# Bulk changes in posterior scleral collagen microstructure in high myopia

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**Abstract**

Purpose: We aimed to characterise any bulk changes in posterior scleral collagen fibril bundle architecture in human eyes with high myopia.

Methods: Wide-angle X-ray scattering (WAXS) was employed to map collagen orientation at 0.5mm x 0.5mm spatial intervals across the posterior sclera of seven non-myopic human eyes and two eyes with high myopia (>6D of refractive error). At each sampled point, WAXS provided thickness-averaged measures of 1) the angular distribution of preferentially-aligned collagen fibrils within the tissue plane and 2) the anisotropic proportion (ratio of preferentially aligned to total collagen scatter).

Results: Non-myopic specimens featured well-conserved microstructural features, including strong uniaxial collagen alignment along the extraocular muscle insertion sites of the midposterior sclera and a highly anisotropic annulus of collagen circumscribing the nerve head in the peripapillary sclera. Both myopic specimens exhibited notable alterations in the peripapillary sclera, including a partial loss of circumferential collagen alignment and a redistribution of the normally observed regional pattern of collagen anisotropic proportion.

Conclusions: Bulk alterations to the normal posterior scleral collagen microstructure occur in human eyes with high myopia. Myopic alteration of the peripapillary scleral architecture may impact the mechanical environment of the optic nerve head, with possible implications for glaucoma damage susceptibility.

## Introduction

Myopia is the most common visual disorder, affecting 23% of the world's population, with the number expected to reach 50% by 2050 [1]. Myopia is a type of refractive defined by the inability to see at greater distances and is caused, in major part, by an abnormal axial lengthening of the globe, placing the eye's focal plane in front of the retina. Individuals with myopia exceeding 6D are classified as having high myopia and are at increased risk of developing further complications that can lead to temporary or permanent loss of vision, including glaucoma, cataract, macular degeneration and retinal detachment [2]. As its prevalence continues to rise, gaining control of the escalating myopia problem is becoming a growing global concern [3].

Myopic lengthening of the eye involves remodelling and biomechanical changes to its main load-bearing tissue – the sclera, the white fibrous tissue that comprises about 85% of the ocular tunic [4]. The sclera consists predominantly of densely woven fibrils of the complex protein collagen that impart the tissue with mechanical rigidity and which, in turn, helps maintain the eye's structural integrity and shape [5]. In the human sclera, about 90% of the dry weight is due to collagen. After being secreted into the extracellular space, collagen molecules assemble into fibrils, which have a wide range of diameters, from 25 to 230nm [6] and span many hundreds of microns in length in mature tissues [7]. The collagen fibril bundles in the sclera are more complex and generally more disorganised than in the neighbouring cornea and show a high degree of regionally variability in their bulk orientation between different areas of the tunic [8-10]. The collagen architecture of the posterior sclera plays a major role in governing tissue deformation in response to changes in intraocular pressure (IOP), and scleral stresses are readily transmitted to the more compliant tissues of the optic nerve head (ONH) [9, 11]. The ONH may be considered a "weak spot" in the scleral tunic, where the sieve-like lamina cribrosa (LC) supports the exiting nerve axons, and where deformation forces are accumulated – making it an area of particular mechanical interest [12, 13].

A number of alterations to both the scleral structure and neighbouring tissues have been

noted to occur with high myopia. With axial elongation of the eye globe the sclera, lamina cribrosa and choroid have been noted to become thinner [14-16]. Sclera growth and remodelling in the myopic eye is considered to be a dual process [17, 18]. The amount of collagen decreases by both a down-regulation in the synthesis of the type I collagen and concomitant stimulation of collagen degradation [19, 20]. The end result is a decline in existing collagen bundles and a prevention of the formation of new ones. A decrease in collagen fibril diameter, particularly near the posterior pole, has also been noted [21]. Studies in mammal models further confirm that the changes during myopia development are the result of active tissue remodelling rather than just passive stretching of the sclera, contributing to a compromise in the mechanical stability and integrity of the tissue [22, 23]. However, while there is substantial evidence that collagen remodelling underlies the axial elongation of the myopic sclera, it is not known the extent to which this process manifests in terms of bulk changes to the orientation of collagen in the tunic – a key determinant of its direction-dependent biomechanical properties. Previously we have applied wide-angle X-ray scattering (WAXS) to map the collagen fibrillar architecture in both normal and glaucomatous posterior scleral shells [9, 10]. The goal of the current study was to apply these methods to evaluate any bulk changes to collagen orientation in the posterior sclera of highly myopic human eyes.

