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**Anterior scleral thickness changes with accommodation in
myopes and emmetropes**

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1 **Abstract**

2 Although a range of changes in anterior segment structures have been documented
3 to occur during accommodation, the quantification of changes in the structure of the
4 anterior sclera during the accommodation process in human subjects has yet to be
5 examined. This study therefore aimed to investigate the presence of short-term
6 changes in anterior scleral thickness associated with accommodation in young adult
7 myopic and emmetropic subjects. Anterior scleral thickness was measured in 20
8 myopes and 20 emmetropes (mean age 21 ± 2 years) during various
9 accommodation demands (0, 3 and 6 D) with anterior segment optical coherence
10 tomography (AS-OCT). A Badal optometer was mounted in front of the objective
11 lens of the AS-OCT to allow measurements of the anterior temporal sclera (1, 2 and
12 3 mm posterior to the scleral spur) to be obtained while fixating on an external
13 accommodation stimulus. Anterior scleral thickness was not statistically different
14 between refractive groups at baseline, but thinned significantly with the 6 D
15 accommodation demand ($-8 \pm 21 \mu\text{m}$, $p < 0.05$), and approached a statistically
16 significant change with the 3 D demand ($-6 \pm 20 \mu\text{m}$, $p = 0.066$). While both
17 refractive groups thinned by a statistically significant amount at the 1 mm location
18 with the 3 D demand; significant ($p < 0.001$) refractive group differences occurred at
19 3 mm, where the thinning found in the myopic group reached statistical significance
20 with both the 3 D ($-12 \pm 21 \mu\text{m}$) and 6 D ($-19 \pm 17 \mu\text{m}$) demands, and the
21 emmetropes showed no significant changes. This demonstrates the first evidence of
22 a small but statistically significant thinning of the anterior sclera during
23 accommodation. These changes were more prominent in myopes, particularly 3 mm
24 posterior to the scleral spur. These regional differences may be associated with
25 previously reported regional variations in ciliary body thickness between refractive

26 groups, regional differences in the contraction of the ciliary muscle with
27 accommodation, or differences in the response of the sclera to these biomechanical
28 forces.

29 **Keywords**

30 Sclera, accommodation, myopia, optical coherence tomography.

31 **1. Introduction**

32 The tough, fibrous sclera is one of the principal determinants of the size and shape
33 of the eye. The biomechanical properties of the normal sclera also allow the eye to
34 resist deformation associated with external forces such as extraocular muscle
35 contraction, and internal forces such as intraocular pressure and accommodation.
36 There is evidence however, that the normal biomechanical properties of the sclera
37 may be compromised by the development of myopia through a generalised and
38 localised thinning of the sclera (particularly at the posterior pole) (Avetisov et al.,
39 1983; Elsheikh et al., 2010; Norman et al., 2010; Vurgese et al., 2012), associated
40 with elongation of the globe (Rada et al., 2006) which is thought to render the myopic
41 sclera more susceptible to deformation from otherwise normal ocular forces (Norton
42 and Essinger, 1994; Troilo and Wallman, 1991; Wallman and Adams, 1987).

43 In the anterior eye, the sclera is located adjacent to the ciliary body, and these two
44 structures maintain close anatomical and functional relationships. The longitudinal
45 ciliary muscle fibres run adjacent and parallel to the sclera, originating posteriorly
46 from the ora serrata where their tendons connect with the equatorial sclera and
47 choroid, and extend anteriorly to their attachment at the scleral spur (Ishikawa,
48 1962). As the ciliary muscle contracts during accommodation, it draws the mass of
49 the ciliary body inward and forward, allowing the lens to become thicker and more
50 optically powerful (Ishikawa, 1962). Given the sclera's close relationship with the
51 ciliary body, it is conceivable that the forces of contraction during accommodation
52 could influence the structure of the overlying anterior sclera.

53 The apparent link between near work and myopia (see Huang et al., 2015 and
54 Morgan et al., 2018 for comprehensive reviews), has been the catalyst for numerous

55 studies that have examined the changes in a variety of ocular parameters during
56 accommodation (Drexler et al., 1998; Mallen et al., 2006; Ostrin et al., 2006; Read et
57 al., 2010; Woodman et al., 2012; Woodman et al., 2011; Zhong et al., 2014),
58 however only qualitative assessment of scleral changes during accommodation have
59 been reported previously (Croft et al., 2013). Since the relatively recent introduction
60 of anterior segment optical coherence tomography (AS-OCT), it has been used to
61 investigate regional variations in anterior scleral thickness (Buckhurst et al., 2015;
62 Ebnetter et al., 2015; Pekel et al., 2015; Read et al., 2016b), and variation in anterior
63 scleral thickness with time of day (Read et al., 2016a), contact lens wear (Alonso-
64 Caneiro et al., 2016b), ocular disease (Schlatter et al., 2015; Shoughy et al., 2015;
65 Yoo et al., 2011), and ocular therapeutic procedures (Taban et al., 2010; Zinkernagel
66 et al., 2015). Earlier studies relating to the *in vivo* determination of human scleral
67 thickness employed ultrasound biomicroscopy (UBM) and magnetic resonance
68 imaging (MRI), which are limited by the requirement for contact with the ocular
69 surface with UBM measures (Oliveira et al., 2006), and lower axial resolution and
70 propensity for motion artefact with MRI measurements (Norman et al., 2010). AS-
71 OCT allows for rapid, non-contact imaging of the anterior segment at the higher
72 resolution necessary to detect short-term variations in anterior scleral thickness, and
73 provides excellent repeatability and reliability in the measurement of anterior scleral
74 thickness (Buckhurst et al., 2015; Read et al., 2016a; Read et al., 2016b).

