

Markov, Eliasy, Pijanka, Patterson, Sorensen, Elsheikh, Boote

1 **Bulk changes in posterior scleral collagen microstructure in high myopia**

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3 Petar Markov¹, Ashkan Eliasy², Jacek Pijanka¹, Neil Patterson³, Thomas Sorensen³, Ahmed
4 Elsheikh^{2,4}, Craig Boote¹

5

6 ¹ Structural Biophysics Group, School of Optometry and Vision Sciences, Cardiff University,
7 Cardiff, UK.

8 ² Biomechanical Engineering Group, School of Engineering, University of Liverpool,
9 Liverpool, UK.

10 ³ Diamond Light Source, Harwell Science and Innovation Campus, Harwell, UK.

11 ⁴ NIHR Biomedical Research Centre for Ophthalmology, Moorfields Eye Hospital NHS
12 Foundation Trust and UCL Institute of Ophthalmology, UK

13

14

15 **Correspondence:**

Craig Boote

16

Cardiff University

17

School of Optometry and Vision Sciences

18

Maindy Road

19

Cardiff CF24 4HQ

20

UK

21

BooteC@cardiff.ac.uk

22

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25 **Abstract**

26

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

29

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

36

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

43

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

48

49

50 **Introduction**

51

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

61

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

79

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

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

99

100 **Methods**

101

102 Tissue details and sample preparation

103

104 All experimental procedures were conducted in accordance with the Declaration of Helsinki.
105 Eight human ocular globes (seven non-myopic and one highly myopic) were obtained within
106 48 hours post-mortem from the Fondazione Banca degli Occhi del Veneto, Italy. In addition,
107 one further highly myopic eye was obtained from the Department of Ophthalmology,
108 University of Hong Kong. Following removal of the ocular contents, the intact scleral shells
109 were stored in 4% paraformaldehyde at 277K. The eyes were designated their
110 myopic/normal status ($> 6D$ for highly myopic) via examination by an ophthalmologist and
111 none had a history of previous surgery involving the posterior sclera. Furthermore, using the

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

126

127 X-ray scattering data collection

128

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

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

144

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

158

159 X-ray scattering data processing

160

161 By analysing the angular distribution of intensity around the 1.6nm WAXS reflection (Figure
162 3A) a quantitative measure of the relative number of collagen fibrils orientated at a given
163 angle within the scleral plane can be acquired. We obtained from all specimens, at each
164 sampled point in the tissue: 1) the relative number of preferentially aligned fibrils at a given
165 angle over and above the underlying isotropic population, referred to as the *collagen*
166 *orientation distribution*, with the magnitude of the principal direction, referred to as the
167 *collagen anisotropy*. 2) the scatter due to preferentially aligned collagen divided by that from
168 the total fibrillar collagen content, referred to as the *anisotropic proportion*.

169

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

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

187

$$188 \quad \text{Anisotropic_proportion} = \frac{\int_0^{2\pi} I_a d\phi}{\int_0^{2\pi} (I_a + I_i) d\phi} \quad (1)$$

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

202

203 **Results**

204

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

217

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

230

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

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

254

255 In order to further quantify the structural differences between the non-myopic control group
256 and the two highly myopic eyes, we compared the angular displacement of the collagen
257 vector plots from an idealized circumferential distribution (Figure 4). The right eye was
258 chosen as default and for left eyes a mirror image of the polar vector maps was taken. Figure
259 9 shows maps of the angle difference between the idealized circumferential distribution and
260 A) averaged control, B) myopic specimen HM1 and C) myopic specimen HM2. The results
261 indicate how closely the non-myopic structure follows the idealized circumferential
262 orientation around the ONH (Figure 9A). HM1 followed the pattern to a lesser degree and
263 diverged markedly from the idealized distribution in the ST quadrant with a maximum

264 deviation of 74° (Figure 9B). For HM2 the differences were most pronounced on the outer
265 parts of the peripapillar region in the SN quadrant, with a maximum deviation of 83° (Figure
266 9C).

267

268 **Discussion**

269

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

280

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

293

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

314

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

325 wide-spread nature of the structural deformations present in this specimen precluded the use
326 of the inferior oblique muscle insertion as a reliable landmark. Nonetheless, the specimen
327 was confirmed to be highly myopic in the clinic, with >6D of refractive error. Thirdly, there
328 are inherent limitations to the WAXS method itself. As mentioned, WAXS yields thickness-
329 averaged results and cannot provide clarity to the structural composition throughout the
330 tissue depth. Pijanka et al. (2015) showed that the circumferentially aligned collagen fibers
331 do not persist through the entire tissue depth but rather the outer two-thirds of the stroma
332 [27]. Thus it remains unknown if the observed changes in myopic specimens are present
333 through the entire depth of the scleral tissue. Secondly, flattening of the scleral coat may
334 have released some of the residual stress that is present in the intact tissue, potentially
335 causing changes in the typical collagen fibril orientation. It has been shown, however, that
336 this effect is more profound at a macro (organ) level and less prominent at the collagen
337 microstructure level [48]. Moreover, the relaxing incisions used to flatten the tissue did not
338 penetrate the peripapillary tissue where the quantitative analysis in this paper was
339 concentrated. In addition, original fixation of the eye tunic in its natural curvature should
340 have further limited the extent of any fibrillar reorganization upon subsequent dissection.

341

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

352

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354

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358

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477 **Figure Legends**

478

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

486

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

493

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

511

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

516

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

524

525 **Figure 6:** WAXS polar plot vector maps comparing one non-myopic (A-B) and two highly
526 myopic (C-F) posterior scleras. (A) Full map of non-myopic specimen N4; (B) 30 x 30
527 vector plot zoom of N4; (C) Full map of highly myopic specimen HM1; (D) 30 x 30 vector
528 plot zoom of HM1; (E) Full map of highly myopic specimen HM2; (F) 30 x 30 vector plot
529 zoom of HM2. The zoomed regions are denoted by a red square on the full maps.

530 Peripapillary scleral region is shown bounded by black lines, in which largely
531 circumferential collagen alignment is observed. Arrows: interruption of the circumferential
532 collagen orientation (normally limited to the SN quadrant in non-myopic eyes) is more
533 extensive in highly myopic specimens. S, N, I and T denote superior, nasal, inferior and
534 temporal directions, respectively.

535

536 **Figure 7:** WAXS contour maps of collagen anisotropy for one non-myopic (A-B) and two
537 highly myopic (C-F) posterior scleras. (A) Full map of non-myopic specimen N4; (B) 30 x
538 30 point zoom of N4; (C) Full map of highly myopic specimen HM1; (D) 30 x 30 point
539 zoom of HM1; (E) Full map of highly myopic specimen HM2; (F) 30 x 30 point zoom of
540 HM2. The zoom regions are denoted by a red square on the full maps. Peripapillary scleral
541 region is shown bounded by black lines. S, N, I and T denote superior, nasal, inferior and
542 temporal directions, respectively.

543

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

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

550

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

557

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

563

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

567

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

576

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

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

585

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