## Methods

### Tissue details and sample preparation

All experimental procedures were conducted in accordance with the Declaration of Helsinki. Eight human ocular globes (seven non-myopic and one highly myopic) were obtained within 48 hours post-mortem from the Fondazione Banca degli Occhi del Veneto, Italy. In addition, one further highly myopic eye was obtained from the Department of Ophthalmology, University of Hong Kong. Following removal of the ocular contents, the intact scleral shells were stored in 4% paraformaldehyde at 277K. The eyes were designated their myopic/normal status (> 6D for highly myopic) via examination by an ophthalmologist and none had a history of previous surgery involving the posterior sclera. Furthermore, using the

polar vector plot maps of collagen orientation from the conducted WAXS experiments, we measured the distance between landmarks of the optic nerve canal edge and the insertions of the inferior oblique muscle, as a measure of the degree of scleral lengthening (Figure 1, Table 1). Scleral specimens were prepared based on previously established protocols. The surrounding fat, muscle and episcleral tissues were carefully removed before the optic nerve was excised with a razor blade flush to the sclera [10]. The cleaned globes were dissected around the equator and the internal lens, retina and choroid and subsequently removed. To prevent the formation of creases when flat mounting the posterior cups, relaxing meridional incisions were made in the posterior sclera from the equator to just outside the peripapillary region. The specimens were then returned to 4% paraformaldehyde until the time of the X-ray experiments. As shown in our previous work, this mild fixation does not affect WAXS orientation measurements [24]. Details of the eyes used in this study are provided in Table 1. The mean donor age for the control group of seven non-myopic eyes was 66.3±7.1 years, while the donor ages of the two highly myopic specimens were 60 and 64.

X-ray scattering data collection

Previously our group has developed a method for quantifying the bulk collagen fiber orientation of the sclera using WAXS [9, 10]. When incident monochromatic X–rays pass through the sclera, a portion of them are scattered at different angles and their direction will reflect the sclera's intrinsic microstructure. A well-resolved single diffraction peak is formed perpendicular to the fibril axis - referred to as the equatorial direction. This scatter pattern arises from the regular ~1.6nm lateral packing of the collagen molecules that make up the fibrils [25]. The angular intensity distribution can be analysed to quantify the number of fibrils in each direction within the tissue plane. A key advantage of this approach is that the scleral tissue is not required to be sectioned, embedded, or stained for the experiments, thus preventing artificial disruptions in the microstructure. Moreover, irrespective of the varying diameter and packing of scleral collagen fibrils across the eye tunic, the diameter and packing of the constituent collagen molecules from which the WAXS signal originates is highly uniform, which gives rise to a sharp well-resolved signal that is relatively impervious

to variations in tissue hydration [9]. The technique provides quantification of the collagen orientations as an average of the tissue thickness [26].

WAXS experiments were conducted at the Diamond Light Source (Harwell, UK), the UK's national synchrotron facility. The specimens were measured using macromolecular crystallography beamlines I02 and I03, which have identical capabilities. The beamlines were operated in a custom-modified fiber-diffraction set-up to record WAXS patterns across each scleral sample at 0.5mm (horizontal) × 0.5mm (vertical) intervals using an integrated x-y motor stage (Figure 2) [9, 27]. To prevent tissue dehydration during data collection, the specimens were wrapped in polyvinylidene chloride film and mounted inside Perspex (Lucite Group Ltd, Southampton, UK) chambers with Mylar (DuPont-Teijin, Middlesbrough, UK) windows. The incident X-ray beam was directed perpendicular to the specimen surface, with an exposure time of 1s or 0.5s and recorded electronically on a Pilatus-6MF silicon pixel detector (Dectris Ltd, Baden, Switzerland) placed 350mm behind the specimen. The wavelength of the focused beam was 0.09795nm with a 150μm × 80μm cross-sectional size.