75 This study aimed to characterise the changes occurring in anterior scleral thickness
76 associated with accommodation using AS-OCT. Given the documented changes in
77 scleral properties with refractive error (Avetisov et al., 1983; Elsheikh et al., 2010;
78 Norman et al., 2010; Rada et al., 2006; Vurgese et al., 2012), both myopic and
79 emmetropic subjects were examined.

80 2. Material and Methods

81 2.1 Participants

82 Forty healthy young adult participants aged 18-25 years (mean 21 ± 2 years) were
83 recruited from the students of the Queensland University of Technology School of
84 Optometry and Vision Science. Ethics approval was sought from, and granted by
85 the University Human Research Ethics Committee prior to subject recruitment. All
86 participants provided written informed consent before study commencement, and
87 were treated in accordance with the tenets of the Declaration of Helsinki.

88 Prior to study enrolment, each subject's refractive error, visual acuity, and binocular
89 vision characteristics were determined, to ensure all study participants had a best
90 corrected visual acuity of at least logMAR 0.00, and amplitudes of accommodation
91 greater than 6 D. An anterior and posterior ocular health screening was also
92 conducted for all subjects, to identify and exclude any participant with a history of
93 significant systemic or ocular disease, injury or surgery. A significant portion of the
94 study population wore soft contact lenses regularly (40%), and were required to
95 cease lens wear at least 24 hours prior to data collection. Any potential participants
96 who wore rigid contact lenses were excluded from the study.

97 Subjects were categorised into one of two refractive error groups (emmetropic or
98 myopic) by their spherical equivalent refraction (SER), which was obtained via non-
99 cycloplegic subjective refraction. Those with SER between -0.25 and $+0.75$ D (and
100 ≤ 1 DC) were considered emmetropic ($n = 20$, mean SER $+0.38 \pm 0.22$ D), and those
101 with a SER between -0.75 and -6.00 D (and ≤ 1 DC) were classified as myopic ($n =$
102 20 , mean SER -2.83 ± 1.50 D). The refractive groups were well matched in terms of
103 age (emmetropes 21 ± 1 years, myopes 22 ± 2 years), gender ratio (each group

104 comprised of 50% female, 50% male), and monocular amplitude of accommodation
105 (emmetropes 11 ± 1 D, myopes 11 ± 2 D). Of the 40 subjects recruited for the study,
106 21 were of Caucasian ethnicity (53%), 15 were of East/South East Asian ethnicity
107 (37%), and the remaining 4 subjects (10%) were from other ethnic origins. The
108 ethnicity of the refractive error groups was also approximately matched (myopes
109 50% Caucasian, emmetropes 55% Caucasian).

110 **2.2 Instrumentation**

111 Following the screening and classification of participants, each subject had
112 measures of their left eye's anterior temporal scleral thickness taken during 0, 3 and
113 6 D of accommodation with the Spectralis spectral domain AS-OCT (Heidelberg
114 Engineering, Heidelberg, Germany) utilising the instrument's enhanced depth
115 imaging mode (EDI) to optimise the visualisation of the sclera (Spaide et al., 2008).
116 The Spectralis AS-OCT uses a super luminescent diode (870nm central wavelength)
117 to capture fast (40,000 A-scans per second), high resolution images (3.9 μm axial
118 resolution, 11 μm lateral resolution) to a scanning depth of 1.9 mm. A scanning laser
119 ophthalmoscope (SLO) is also employed by the instrument to provide en-face
120 images tracked in real-time to enable reliable averaging of the OCT B-scans,
121 improving scan contrast and fine detail.

122 A Badal optometer was custom built and mounted on the OCT in front of the anterior
123 segment objective lens to allow measurements to be collected while an external
124 fixation target (an LCD screen from an iPhone 4S, Apple Inc., California, USA,
125 screen resolution 326 ppi, screen luminance approximately 20 cd/m^2) provided
126 varying accommodative demands (Figure 1). The Badal optometer was mounted at
127 a 42° angle to the measurement axis so that the temporal sclera could be imaged

128 while the subject viewed a target on the external LCD screen. The use of a Badal
129 optometer allowed for the correction of each individual's spherical equivalent
130 refractive error, in addition to providing 0, 3 and 6 D of accommodative demand.
131 Each subject's right eye was occluded for the duration of the experiment to eliminate
132 the potential confounding effects of convergence in the eye being measured.

