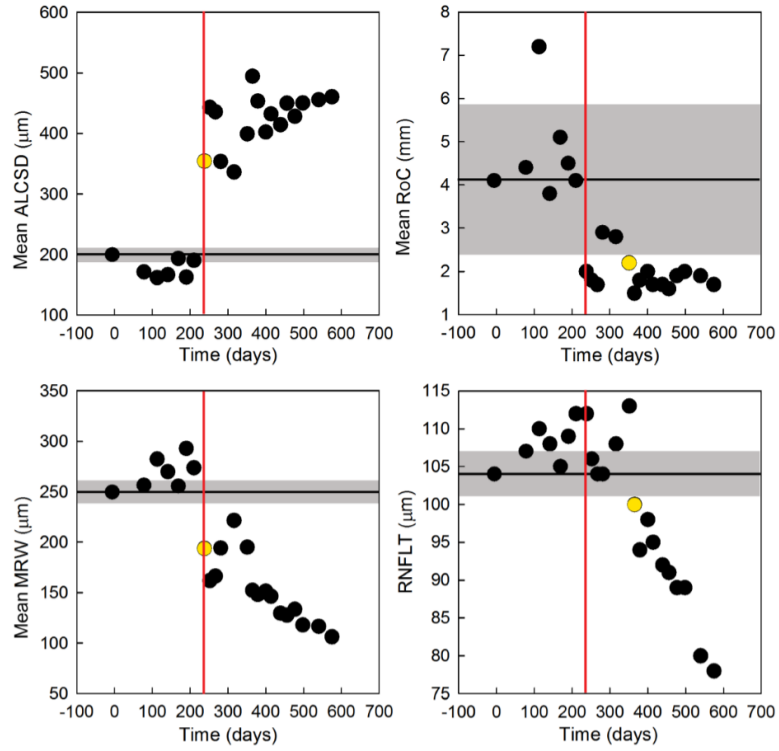


Figure 4.16: First significant change in beam orientation (red line) preceded or coincided with the first significant change in ONH parameters (yellow dot) for OHT-64

compute the dominant orientation as it is fast, works directly on AOSLO grayscale images of the monkey eye which consists of beams in diverse orientations, and is robust to noise and blur in the image.

To compute the dominant orientation in a local region we defined an empirically chosen window of size $150\mu m \times 150\mu m$ based on the nearest neighbor distance of the lamina pores. A more objective method to obtain the window size is desirable. Ivers et al. assessed the repeatability of AOSLO imaging and measurement of lamina cribrosa pore geometry using manual segmentation of the pores [28]. However, it is important to have an objective and accurate representation of the anterior lamina cribrosa microarchitecture to

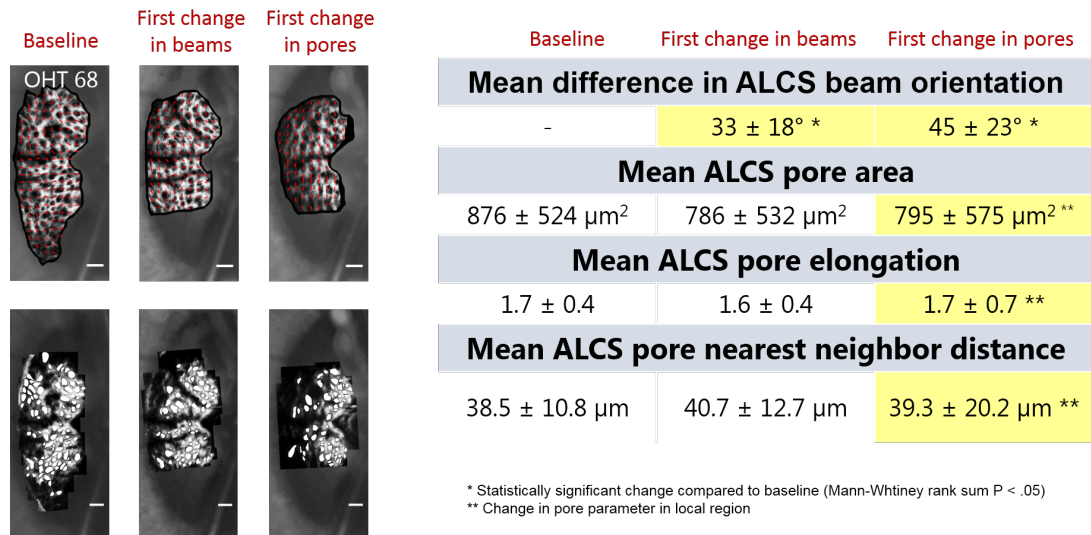


Figure 4.17: First significant change in beam orientation coincided with the first significant change in manual measure of ALCS pore geometry for OHT-68. Scale bar = $150 \mu\text{m}$.