X-ray scattering data processing

By analysing the angular distribution of intensity around the 1.6nm WAXS reflection (Figure 3A) a quantitative measure of the relative number of collagen fibrils orientated at a given angle within the scleral plane can be acquired. We obtained from all specimens, at each sampled point in the tissue: 1) the relative number of preferentially aligned fibrils at a given angle over and above the underlying isotropic population, referred to as the *collagen orientation distribution*, with the magnitude of the principal direction, referred to as the *collagen anisotropy*. 2) the scatter due to preferentially aligned collagen divided by that from the total fibrillar collagen content, referred to as the *anisotropic proportion*.

The quantification of scleral fiber collagen orientation from WAXS patterns is described in detail elsewhere [9, 25]. The scatter profiles were analysed using a bespoke MATLAB software script (MATLAB; The MathWorks, Natick, MA) that adapted a previously used

approach [9, 28]. 720 radial profiles (one every 0.5°) were extracted from each WAXS pattern and a unique power-law background function was fitted and subtracted from each (Figure 3B) [9, 10, 27]. The isolated scatter profiles along each direction were normalised against X-ray beam current fluctuations and exposure time, radially integrated and the values extracted to angular bins. The resulting angular intensity profiles were divided into two components: isotropic and anisotropic scatter (Figure 3C) and the latter plotted in polar vector coordinates. To take into account the fact that equatorial scatter occurs at right angles to the collagen axis a 90° shift in the total collagen scatter distribution was performed. For each sampled point in the scleral tissue the collagen orientation distribution could be represented by a polar vector plot (Figure 3D). Individual plots were then assimilated into montages and the anisotropy assigned color codes in MATLAB, representative of the highest degree of alignment (maximum vector length per plot). Contour maps of collagen anisotropic proportion were generated in MATLAB, by calculating the ratio of aligned against total integral collagen scatter, (Equation 1):

$$Anisotropic\_proportion = \frac{\int_0^{2\pi} I_a d\phi}{\int_0^{2\pi} (I_a + I_i) d\phi} \quad (1)$$

where $I_a$ and $I_i$ are the aligned and isotropic collagen scatter at angle φ (Figure 3C). To compare bulk collagen structural changes between myopic and non-myopic individuals we selected a fixed region of 64 sampling points within a 1.5mm radius of the optic nerve canal edge, representative of the peripapillary scleral region [9]. Sampling points outside of this region were considered to be part of the mid-posterior sclera. The peripapillary sclera was further divided into 4 quadrants based on their position: Superior-Nasal (SN), Superior-Temporal (ST), Inferior-Temporal (IT) and Inferior-Nasal (IN), and for all of the sub-regions an average for the collagen anisotropy was calculated. To quantify any distortion in the alignment direction of preferentially aligned collagen bundles in the peripapillary sclera, we compared the angular displacement of the main direction revealed by the polar vector plots (for individual myopic specimens and the averaged control) to an idealized angle distribution representative of the circumferential collagen fiber structure circumscribing the optic nerve that characterizes the normal human sclera (Figure 4) [9, 10, 29].

## Results

In Figure 5 a polar plot map of collagen orientation is presented. The map is overlaid on top of a photograph of the scanned posterior sclera of a non-myopic right human eye (sample N4). In accordance with previous WAXS studies, reproducible structural features characteristic of the non-myopic sclera and were found [9, 10]. These included the tendon insertions of the inferior oblique muscle in the midposterior region, which were found to be consistent in position from landmark of the optic nerve canal (Table 1). Around the optic nerve, the collagen bundles were preferentially aligned in a circumferential direction and this feature exhibited noticeably higher collagen anisotropy. Another consistent feature was two symmetrical linear fiber bands that radiate tangentially from the peripapillary ring of aligned collagen outwards into the mid-posterior scleral region [30]. All of these features were found to be present in the other six non-myopic specimens from the control group (see supplementary material).