133 For the duration of the experiment, subjects were required to turn their head 42° to
134 their right to view the LCD screen with their left eye through the Badal optometer
135 system. This head angle allowed for the subject's visual axis to be aligned with the
136 centre of the Badal system, while the OCT was aligned to image the temporal
137 anterior sclera of the left eye. Since no eye movements were required to view the
138 target (only head movement), the potential confounding influence of extraocular
139 muscle forces upon the anterior sclera was reduced. The angle of head turn was set
140 and monitored throughout the task with a smartphone application (AngleFinder,
141 version 1.1, developer Pool Night Studios, LLC) which was used on another iPhone
142 device mounted on a headband and worn on the subject's head. The AngleFinder
143 application utilises the iPhone's in-built gyroscope to measure angles, displaying the
144 roll, pitch and yaw of the device relative to the previous orientation. The yaw was
145 monitored to make sure this did not vary substantially between measurement
146 sessions, thus ensuring consistency in head turn (mean difference in yaw between 0
147 and 3, and 0 and 6 D measurements for all subjects was found to be $+1.5 \pm 3^\circ$ and
148 $+2 \pm 3^\circ$ respectively).

149 Measures of axial ocular dimensions were also obtained with the Lenstar LS 900
150 biometer (Haag-Streit AG, Koeniz, Switzerland) during 0, 3 and 6 D of
151 accommodation (using a custom built Badal system and cold mirror to allow fixation
152 of the same Maltese cross target used when imaging the sclera with the AS-OCT) to

153 investigate any relationship between changes in the sclera and changes in other
154 ocular dimensions.

155 **2.3 Methods**

156 To eliminate the potential effects of prior near work, subjects were required to watch
157 a movie playing on the LCD screen for 10 minutes, which was imaged through the
158 Badal system at optical infinity. Following this wash-out period, the movie was
159 paused and the screen's display was changed to present a Maltese cross fixation
160 target. The subject was required to fixate the centre of the Maltese cross with their
161 left eye and keep it in sharp focus for the duration of the measurement.

162 Images of the left anterior temporal sclera were obtained (using the scleral/EDI mode
163 of the OCT), with two $20^\circ \times 5^\circ$ (11.1 mm x 2.8 mm) volume scans consisting of 21
164 lines of 30 averaged B-scans (Figure 2A) taken for each accommodation level, using
165 the instrument's high resolution scanning protocol (1536 x 496 pixels per B-scan).

166 Any scans with a quality index (QI) score ≤ 25 dB were discarded and retaken.

167 Since the instrument's automatic follow-up function (to register repeated
168 measurements to the same location) is not available during anterior segment
169 imaging, care was taken to position the volume scan in approximately the same
170 vertical and horizontal location on the anterior eye for all repeated image
171 acquisitions. Scleral thickness measures were then derived from the OCT images,
172 defined as the axial distance between the anterior scleral and the posterior scleral
173 boundary (Figure 2B and 2C). Although an axial thickness measure is commonly
174 used in OCT analyses, significant B-scan tilts could potentially bias these thickness
175 measures (Alonso-Caneiro et al., 2016a). However analysis of each B-scan
176 revealed minimal tilt between conditions in the images collected (mean tilt of $0.7^\circ \pm$

177 0.8°), which would result in less than a micron of measurement error in determining
178 scleral thickness in the majority of scans, which is well below the axial resolution of
179 the instrument (3.9 microns). The images were scaled using the instrument's
180 proprietary algorithm which assumed a homogenous refractive index of 1.40 for the
181 sclera to convert optical distances into geometrical measures of tissue thickness
182 (Read et al., 2016b).

183 After the baseline images were obtained, the Maltese cross target was minimised,
184 and the LCD screen was repositioned to provide a 3 D accommodation demand.
185 The subjects then resumed watching the movie on the screen for a further 10
186 minutes before it was again paused, and the screen was changed to display the
187 Maltese cross fixation target, and two more OCT measurements were taken with a 3
188 D demand. The subjects then underwent another wash-out period, resuming the
189 movie viewing for another 10 minutes with the screen imaged once again at optical
190 infinity. And for the final task, the screen was repositioned to provide a 6 D
191 accommodation stimulus, and the subjects continued watching the movie for 10
192 minutes, before the OCT measurements were taken again. This protocol was
193 repeated with the Badal optometer mounted on the Lenstar device, allowing 5
194 measures of ocular biometry of the subjects' left eyes at each of the 3
195 accommodation levels (0, 3 and 6 D).

196 To reduce the potential confounding influence of diurnal variation in ocular
197 parameters, the measurement sessions were restricted to a 4 hour window each day
198 between 0800-1200 hours (Brown et al., 2009; Chakraborty et al., 2011; Read et al.,
199 2016a; Read et al., 2008). Randomisation of the order of instrument measurements
200 (OCT and Lenstar biometer) was performed prior to the subjects' recruitment to

201 ensure any order effects did not confound the results, and all data for an individual
202 subject was collected within the same measurement session on a single day.