better understand and track structural changes in the ALCS during glaucoma progression. Roberts et al. used mean intercept length to characterize the lamina cribrosa beam orientation, which works well in binary segmented histological monkey eyes [58]. We used principal component analysis to quantify the lamina cribrosa microstructure as manifest in predominant lamina beam orientation and assess the repeatability of lamina cribrosa structure directly from gray scale *in vivo* normal monkey eyes without the need for binary segmentation. This automated method is more objective and will help us analyze lamina cribrosa microarchitecture on a more rapid time-scale.

This study also characterized the longitudinal changes in anterior lamina cribrosa surface microarchitecture concurrent with structural changes in the ONH and RNFL during early experimental glaucoma. In the experimental model, IOP was elevated unilaterally via a

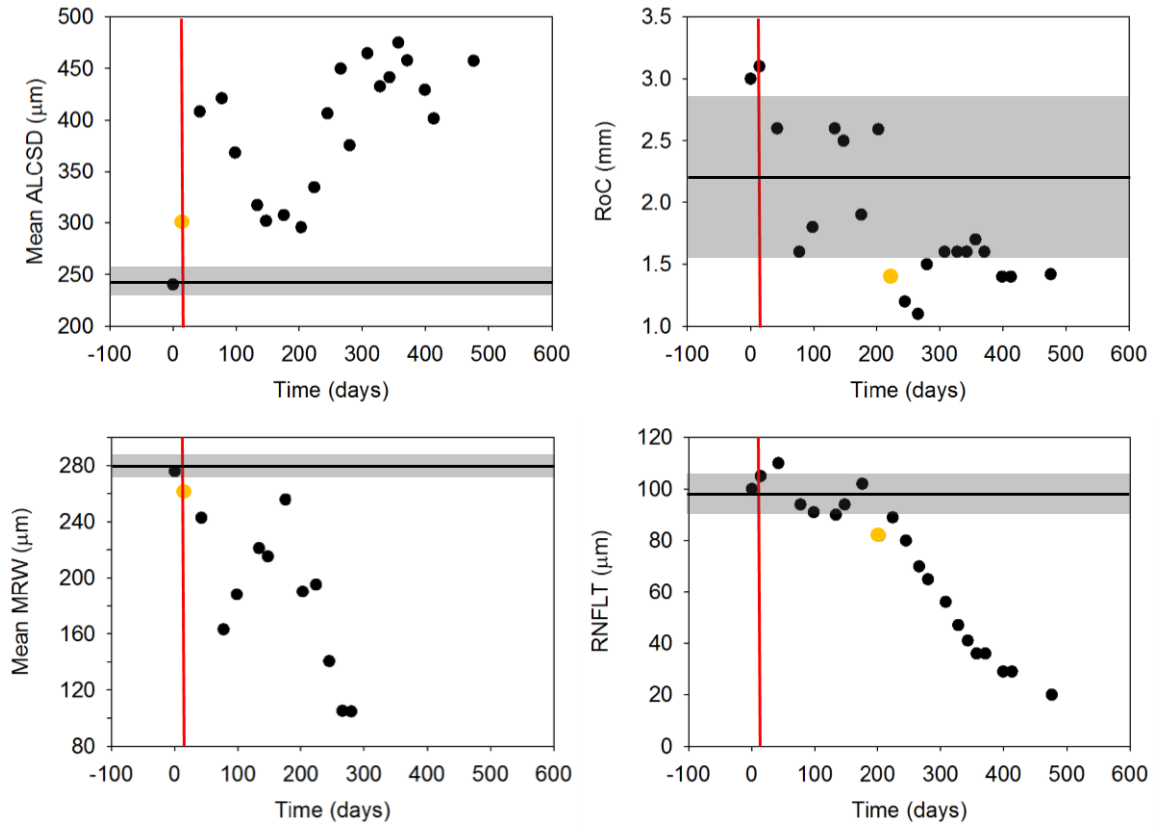


Figure 4.18: First significant change in beam orientation (red line) coincided with the first significant change in ONH parameters (yellow dot) for OHT-68. Scale bar = $150 \mu\text{m}$.

laser treatment of the trabecular meshwork. The first significant changes in beam orientation and ONH structural changes occurred prior to change in RNFL thickness. The first significant changes in beam orientation estimated using our method coincided or preceded manually measured lamina pore parameters.