In Figure 6 a comparison between a typical non-myopic scleral polar vector map and the two highly myopic specimens is presented, and reveals several marked differences in the bulk collagen orientation. In non-myopics, there is consistently a disruption in the circumferential collagen orientation in the SN quadrant of the peripapillary sclera, as found in previous studies [9, 10] (Figure 6B). However, for myopic specimen HM1 two such regions of disruption were observed instead in the ST and IN regions (Figure 6D). HM2 exhibited more widespread differences: the ONH appears wider in size and the surrounding annulus of collagen, which had a noticeably larger interruption in its circumferential structure in the SN quadrant, was spread over a larger radial distance  extending well into the midposterior sclera. Collagen anisotropy was markedly lower in the peripapillary region for HM2 than for HM1 and the controls, but featured higher values in the midposterior region of the collagen annulus (Figure 6F).

For each sampling point of the posterior sclera, a value for the ratio of aligned to total collagen (anisotropic proportion) was also extracted and plotted (Figure 7). The anisotropic

proportion values of the peripapillary sclera for the seven non-myopic posterior scleral specimens were combined into an averaged control. This was justified based on the highly conserved collagen structure of the posterior sclera in non-diseased eyes, as shown herein and previously [9]. Regional quantification of this data is shown in Figure 8 and Table 2. For all seven non-myopic specimens the minimum collagen anisotropic proportion was consistently observed in the SN quadrant and the maximum value observed in the IN quadrant (Table 2 and Supplementary Table 1). This pattern was not exhibited in the highly myopic specimens HM1 and HM2, where the minimal value was in the ST and IT, and maximum in the IT and ST quadrants, respectively (Table 2). The atypical results for the myopic sclera are highlighted in Figure 8, where the myopic specimen values are clearly identifiable as outliers to the box-plot data. The anisotropic proportion for the peripapillary sclera in specimen HM2 generally demonstrated higher values than both the controls and HM1 (Figure 7B, D, F). This appeared initially at odds with the vector plot maps, that indicated overall lower collagen anisotropy for HM2 around the nerve head (Figure 6B, D, F). However, the two observations may be reconciled if we consider that the collagen anisotropy will scale directly with tissue thickness (and hence total collagen scatter), whereas the anisotropic proportion will scale inversely with thickness. Hence, it is likely that excessive tissue thinning around the posterior pole in myopic specimen HM2 would have manifested in a lower total collagen scatter and hence higher anisotropic proportion, while the absolute number of fibrils along the preferred direction (defining the collagen anisotropy) was relatively low.

In order to further quantify the structural differences between the non-myopic control group and the two highly myopic eyes, we compared the angular displacement of the collagen vector plots from an idealized circumferential distribution (Figure 4). The right eye was chosen as default and for left eyes a mirror image of the polar vector maps was taken. Figure 9 shows maps of the angle difference between the idealized circumferential distribution and A) averaged control, B) myopic specimen HM1 and C) myopic specimen HM2. The results indicate how closely the non-myopic structure follows the idealized circumferential orientation around the ONH (Figure 9A). HM1 followed the pattern to a lesser degree and diverged markedly from the idealized distribution in the ST quadrant with a maximum

deviation of 74° (Figure 9B). For HM2 the differences were most pronounced on the outer parts of the peripapillar region in the SN quadrant, with a maximum deviation of 83° (Figure 9C).

## Discussion

This paper presents the first application of WAXS mapping to determine bulk collagen orientation changes in human eyes with high myopia. The results verify that in non-myopic human posterior sclera the collagen orientation distribution is highly conserved between individuals, while in specimens with high myopia a marked loss of the normal microstructural organisation was observed. Previous research has provided evidence on remodelling of the scleral extracellular matrix with myopia progression [18, 31]. However, until now it has remained unknown how bulk scleral collagen fibril orientation is affected in myopia. The presented results provide evidence that highly myopic posterior sclera do not follow the normal fibrillar organisation, with both myopic specimens exhibiting marked changes in the peripapillary sclera.