203 **2.4 Data Analysis**

204 The axial biometry data at each measurement session were analysed using a
205 previously described method (Read et al., 2010; Woodman et al., 2012), and then
206 averaged. The OCT images were exported and analysed using custom written
207 software. Analysis of the en-face SLO images was performed to ensure that the
208 thickness data at each measurement session were registered to the same anterior
209 eye locations. Three common points (e.g. anatomical landmarks such as vessel
210 crossings/bifurcations) were chosen in the en-face image from each volume scan,
211 and the horizontal and vertical translation and rotation required to register each
212 subsequent scan with the baseline scan was derived (Figure 3A). The measurement
213 with better image quality at baseline (0 D) was chosen, and the image at each
214 accommodation level showing the least rotation with respect to this image was
215 chosen for further analysis. Any scans with excessive rotation ($> 2.9^\circ$, which
216 corresponds to rotation that would extend beyond two adjacent scan lines) were
217 excluded to reduce the potential confounding influence of large amounts of data
218 interpolation upon the thickness measurements. One volume scan with acceptable
219 alignment at each accommodation level (0, 3 and 6 D) was required for the subjects
220 to be included in the analysis. The average absolute rotation required for the
221 registration of the scans was $0.2 \pm 1^\circ$ for all subjects and all conditions.

222 Once the rotational and translational alignment between the measurements was
223 determined, the scan corresponding to the position of the central scan line of the
224 baseline scan was identified in the 3 D and 6 D scans (Figure 3B). The central

225 scans and the two consecutive scans either side of this (i.e. the 5 central scans) in
226 each measurement were then analysed to segment the posterior scleral boundary,
227 the anterior scleral boundary and anterior conjunctival boundary in each scan (Figure
228 2C and 3C). Each scan was initially segmented using an automated algorithm
229 which delineated the anterior conjunctival surface and the posterior scleral surface.
230 One experienced observer, masked to the subjects' refractive error and
231 accommodation level then checked the integrity of the automated segmentation, and
232 manually corrected any errors. The masked observer then manually segmented the
233 boundary between the anterior sclera and episclera, which was identified based
234 upon the position of the episcleral blood vessels in the scan. In accordance with
235 previous studies using AS-OCT to examine anterior scleral thickness, the deep
236 episcleral vascular plexus was identified as a thin, hyporeflective region external to
237 the solid scleral tissue (Alonso-Caneiro et al., 2016b; Ebnetter et al., 2015; Read et
238 al., 2016a; Read et al., 2016b; Shoughy et al., 2015). The position of the scleral
239 spur in each OCT image was also manually marked, according to previously defined
240 criteria (Day et al., 2013; Sakata et al., 2008).

241 The data obtained via the segmentation of the five central scan lines in each
242 measurement were then used to derive a thickness volume map of both the temporal
243 total anterior wall thickness (from the anterior conjunctiva to the posterior sclera) and
244 the scleral thickness (from the anterior sclera to the posterior sclera) for each subject
245 at each accommodation level (Figure 3C).

246 Each thickness volume (for 3 D and 6 D) was then rotated using custom written
247 software in order to precisely align/register each thickness volume to the baseline (0
248 D) measurement using the rotational information derived from the analysis of the en-
249 face images (Figure 3D). The thickness profile from the central line, in each of the

250 final rotated thickness volumes was then extracted and analysed, thus ensuring that
251 the thickness profile from each condition was derived from the same anterior eye
252 location. The total anterior wall thickness and scleral thickness at discrete points 1,
253 2 and 3 mm posterior to the scleral spur were calculated, measured in the
254 axial/vertical direction of the OCT images from the anterior conjunctival boundary to
255 the anterior scleral boundary, and the anterior scleral boundary to the posterior
256 scleral boundary, respectively. These locations were chosen for comparability to
257 previous OCT studies examining regional scleral thickness (Buckhurst et al., 2015;
258 Pekel et al., 2015; Read et al., 2016a; Read et al., 2016b), and changes in the ciliary
259 body associated with accommodation and refractive error (Buckhurst et al., 2013;
260 Kuchem et al., 2013; Lewis et al., 2012; Lossing et al., 2012; Pucker et al., 2013;
261 Richdale et al., 2013) that also used fixed distances from the scleral spur.

262 Reliability and repeatability of the segmentation of the sclera, episclera and
263 conjunctiva was assessed by having the experienced observer analyse five
264 randomly selected scans three times each. The average within session standard
265 deviation for the determination of the location of the scleral spur was 2 μm . For the
266 total wall thickness the within session SD across all measurement locations ranged
267 between 3 μm and 5 μm ; and for the scleral thickness ranged between 4 μm and 7
268 μm . The intraclass correlation coefficient (two-way mixed model, absolute
269 agreement) for intra-observer reliability, was greater than 0.96 for all parameters
270 tested (Shrout and Fleiss, 1979).