Table 4.2: Summary of first structural change(s) to occur in each EG eye, where (X) indicates the first ONH parameter(s) to change

Subject ID	Mean ALCSD	Mean MRW	ALCS Beam Orientation	ALCS Pore Parameters	Mean RoC	RNFLT
OHT-63		(X)				
OHT-64	(X)	(X)	(X)	(X)		
OHT-65	(X)	(X)	(X)	(X)		
OHT-66	(X)					
OHT-68	(X)	(X)	(X)			
OHT-69	(X)	(X)				

4.5 Conclusions

As evidenced by low errors in estimating the dominant orientations of synthetic images of a single known orientation, robustness to noise and blur, and presence of diverse orientations, PCA can accurately compute the dominant orientation of the lamina cribrosa beams in monkey eyes. This method can operate directly on grayscale AOSLO images thereby eliminating the need to binary segment the lamina cribrosa. This method is simple and computationally less intense. Quantification of laminar beam orientation *in vivo* using PCA will provide the opportunity to track longitudinal changes in beam orientation in glaucoma and better understand laminar remodeling due to biomechanical changes during disease progression.

Chapter 5

Automated segmentation of lamina cribrosa pores in non-human primates using AOSLO

5.1 Introduction

Glaucoma is a complex group of eye diseases that results in irreversible death of retinal ganglion cells (RGC) leading to permanent blindness. However, the mechanisms responsible for development and progression of glaucoma is not fully understood. It is now widely accepted that glaucoma is said to be present when there is both structural and functional defects present in the eye. Structural defects like loss of axons are traditionally assessed by examining the cup-to-disc ratio. Typically, the cup-to-disc ratio progressively increases in eyes affected by glaucoma. Hence, it is essential to detect glaucoma at an early stage prior to loss of ganglion cell axons. Substantial evidence suggests that axonal damage initially occurs in the optic nerve head (ONH) at the level of lamina cribrosa [3, 53, 55, 51].

The lamina cribrosa is a three-dimensional porous meshwork in the optic nerve head, consisting primarily of collagenous beams that provide functional and structural support to the retinal ganglion cell axons passing through the ONH from the retina to the brain. Several *ex vivo* studies reported permanent posterior bowing and stretching of lamina cribrosa tissue due to chronic increase in intraocular pressure to different levels in an experimental model of glaucoma [5, 6, 74]. Structural changes of the lamina cribrosa due to increase in intraocular pressure (IOP) may potentially damage the laminar capillaries and astrocytes that may compromise axonal structure and function. These histological observations were confirmed *in vivo* in human glaucoma patients who have shown significantly larger mean anterior lamina cribrosa surface depths (ALCSDs) compared to normal eyes. *In vivo* images of the anterior lamina cribrosa in human glaucomatous eyes using confocal scanning laser ophthalmoscope (SLO) showed that pore area and elongation were larger

in eyes with sever glaucoma [16]. With the advent of adaptive optics technology, the resolution and contrast of the lamina cribrosa pores were improved [70, 28, 68, 76]. These studies support the need for objective and accurate *in vivo* analysis and examination of microstructural characteristics of lamina cribrosa.

Lamina cribrosa pore microarchitecture was quantified *in vivo* by using a pixel-pixel classification Niblack's adaptive thresholding method [40, 41]. The purpose of this study is to develop an automated method to segment lamina cribrosa pores using a boundary evolution technique to identify the closed boundary of the lamina cribrosa pores [45]. To validate the method, we established a reference standard by taking a combination of segmentation by three human raters. A voting rule was employed which selects pixels where a majority of raters agreed on the presence of the structure. To compare the accuracy of the automatic segmentation, spatial overlap metrics such as Dice-Jaccard similarity coefficient, sensitivity and specificity values were measured. Repeatability of the automatic segmentation method was assessed by performing statistical tests on the segmentation results of the same eyes imaged at different time points. This work provides an automated and repeatable method for determining lamina cribrosa pore geometry and can be extended to track longitudinal changes in pore geometry in experimental glaucoma.