The existence of a distinct ring of peripapillary collagen fibers around the optic nerve was reported for the first time less than a decade ago and since then has been documented to exist in humans as well as a number of animals [24, 29, 32-35]. The circumferentially orientated fibris bundles provide mechanical stability to the ONH as they limit the IOP-related expansion of the scleral canal and reduce the in-plane tensile strains within the lamina cribrosa [10, 11, 13, 26, 36, 37]. As such, changes to the peripapillary collagen architecture may be linked to an increased susceptibility to ONH damage in glaucoma [9, 38, 39]. Both highly myopic specimens in this study displayed noticeable disruption in the preferential orientation of the collagen fibrils around the ONH. It is possible that remodelling of the extracellular matrix has occurred as a result of myopic progression and that, given the mechanical role of the peripapillary sclera, that this may, in turn, affect the mechanical environment of the ONH and its physical response to IOP fluctuations [11, 38, 40, 41].

A number of studies have linked a significant increase in the prevalence of glaucoma with high myopia [42-44]. Studies conducted by Jonas et al. (1988), Saw et al. (2005) and Kimura et al. (2014) indicate that highly myopic patients have larger optic discs [2, 45, 46]. Jonas et al. (1988) described them as "secondary acquired macrodisks", which are accompanied by larger peripapillary atrophic region [45]. Saw et al. (2005) added to the list of abnormalities a tilt to the optic disc as well as a thinner LC [2]. Bellezza et al. (2000) concluded that a larger optic disc is more susceptible to IOP-related damage, which could link to the pathological changes to the scleral architecture presented here [47]. Specifically, in specimen HM2 the scleral canal was noticeably enlarged, with the width of the aligned collagen ring spanning a larger radius than in the control specimens. This could be a direct result of elongation of the eye. Based on the polar vector plot map for HM1 the optic nerve canal appears to be stretched in the ST-IN direction, in which there also a smaller amount of preferentially aligned collagen. This is reminiscent of the findings of Pijanka et al. (2012) for glaucomatous specimens, which showed a significantly lower degree of peripapillary collagen alignment in glaucomatous eyes [9]. Furthermore, the Beijing eye study found that, while there was no significant difference in IOP between highly-myopic and non-myopic eyes, the former group exhibit a significantly higher onset of glaucoma [42]. This could further suggest that a greater risk of developing glaucomatous damage might be linked with structural changes occurring with high myopia, such as those in the peripapillary sclera noted herein.

Several limitations must be taken into account in the present study. Firstly, the number of highly myopic specimens available to the study was small (2) due to the limited access to posterior scleral tissue from donors of known clinical status. However, the structure of the both myopic eyes did noticeably deviate from the non-myopic eyes, whose structural features were, in contrast, highly reproducible between specimens. Secondly, the axial length of the specimens was not determined. This, however, was compensated by calculating the distance from the edge of the optic nerve canal to the insertion of the inferior oblique muscle for each posterior shell, as a measure of the scleral tissue elongation. Notably, the results were highly consistent between controls (Table 1), with a marked increase for myopic specimen HM2. This calculation was not possible to do accurately for HM1 because the

wide-spread nature of the structural deformations present in this specimen precluded the use of the inferior oblique muscle insertion as a reliable landmark. Nonetheless, the specimen was confirmed to be highly myopic in the clinic, with >6D of refractive error. Thirdly, there are inherent limitations to the WAXS method itself. As mentioned, WAXS yields thickness-averaged results and cannot provide clarity to the structural composition throughout the tissue depth. Pijanka et al. (2015) showed that the circumferentially aligned collagen fibers do not persist through the entire tissue depth but rather the outer two-thirds of the stroma [27]. Thus it remains unknown if the observed changes in myopic specimens are present through the entire depth of the scleral tissue. Secondly, flattening of the scleral coat may have released some of the residual stress that is present in the intact tissue, potentially causing changes in the typical collagen fibril orientation. It has been shown, however, that this effect is more profound at a macro (organ) level and less prominent at the collagen microstructure level [48]. Moreover, the relaxing incisions used to flatten the tissue did not penetrate the peripapillary tissue where the quantitative analysis in this paper was concentrated. In addition, original fixation of the eye tunic in it is natural curvature should have further limited the extent of any fibrillar reorganization upon subsequent dissection.