271 Of the 40 subjects examined, seven were excluded from analysis of the OCT images
272 (2 myopes, 5 emmetropes). Five of the subjects were excluded due to large
273 amounts of rotation/cyclotorsion between measurement conditions, meaning that
274 identical regions of the sclera were not imaged. The quality of all of the OCT B-scan

275 images were also checked, and one subject was excluded due to prominent
276 shadows on the OCT images from their lashes which obscured much of the detail in
277 their images, and one subject was excluded because they were unable to keep their
278 fixation steady enough to have all their measurements taken. Of the remaining 33
279 subjects, the average QI of the scans was 40 ± 3 dB out of a possible 50 dB.

280 **2.5 Statistical Analysis**

281 A repeated measures ANOVA was performed to examine the influence of
282 accommodation level (0, 3, 6 D) and measurement location (1 mm, 2 mm, 3 mm
283 from the scleral spur) on both scleral thickness and total wall thickness (within
284 subject factors), and for any differences associated with refractive error group
285 (between subject factor). If significant differences were identified ($p < 0.05$), post-
286 hoc testing with Bonferroni correction was performed. An analysis of covariance
287 (ANCOVA) was also performed to identify any associations between the changes in
288 total wall or scleral thickness and the changes in other measured biometric
289 parameters (e.g. axial length) during accommodation using the methods of Bland
290 and Altman (Bland and Altman, 1995) for the analysis of repeated measures.

291

292 **3. Results**

293 The baseline scleral and total anterior wall thicknesses were not significantly
294 different between the refractive groups, but did show significant variation with
295 distance from the scleral spur ($p < 0.001$) (Table 1). Anterior scleral thickness was
296 significantly greater at the 3 mm location (543 ± 67 μm) compared to the 1 mm (512
297 ± 52 μm) ($p < 0.05$) and 2 mm locations (504 ± 60 μm) ($p < 0.001$). Likewise, the

298 total anterior wall thickness varied significantly with measurement location ($p < 0.05$),
299 although in this case it was the 2 mm region ($730 \pm 87 \mu\text{m}$) that was significantly
300 thinner than the 1 mm ($751 \pm 81 \mu\text{m}$) ($p < 0.05$) and 3 mm ($752 \pm 91 \mu\text{m}$) ($p < 0.001$)
301 regions.

302 Anterior scleral thickness changed significantly with accommodation, with pairwise
303 comparisons revealing significant scleral thinning with accommodation to the 6 D
304 stimulus (mean change $-8 \pm 21 \mu\text{m}$, $p < 0.05$), and thinning which approached
305 significance during accommodation to the 3 D stimulus ($-6 \pm 20 \mu\text{m}$, $p = 0.066$)
306 (Figure 4).

307 The amount of scleral thickness change with accommodation varied significantly with
308 refractive group, with significant thickness changes in the myopic sclerae recorded at
309 both the 3 and 6 D accommodation demands ($p < 0.05$), but no significant change in
310 the emmetropic group at either accommodation level. A significant difference
311 between the refractive groups' mean scleral thickness change was observed at the 3
312 D level ($p < 0.05$), with a scleral thinning seen in the myopes ($-12 \pm 18 \mu\text{m}$), while
313 the emmetropic mean scleral thickness remained unchanged ($+1 \pm 20 \mu\text{m}$, $p < 0.05$).
314 However, during accommodation to the 6 D stimulus statistically significant refractive
315 group differences were not evident ($p = 0.199$), with both the myopes ($-11 \pm 24 \mu\text{m}$)
316 and emmetropes ($-5 \pm 17 \mu\text{m}$) thinning on average by similar magnitudes.

317 Change in anterior scleral thickness during accommodation also varied significantly
318 ($p < 0.05$) with measurement location (1, 2, 3 mm posterior to the scleral spur).

319 When all subjects were considered, a highly significant thinning was observed at the
320 1 mm location during accommodation to the 3 D demand ($-13 \pm 16 \mu\text{m}$, $p < 0.001$),

321 and a significant thinning observed at the 3 mm location with the 6 D demand ($-11 \pm$
322 $18 \mu\text{m}$, $p < 0.05$) (Figure 5A and 5B).

323 The change in mean scleral thickness with accommodation by region by refractive
324 group interaction approached significance ($p = 0.055$). Both the myopic (-15 ± 17
325 μm) and emmetropic ($-10 \pm 15 \mu\text{m}$) participants thinned in the 1 mm region during
326 accommodation to the 3 D stimulus, and the myopes additionally thinned in the 3
327 mm region during accommodation to both the 3 D ($-12 \pm 21 \mu\text{m}$) and 6 D (-19 ± 17
328 μm) stimuli. Significant mean scleral thickening was observed in the emmetropic
329 participants in the 3 mm region with the 3 D ($+11 \pm 15 \mu\text{m}$, $p < 0.05$), but not 6 D (-1
330 $\pm 3 \mu\text{m}$, $p > 0.05$) accommodation demands. These refractive group differences in
331 the change in mean scleral thickness were significant ($p = 0.001$) in the 3 mm region
332 with accommodation to both the 3 and 6 D stimuli (Figure 5A and 5B).