5.2 Methods

All animal care experimental procedures were approved by the University of Houston's Institutional Animal Care and Use Committee, and adhered to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

The adaptive optics scanning laser ophthalmoscope (AOSLO) montages of anterior lamina cribrosa were examined *in vivo* in eight normal rhesus monkey eyes, two normal human eyes, and four monkey eyes with experimental glaucoma. Argon laser treatment was performed in stages around the trabecular meshwork to elevate the intraocular pressure of each monkey's right eye, while the left eye served as controls. Monkeys were anesthetized with 20-25 mg/kg ketamine, 0.8-0.9 mg/kg xylazine, and 0.04 mg/kg atropine sulfate to minimize eye movements during *in vivo* imaging. Monkey pupils were dilated with 2.5% phenylephrine and 1% tropicamide [18]. A lid speculum was used to hold the monkey eyelid open and a contact lens was placed on the monkey eye to prevent corneal dehydration during imaging. A head mount with tip, tilt, and rotation capabilities was attached to an XYZ translation stage used to stabilize the monkey's head and align the eye's pupil with the AOSLO system for lamina imaging.

5.2.1 Biometric measurements and image scaling

We measured axial length, anterior chamber length, and anterior corneal curvature in all eyes at every time-point using an ocular biometer (IOLMaster; Carl Zeiss Meditec, AG, Jena, Germany). These biometric measurements were incorporated into a model eye to laterally scale AOSLO images using methods described by [28].

5.2.2 *In vivo* imaging using spectral domain optical coherence tomography (SD-OCT)

Wide field (20°) scanning laser ophthalmoscope (SLO) images of the ONH were acquired in fellow eyes of 3 monkeys with unilateral experimental glaucoma using a Spectralis HRA+OCT (Heidelberg Engineering, Germany). *En face* SLO images were taken such that the anterior lamina cribrosa was at best focus. The same instrument was used to acquire 24-48 SDOCT cross sectional radial B-scans (20° in extent) centered on the ONH [Fig. 4.1]. The termination of the retinal pigment epithelium (RPE) / Bruchs membrane (BM) interface (defining Bruchs Membrane Opening [BMO]) and ALCS were manually marked on SDOCT B-scans [Fig. 4.1] using custom MATLAB code (The MathWorks, Inc., Natick, MA) to generate a point cloud in 3-dimensional space. The radius of curvature of ALCS, BMO area and the mean anterior lamina cribrosa surface depths were computed using thin-plate spline [61]. The internal limiting membrane (ILM) was automatically segmented in each B-scan using the SD-OCT software and manually corrected wherever necessary. The mean minimum rim width (MRW), which is the average of the minimum distances between each RPE/BM termination point to the ILM, were calculated for all marked B-scans. The peripapillary retinal nerve fiber layer (RNFL) was automatically segmented from the 12° circular scans centered on the ONH at every time-point and manually corrected wherever necessary. Subsequently, the mean RNFL thickness (RNFLT) was measured.

5.2.3 *In vivo* imaging using adaptive optics scanning laser ophthalmoscope

En face images of the anterior lamina cribrosa at best-focus was acquired using an AOSLO over a field of (1.5°) at a rate of 25Hz using an 840 nm superluminescent diode as described previously. The power of the 840 nm SLD at the corneal plane conformed to the ANSI standards and was set to $300 \mu W$ [33]. Individual frames from the AOSLO videos were registered offline using a custom cross-correlation technique and averaged to reduce the noise in the image [28]. These registered frames were then stitched manually to form a montage. The contrast of the lamina cribrosa montage was improved using contrast limiting adaptive histogram equalization (CLAHE) technique, and a Gaussian filter ($\sigma = 15$) was applied to filter noise in the image. A mask was applied to regions where lamina cribrosa signal strength was weak due to occlusion caused by blood vessels.

5.2.4 Estimating lamina cribrosa pore boundary

We developed a hierarchical method to estimate lamina pore boundary. The first step was to identify the location of the lamina cribrosa pores. We used Wellner's adaptive thresholding method to identify anterior lamina cribrosa pore locations in the montage. The contour of the pores identified using this method serves as the initial contour for identifying the pore boundaries using level sets method.