In conclusion, using WAXS we have mapped the bulk posterior scleral collagen structure of two human eyes with high myopia. In comparison to non-myopic eyes, the highly myopic specimens showed disruptions in the alignment of the characteristic circumferential collagen fibril organisation in the peripapillary sclera, as well as changes in the normally well-conserved regional pattern of anisotropic proportion. The results support the idea that pathological structural remodelling takes place with high myopia that accompanies axial lengthening and mechanical alteration of the scleral tissue. The structural changes that occur with high myopia in the peripapillary region may provide further insight into the increased susceptibility of myopic eyes to glaucoma development, and enhance future modelling studies of ocular biomechanical changes in myopia.

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## Figure Legends

**Figure 1:** Calculating the distance between the edge of optic nerve canal and the tendon insertions of the inferior oblique muscle. WAXS polar vector plots reveal circumferential collagen annulus around the canal and oblique uniaxial alignment of muscle insertion region. Canal edge is denoted by curved line. Three individual measurements (line lengths) were performed and a mean taken as the representative value. (A) Non-myopic posterior sclera N6. (B) Highly myopic specimen HM2. Note marked increase in line length for myopic specimen, indicative of axial lengthening of globe.

**Figure 2:** Beamline I03 at the Diamond Light Source operating in a custom fiber-diffraction set-up. The goniometer (A) provides directional translation of the sample holder (B) between X-ray exposures. A flat-mounted posterior sclera is shown mounted between Mylar sheets. After the specimen is positioned a further Mylar sheet (C) in which a lead beam stop (D) is attached, preventing undiffracted X–rays from reaching and damaging the detector positioned out of shot.

**Figure 3:** X-ray scattering data analysis. (A) Typical WAXS pattern from peripapillary human sclera. The area bounded by the two concentric circles corresponds to the collagen scatter. The X-ray scatter intensity spread as a function of the azimuth angle $\phi$ around the collagen peak can be analysed, which provides the distribution of fibril orientations. The presented two-lobed WAXS pattern is indicative of the uniaxial fiber alignment at that point in the tissue. (B) Power-law background function (green line) fitted to a radial intensity profile (red line) through the pattern shown in (A). The blue open circle marks the peak in collagen intensity, while the blue crosses show the fitting points of the background function. For each WAXS pattern, a background function was independently fitted along the 720 equally spaced radial directions, which allows extraction of the collagen signal in two dimensions. (C) Angular X-ray scatter intensity profile for the pattern presented in (A). The collagen scatter intensity may be represented as two components – scatter from the isotropically aligned collagen fibrils (Ii) and anisotropic scatter (Ia) arising from preferentially aligned collagen. (D) Corresponding polar vector plot of the collagen alignment. The anisotropic collagen scatter is displayed in polar coordinates, where the length of vector **r** is proportional to the relative number of collagen fibrils orientated along the preferred direction.

**Figure 4:** Idealized mathematical polar vector distribution for perfect circumferential alignment, used to compare control and myopic collagen orientation in the largely circumferential peripapillary region. Numerical values from 0 to 180 degrees denote the main orientation angle.

**Figure 5:** WAXS polar vector map showing preferential collagen orientation across non-myopic flat-mounted posterior sclera N4, overlaid over a photograph of the tissue before scanning. The superior direction of the specimen is indicated with an arrow. Polar vectors are colour coded according to bar, with warmer colours indicative of higher degrees of collagen anisotropy. Note highly aligned collagen annulus circumscribing the nerve head (black line bounded region), two tangential fiber bands (black arrows) and uniaxial alignment of the ocular muscle insertion regions, with the inferior oblique highlighted (red arrow).