333 On average, the total anterior wall thickness thinned significantly for all subjects with
334 the 6 D (mean change $-6 \pm 14 \mu\text{m}$, $p < 0.05$), but not the 3 D (mean change -4 ± 14
335 μm , $p = 0.088$) accommodation demand (Figure 4). A significant accommodation by
336 region interaction was observed, with both the 2 mm ($-6 \pm 12 \mu\text{m}$, $p < 0.05$) and 3
337 mm ($-7 \pm 14 \mu\text{m}$, $p < 0.05$) locations thinning significantly from baseline with the 6 D
338 accommodation demand. Both refractive groups showed a similar amount of total
339 anterior wall thinning with accommodation ($p = 0.471$) (Figure 5C and 5D).

340 All subjects exhibited significant ($p < 0.001$) anterior chamber depth (ACD)
341 shallowing (mean change of $-0.10 \pm 0.05 \text{ mm}$ and $-0.29 \pm 0.06 \text{ mm}$, for the 3 D and
342 6 D demands respectively) and increased lens thickness (LT) with accommodation
343 (mean change of $+0.11 \pm 0.05 \text{ mm}$ and $+0.30 \pm 0.06 \text{ mm}$), confirming the
344 accommodative response for the 3 D and 6 D demands. ANCOVA revealed that the

345 scleral thinning seen at the 3 mm location during accommodation was significantly
346 positively correlated with the ACD shallowing ($p < 0.05$, $r^2 = 0.138$, slope $\beta = 3.74$)
347 and significantly negatively correlated with the lens thickening ($p < 0.05$, $r^2 = 0.13$,
348 slope $\beta = -3.797$). The scleral thinning at the 3 mm location was also weakly
349 negatively correlated with the change in AL with accommodation ($p = 0.058$, $r^2 =$
350 0.054 , slope $\beta = -0.18$), although this association only approached significance.

351

352 **4. Discussion**

353 This study provides the first quantitative evidence that the anterior sclera undergoes
354 a small but statistically significant thinning with accommodation in young adults. The
355 similarities between the thickness changes seen in the sclera and total anterior wall
356 with 3 and 6 D of accommodation (Figure 4) suggest that the changes observed with
357 accommodation appear to be primarily confined to the sclera, with minimal change
358 seen in the overlying conjunctiva and episclera in the majority of measurement
359 locations. These changes in anterior scleral thickness appeared more prominent in
360 the myopic than emmetropic subjects. While the myopes exhibited statistically
361 significant thinning at both 3 and 6 D accommodation demands, the emmetropes
362 demonstrated less magnitude of change, and their overall scleral thickness
363 (averaged across the 3 measurement locations) was not significantly different from
364 baseline with any level of accommodation. The scleral changes with
365 accommodation also varied dependent upon the region of measurement, with a
366 statistically significant thinning seen 1 mm posterior to the scleral spur in both the
367 myopes and emmetropes, and the myopes also showing statistically significant
368 thinning 3 mm posterior to the scleral spur with increased accommodation levels.

369 Previous studies utilising time-domain AS-OCT have reported that the ciliary muscle
370 of both children (Lewis et al., 2012) and adults (Lossing et al., 2012; Sheppard and
371 Davies, 2010) undergoes an overall shortening during accommodation, with the
372 anterior portion of the muscle thickening and the ciliary body moving forwards and
373 inwards. While the ciliary muscle thickens in the anterior region 1 mm posterior to
374 the scleral spur, the posterior ciliary muscle is reported to thin, particularly in the
375 location 3 mm posterior to the scleral spur (Lewis et al., 2012; Lossing et al., 2012).
376 Since the longitudinal ciliary muscle fibres are firmly attached to the overlying sclera
377 at both the scleral spur and ora serata (Ishikawa, 1962), it is possible that the
378 mechanical force from the ciliary muscle could directly or indirectly influence the
379 scleral structure and lead to the scleral thickness changes that we observed during
380 accommodation.

381 Given that the ciliary body transfers its mass anteriorly during contraction, it is
382 possible that the thinning observed in the most anterior portion of the sclera that we
383 examined (1 mm location) during accommodation to the 3 D stimulus, is occurring in
384 response to the ciliary body movement adjacent to this region of the sclera.

385 However, the change in scleral thickness at the 1 mm location did not increase in
386 magnitude with the larger accommodation demand, which would be expected if
387 direct mechanical force from the ciliary muscle acting on the sclera was the only
388 mechanism involved.