5.2.5 Wellner's method to identify lamina cribrosa pores

Wellner's method is an adaptive thresholding algorithm [72]. Conventional thresholding algorithms use a global threshold to binarize a gray image. Whereas, adaptive thresholding algorithm computes the intensity threshold dynamically at each pixel [44, 31, 29, 41]. The dynamic threshold value is determined based on the intensities in a local region around the point of interest. Wellner's method computes the threshold value based on the local mean and standard deviation of pixel intensities in a local region given by the following equation.

$$T = m + k * B \quad (5.1)$$

where,

$$B = \frac{(\sum p_i^2 - m^2)}{N}$$

k is the Wellner factor

m = mean intensity

p_i = pixel value of grayscale image

N = number of pixels

Fig. 5.1 shows the output of pore localization algorithm.

5.2.6 Distance regularized level sets for identifying pore boundaries

For computing the lamina cribrosa pore boundaries we used the level sets function (LSF) method [34, 43, 9, 78]. The advantage of level set method is that it can identify complex

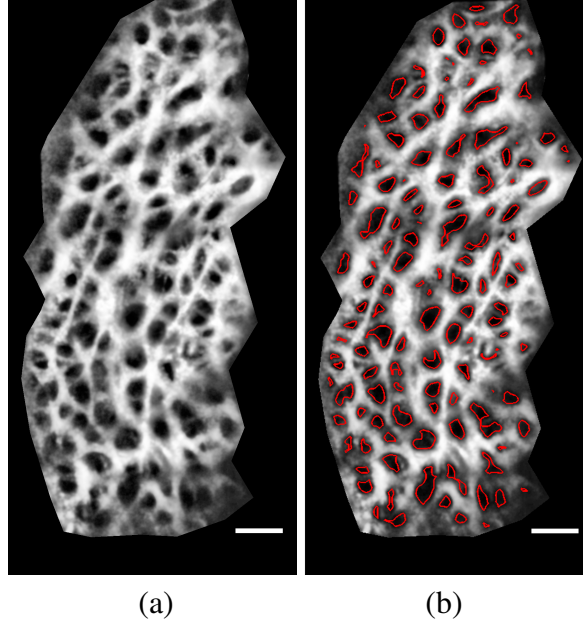


Figure 5.1: Pore localization. (a) Original gray scale lamina cribrosa montage. (b) The red contours represent the pores identified using Wellner's method. Scale bar = 150 μm .

contours and also segment regions that split and merge more efficiently than pixel-by-pixel classifiers. A level set is a function $\phi(x, y) : \Omega \rightarrow \mathbb{R}$, where Ω is the domain of image $I(x, y)$, where the function represents a contour C on the image plane when the function is zero.

$$C \equiv \{(x, y) : \phi(x, y) = 0\}, \forall (x, y) \in \Omega$$

The binary image obtained after adaptive thresholding method serves as the initial level set function $\phi_0(x, y) = 0$ at the boundaries of the initial pore locations. The next step is to define an edge indicator function g whose values are smaller at boundaries.

$$g = \frac{1}{1 + |\nabla G_\sigma * I|^2} \quad (5.2)$$

where G_σ is a Gaussian with standard deviation σ . The image is convolved with a Gaussian kernel to reduce noise in the edge indicator. The energy function of the LSF is defined as

$$E(\phi) = \mu R_p(\phi) + \lambda L_g(\phi) + \alpha A_g(\phi) \quad (5.3)$$

The first term is the level set regularization term. This term ensures the smoothness of the contour, which is necessary for continuous curve evolution.

$$R_p(\phi) = \int_{\Omega} p(|\nabla \phi|) dx \quad (5.4)$$

where p is potential function $p(s) = s^2$.

The second term ensures the energy is minimum at the object boundary and the third term controls the area of the objects. The constants $\mu > 0$, $\lambda > 0$ and $\alpha \in \mathbb{R}$ are coefficients of the energy functionals R_p , L_g and A_g . The energy terms $L_g(\phi)$ and $A_g(\phi)$ are defined as,

$$L_g(\phi) = \int_{\Omega} g\delta(\phi) |\nabla \phi| dx \quad (5.5)$$

and

$$A_g(\phi) = \int_{\Omega} gH(-\phi) dx \quad (5.6)$$

where δ and H are the Dirac delta function and the Heaviside function, respectively.