**Figure 6:** WAXS polar plot vector maps comparing one non-myopic (A-B) and two highly myopic (C-F) posterior scleras. (A) Full map of non-myopic specimen N4; (B) 30 x 30 vector plot zoom of N4; (C) Full map of highly myopic specimen HM1; (D) 30 x 30 vector plot zoom of HM1; (E) Full map of highly myopic specimen HM2; (F) 30 x 30 vector plot zoom of HM2. The zoomed regions are denoted by a red square on the full maps. Peripapillary scleral region is shown bounded by black lines, in which largely circumferential collagen alignment is observed. Arrows: interruption of the circumferential collagen orientation (normally limited to the SN quadrant in non-myopic eyes) is more extensive in highly myopic specimens. S, N, I and T denote superior, nasal, inferior and temporal directions, respectively.

**Figure 7:** WAXS contour maps of collagen anisotropy for one non-myopic (A-B) and two highly myopic (C-F) posterior scleras. (A) Full map of non-myopic specimen N4; (B) 30 x 30 point zoom of N4; (C) Full map of highly myopic specimen HM1; (D) 30 x 30 point zoom of HM1; (E) Full map of highly myopic specimen HM2; (F) 30 x 30 point zoom of HM2. The zoom regions are denoted by a red square on the full maps. Peripapillary scleral region is shown bounded by black lines. S, N, I and T denote superior, nasal, inferior and temporal directions, respectively.

**Figure 8:** Box plots of mean collagen anisotropic proportion in the peripapillary sclera by quadrant for the non-myopic control group (SN: Superior-Nasal, ST: Superior-Temporal, IT: Inferior-Temporal, IN: Inferior-Nasal). Median value of each quadrant is represented as a red line, while the whiskers denote outliers. Specimen-specific corresponding values for highly

myopic specimens HM1 and HM2 are shown for comparison and denoted by circles and asterisks, respectively. Note that the myopic data all lie outside the non-myopic range.

**Figure 9:** Variation from idealized circumferential angle distribution (with respect to the nerve canal edge) of the polar vector plots from the peripapillary sclera. Averaged control is shown alongside the two highly myopic specimens HM1 and HM2 following the orientation of a right eye viewed from the back: Top – Superior, Left – Nasal, Bottom – Inferior, Right – Temporal. Marked deviations from circumferential alignment show up as hot-spots in the myopic maps.

**Table 1:** Details of the eye specimens used in the current study. Optic nerve head (ONH) canal edge to inferior ocular (IO) muscle insertion distance is included as a measure of relative axial globe elongation for all specimens, apart from HM1 which was not measurable (as denoted by an asterisk). Note the consistent ONH-IO distance for normal (non-myopic) specimens, which was markedly increased for highly myopic specimen HM2.

**Table 2:** Comparison of average collagen anisotropic proportion by quadrant for control group (n=7) and individual highly myopic specimens HM1 and HM2. Minimum and maximum mean values are highlighted in blue and red font, respectively.

**Supplementary Figure 1:** WAXS polar plot vector maps of three non-myopic (A-F) posterior scleras. (A) Full map of non-myopic specimen N1; (B) 30 x 30 vector plot zoom of N1; (C) Full map of non-myopic specimen N2; (D) 30 x 30 vector plot zoom of N2; (E) Full map of non-myopic specimen N3; (F) 30 x 30 vector plot zoom of N3. The zoomed regions are denoted by a red square on the full maps. Peripapillary scleral region is shown bounded by black lines. Discontinuations of the circumferential collagen orientation in the SN quadrant are indicated by arrows. S, N, I and T denote superior, nasal, inferior and temporal directions, respectively.

**Supplementary Figure 2:** WAXS polar plot vector maps of three non-myopic (A-F) posterior scleras. (A) Full map of non-myopic specimen N5; (B) 30 x 30 vector plot zoom of

N5; (C) Full map of non-myopic specimen N6; (D) 30 x 30 vector plot zoom of N6; (E) Full map of non-myopic specimen N7; (F) 30 x 30 vector plot zoom of N7. The zoomed regions are denoted by a red square on the full maps. Peripapillary scleral region is shown bounded by black lines. Discontinuations of the circumferential collagen orientation in the SN quadrant are indicated by arrows. S, N, I and T denote superior, nasal, inferior and temporal directions, respectively.

**Supplementary Table 1:** Comparison of average collagen anisotropic proportion by quadrant for non-myopic control group specimens. Minimum and maximum mean values are highlighted in blue and red font, respectively.