389 Another possible mechanism by which the anterior sclera may thin during
390 accommodation is through the action of scleral myofibroblast cells. Myofibroblasts
391 have been identified in the sclera of humans and monkeys and are thought to
392 contract in response to external scleral tissue stresses (e.g. from eye movements,
393 accommodation and fluctuations in intraocular pressure) to prevent deformation of

394 the surrounding extracellular matrix (Poukens et al., 1998). These cells are found
395 within the inner sclera of the posterior pole (Poukens et al., 1998), and in the region
396 of the scleral spur of human eyes (Tamm et al., 1995). The scleral thinning seen at
397 the 1 mm location with accommodation in both the myopes and emmetropes may
398 therefore relate to a contractile response from the myofibroblasts within the scleral
399 spur. As the ciliary body contracts during accommodation, it presumably imposes
400 mechanical force upon on the anterior sclera overlying the ciliary body region. The
401 myofibroblasts within the scleral spur may then contract in response to these forces
402 and result in a slight thinning of the sclera within this region.

403 There are structural differences in the ciliary body of myopic and emmetropic eyes,
404 with numerous studies reporting thicker posterior ciliary muscles in the eyes of
405 myopes compared to emmetropes or hyperopes (Bailey et al., 2008; Kuchem et al.,
406 2013; Lewis et al., 2012; Muftuoglu et al., 2009; Oliveira et al., 2005; Pucker et al.,
407 2013). Most studies report the greatest difference in thickness associated with
408 myopia occurs in the region 3 mm posterior to the scleral spur (Lewis et al., 2012),
409 although some studies also report similar thickening at the 2 mm location (Bailey et
410 al., 2008; Buckhurst et al., 2013; Muftuoglu et al., 2009; Oliveira et al., 2005; Pucker
411 et al., 2013), with longer eyes demonstrating thicker ciliary muscles. These findings
412 show some parallels with the data presented in our study. The greatest changes in
413 scleral thickness with accommodation were observed in the myopic subjects and in
414 the region of the sclera located 3 mm posterior to the scleral spur, which
415 corresponds to the region of the ciliary body that is reportedly thickest in myopes
416 (Bailey et al., 2008; Buckhurst et al., 2013; Lewis et al., 2012; Muftuoglu et al., 2009;
417 Oliveira et al., 2005; Pucker et al., 2013). In the emmetropic subjects however, the
418 greatest scleral thinning was found in the region 1 mm posterior to the scleral spur,

419 which corresponds to the area of the ciliary body which is reported to be
420 comparatively thicker in emmetropes and hyperopes (Kuchem et al., 2013; Pucker et
421 al., 2013). It is therefore possible that the scleral thinning we have found
422 accompanying accommodation is due to contraction of the ciliary muscle adjacent to
423 the sclera, with greater thinning seen in the regions of the sclera which correspond to
424 the thickest regions of the ciliary body for that particular refractive group.

425 The disparity in the scleral thinning response with accommodation between the
426 refractive groups that we found may reflect the reported change in scleral
427 biomechanical properties that accompany myopia. Investigations regarding the
428 biomechanical changes of the sclera in response to myopia development in animals
429 have found an increase in the viscoelasticity of the sclera of form deprived tree
430 shrews, making these myopic sclerae weaker and more extensible, and susceptible
431 to deformation under otherwise normal scleral stressors such as intraocular pressure
432 (Phillips et al., 2000; Siegwart and Norton, 1999). This may explain why scleral
433 thinning was more pronounced in the myopes than the emmetropes at both the 3 D
434 and 6 D accommodation levels, with the sclera of the myopic eyes appearing to be
435 more readily deformed under normal accommodative forces. The regional variation
436 in scleral thinning reported in this study may be due to differences in the
437 biomechanical properties of the anterior sclera as it moves from the limbal to
438 equatorial region, since evidence of a variation in scleral resistance has previously
439 been reported between the anterior scleral quadrants (Patel et al., 2011). The
440 emmetropic group not only demonstrated less overall scleral thinning with
441 accommodation than the myopic group, but also demonstrated a small but
442 statistically significant scleral thickening at the 3 mm location in response to the 3 D
443 accommodation demand. While the exact mechanism underlying this small

444 magnitude scleral thickening is unclear, we speculate that this is possibly due to a
445 redistribution of anterior scleral tissue during accommodation. There could be a
446 complex interaction between the forces imposed on the sclera by contraction of the
447 ciliary body and myofibroblasts within the scleral spur, combined with regional and
448 refractive group variations in the ciliary body and scleral resistance leading to
449 thickening in a region in one group of subjects, and thinning in another group.

450 Structurally the myopic sclera is thought to be generally thinner than non-myopic
451 eyes, particularly in posterior ocular regions, with highly myopic eyes exhibiting
452 areas of localised ectasia through pronounced scleral thinning at the posterior pole
453 (Avetisov et al., 1983; Elsheikh et al., 2010; Norman et al., 2010; Vurgese et al.,
454 2012). Despite these reports, the baseline scleral thickness values of the two
455 refractive groups used in this study were not significantly different. It is possible that
456 our myopic subjects did not have sufficient axial elongation to produce significant
457 anterior scleral thinning. Another recent study utilising AS-OCT also reported no
458 difference in anterior temporal scleral thickness between age-matched high myopes
459 and emmetropic controls (Pekel et al., 2015). This suggests that any structural
460 changes in the anterior sclera associated with myopia are minimal, which is
461 consistent with previous studies reporting scleral thinning associated with myopia
462 largely confined to the posterior eye (Avetisov et al., 1983; Elsheikh et al., 2010;
463 Norman et al., 2010; Vurgese et al., 2012).