The energy term L_g is minimized if the level set function is located at the object boundaries and the term A_g which computes the area of the region speeds up the motion of the level set evolution process, especially when the initial level set contour is placed far from the desired object boundary. Eq. 5.3 can be minimized by solving the following gradient flow:

$$\frac{\partial \phi}{\partial t} = \mu \operatorname{div} (d_p(|\nabla \phi|) \nabla \phi) + \lambda \delta(\phi) \operatorname{div} \left(g \frac{\nabla \phi}{|\nabla \phi|} \right) + \alpha g \delta(\phi) \quad (5.7)$$

where $\text{div}(\cdot)$ is the divergence operator and d_p is a function defined by

$$d_p(s) = \frac{p'(s)}{s}$$

5.2.7 Statistical analysis

The coefficients of repeatability ($1.96 \times SD \times \sqrt{2}$) were calculated for mean ALCSD, mean RoC, mean MRW, and RNFLT. The first time-point of significant change for each ONH parameter in each experimental glaucoma (EG) eye was the first time-point that fell outside the 95% confidence interval (established from the control eyes).

Spatial overlap metrics like sensitivity, specificity, and Dice and Jaccard coefficients were computed to validate the accuracy of the pore segmentation method. In addition, Mann-Whitney rank sum test [35] was used to assess statistically significant differences in pore parameters with respect to baseline measurements.

5.3 Results

5.3.1 Validation

We validated the performance of the method using adaptive optics scanning laser ophthalmoscope (AOSLO) montages of anterior lamina cribrosa *in vivo* in 2 normal human eyes, 8 normal rhesus monkey eyes, and 4 rhesus monkey eyes with experimental glaucoma. The reference standard was established by taking a combination of segmentation

by three human raters. A voting rule was employed which selects pixels where a majority of raters agreed on the presence of pores as shown in [Fig. 5.2]. The color codes

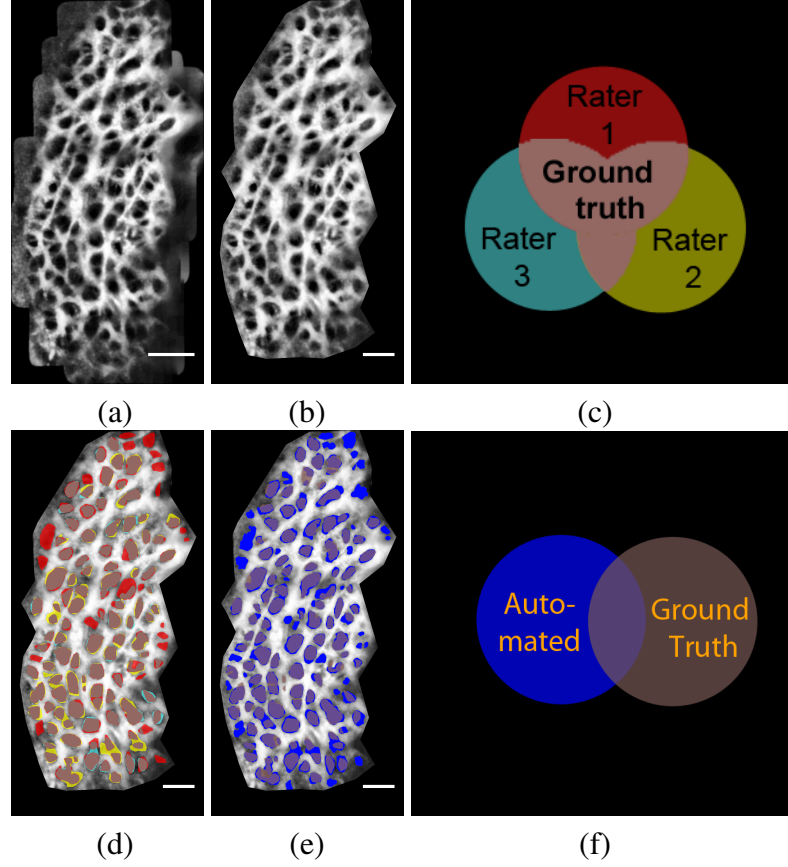


Figure 5.2: Validation of automatic segmentation (a) Original AOSLO montage (b) Masked montage (c) Color codes of raters (d) Pores marked by 3 raters, where the overlap of two or more raters in pink is the ground truth (e) Overlap of ground truth and automatic segmentation (f) Overlap regions of automated and ground truth. Scale bar = $150 \mu\text{m}$.

are shown in [Fig. 5.2(c) and (e)]. The results of automatic segmentation were compared with the ground truth using a spatial overlap-based method. For an image with N pores a confusion matrix C of size $N \times N$ is defined where C_{ij} is pixel automatically segmented as pore number/label i while the true label is j . Spatial overlap measures like sensitivity

(SE), specificity (SP), Dice coefficient and Jaccard coefficient were computed as follows.

$$SE = TN / (TN + FP)$$

$$SP = TP / (TP + FN)$$

where

$$TP = C_{kk} \quad TN = \sum_{i=1}^N \sum_{j=1}^N C_{ij}$$

$$FN = \sum_{j=1}^N C_{ik} \quad FP = \sum_{j=1}^N C_{kj}$$

where $i, j \neq k$

$$Dice = 2TP / (2TP + FP + FN)$$

$$Jaccard = TP / (TP + FP + FN)$$

The mean sensitivity and mean specificity of 14 eyes are 83 ± 4.9 and 96.8 ± 1.6 respectively.

5.3.2 Assessing repeatability of automatic pore segmentation

The repeatability of calculating anterior lamina cribrosa pore area using automated segmentation method was assessed following registration of AOSLO images taken at 2 different time-points in 6 normal rhesus monkey eyes. Only the pores that were segmented in both sessions were analyzed. Mann-Whitney rank sum test [35] produced no significant

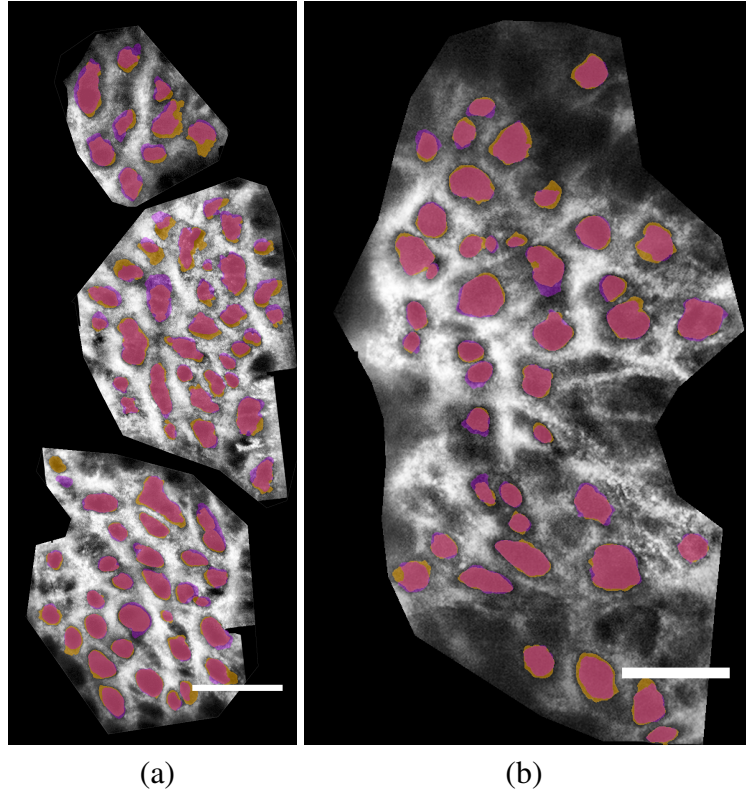


Figure 5.3: Repeatability of automatic segmentation assessed by examining the differences in area at 2 different time-points (a) Overlap of automatic segmentation of normal monkey eye OHT64 (b)Overlap of automatic segmentation of normal monkey eye OHT-66. Scale bar = 150 μm .

differences ($P > .05$) in the lamina cribrosa pore areas of 6 normal monkey eyes at two different time-points. More than 90% of the pores were analyzed at both times points for all monkey eyes. [Fig. 5.3] shows the results of repeatability test in the registered images of a normal eye acquired at two different time-points.

5.3.3 Longitudinal assessment of structural changes in monkeys with unilateral experimental glaucoma

Longitudinal changes were measured in predominant beam orientation in 3 monkeys with EG. The first time-point with statistically significant differences in beam orientation was compared with first time-points of significant changes in RNFLT and ONH parameters like mean ALCSD, mean MRW, and mean RoC in 3 EG eyes. The first significant change in global lamina pore area coincided with changes in beam orientation and other ONH parameter changes in 3/3 EG eyes. The summary of first change can be seen in Table 5.1

Table 5.1: Summary of first structural change(s) to occur in each EG eye, where (X) indicates the first ONH parameter(s) to change

Subject ID	Mean ALCSD	Mean MRW	ALCS Beam Orientation	ALCS Pore Area	Mean RoC	RNFLT
OHT-70	(X)	(X)		(X)		
OHT-71	(X)	(X)	(X)			
OHT-72	(X)	(X)				

5.4 Discussion and Conclusion

In vivo images of the anterior lamina cribrosa were acquired using AOSLO and subsequently segmented using a hierarchical method of pore localization using Wellner’s adaptive thresholding followed by distance regularized level sets for pore boundary identification. Lamina cribrosa pores in ALCS montage of 3 normal human eyes, 6 normal monkey eyes, and 5 monkey eyes with experimental glaucoma were manually marked by

3 raters. The ground truth was established by employing a voting rule which selects pixels where a majority of raters agreed on the presence of pores. The main advantage of a level sets based method is its ability to identify closed contour boundaries more efficiently than pixel-by-pixel classifiers. The sensitivity and specificity of the automated method were 83% and 95%, which is comparable to previous studies [40]. The automated method tended to identify fewer pores than manual raters. The difference maybe due to the automated method merging pores with poor contrast as well as discrepancies with manual raters in regions with blood vessels. Repeatability of calculating ALCS pore area using automated segmentation was assessed following registration of images taken at 2 different time-points in 6 normal eyes. There was no significant differences in the ALCS pore area (Mann-Whitney rank sum test, $P > .05$). As evidenced by spatial overlap metric values, the quantification of ALCS pores via automated segmentation was comparable to manual segmentation results and repeatable over time. First time-point of change in pore area and beam orientation did not coincide for 2/3 EG eyes. This is likely due to estimating beam orientation in 2 dimensions, whereas pore area is computed in 3 dimensions. Our automated segmentation method can be used to characterize lamina cribrosa microarchitecture in normal and EG eyes.

Chapter 6

General Conclusions

6.1 General Conclusions

Lamina cribrosa is thought to play an important role in the onset and progression of glaucoma. This work contributes to developing methods for a more accurate and objective representation of the structural characteristics of lamina cribrosa.

6.1.1 Chapter 3: 3D modeling to characterize lamina cribrosa surface and pore geometries using *in vivo* images from normal and glaucomatous eyes

This study established methods to accurately quantify the native pore geometry of anterior lamina cribrosa. The 3D transformation and tessellation methods can be employed with

en face and axial imaging techniques, such as AOSLO and SDOCT imaging, to better understand 3D laminar geometry. This approach was used *in vivo* to further examine global and regional differences in the 3D laminar geometry of normal eyes, 3D changes in laminar geometry that occur at different stages of the disease in experimental models of glaucoma and in human glaucoma patients, and 3D differences in laminar geometry between normal and glaucomatous eyes.

6.1.2 Chapter 4: Automated characterization of predominant lamina cribrosa orientation in non-human primates using AOSLO

This study established an automated method to objectively assess the structural characteristics of lamina cribrosa beams to better understand and track structural changes in the ALCS during glaucoma progression. The method was validated using synthetic images of known orientation with different levels of noise and blur. As evidenced by low errors in estimating the dominant orientations of synthetic images of a single known orientation, robustness to noise and blur, and presence of diverse orientations, PCA can accurately compute the dominant orientation of the lamina cribrosa beams in monkey eyes. This method can operate directly on grayscale AOSLO images thereby eliminating the need to binary segment the lamina cribrosa. Quantification of laminar beam orientation *in vivo* using PCA will provide the opportunity to track longitudinal changes in beam orientation in glaucoma and better understand laminar remodeling due to biomechanical changes during disease.

6.1.3 Chapter 5: Automated characterization of predominant lamina cribrosa orientation in non-human primates using AOSLO

This study established an automated method to segment lamina cribrosa pores. Ivers et al assessed the repeatability of AOSLO imaging and measurement of lamina cribrosa pore geometry using manual segmentation of the pores [28]. The method was validated using a reference standard obtained from a combination of segmentation by 3 human raters. As evidenced by spatial overlap metric values, the quantification of ALCS pores via automated segmentation was comparable to manual segmentation results and repeatable over time. Our automated segmentation method can be used to characterize lamina cribrosa microarchitecture in normal and EG eyes.

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Appendices

Appendix A

Publications

- [1] C. Li, N. Sredar, H. Queener and J. Porter, "A correction algorithm to simultaneously control dual deformable mirrors in a woofer-tweeter adaptive optics system," *Opt Express* 18(16):671-684, 2010.
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