464 The correlation found between the changes in the scleral thickness at the 3 mm
465 location and the anterior chamber and lens thickness during accommodation
466 indicates that the changes seen in the 3 mm region are related to the magnitude of
467 the accommodation response. The magnitude of anterior chamber shallowing and
468 lens thickening during accommodation observed in our current study are consistent

469 with previous reports of the biometric accommodation response in young adults
470 (Ostrin et al., 2006). While this finding suggests that our subjects demonstrated a
471 consistent accommodation response during the scleral measurements (given that
472 the same Badal system and target was used in both parts of the study), a limitation
473 of this study is that simultaneous measures of accommodative response were not
474 collected during the OCT scleral measurements. Asymmetries have previously been
475 been observed between the nasal and temporal ciliary body in both animals
476 (Glasser, Croft, Brumback, & Kaufman, 2001), and humans *in vitro* (Aiello, Tran, &
477 Rao, 1992) and *in vivo* (Sheppard and Davies, 2011). Sheppard and Davies
478 (Sheppard and Davies, 2011) reported the temporal ciliary muscle is thicker and has
479 a greater ability to thin than the nasal ciliary muscle, and interpreted this to indicate a
480 stronger contractile response in the temporal region. Comparing differences in
481 anterior scleral changes during accommodation between the nasal and temporal
482 regions may strengthen the argument that the force of contraction of the underlying
483 ciliary muscle leads to this transient scleral thinning. While it was not possible to
484 obtain nasal AS-OCT scans with the participants fixating in primary gaze in our study
485 due to the position of the nose, future studies which overcome this limitation would
486 provide additional insights into the mechanism underlying the anterior scleral
487 thickness changes during accommodation.

488 Despite many of the changes in scleral thickness noted during accommodation
489 reaching statistical significance, it is important to acknowledge that a lot of these
490 significant changes were small in magnitude, and close to the axial resolution of the
491 Spectralis AS-OCT instrument. Future work on this topic with instrumentation with
492 higher resolution capabilities may be necessary to confirm our findings.

493

494 **5. Conclusions**

495 In conclusion, this study demonstrates for the first time a thinning of the anterior
496 temporal sclera during accommodation that is more prominent in young adult
497 myopes. Statistically significant regional differences in the scleral response also
498 accompanied accommodation, with greater thinning seen in the region 3 mm
499 posterior to the scleral spur in myopic subjects, which increased with greater
500 accommodation demand. These regional differences may be explained by
501 previously reported regional differences in the ciliary body thickness between
502 refractive groups, and/or regional differences in the biomechanical properties of the
503 sclera between refractive groups.

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510

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680

681 **Figure Captions**

682 **Figure 1.** Aerial view of the Badal optometer which was mounted in front of the anterior
683 segment objective lens of the Spectralis AS-OCT. The temporal sclera of the participant's
684 left eye was measured while they viewed a Maltese cross displayed on an LCD screen
685 imaged through a +13 D Badal optometer which allowed for the correction of each
686 participant's ametropia, and to provide accommodation stimuli at 0, 3 and 6 D. The right eye
687 was occluded for the duration of the task.

688

689 **Figure 2.** A) En-face image showing the scanning protocol for obtaining anterior scleral
690 images with the AS-OCT. A 20° x 5° volume scan with 21 lines of 30 averaged B-scans was
691 used to obtain images of the anterior temporal sclera utilising the instrument's EDI
692 technique. B) A typical B-scan of the anterior temporal sclera with anatomical landmarks
693 labelled. C) B-scan with automated segmentation, showing the anterior conjunctiva marked
694 in red and the posterior sclera in green. The blue line represents the anterior scleral
695 boundary, which required manual segmentation. The vertical red line marks the position of
696 the scleral spur. Scleral thickness was measured between the anterior scleral and posterior
697 scleral boundary, and total wall thickness was measured between the anterior conjunctival
698 boundary and posterior scleral boundary.

699

700 **Figure 3.** Overview of the OCT image analysis procedures used to determine scleral
701 thickness and total wall thickness. For simplicity, the 6 D scan analysis procedure has been
702 omitted. A) Three common anatomical landmarks were chosen on the 0 D (baseline) en-
703 face image and subsequent 3 D and 6 D scans in order to rotationally and translationally
704 align the scans with the baseline image. B) The scan line corresponding to the position of
705 the central line scan of the baseline (0 D) en-face image was identified in the 3 D scan (red
706 line). C) The 5 central line scans in the 0 D scan (and corresponding line scans in the 3 D
707 scan) were then analysed to segment the anterior conjunctival boundary (red), anterior
708 scleral boundary (blue), and posterior scleral boundary (green). A vertical reference line
709 (shown by the solid white line) was placed to mark the position of the scleral spur (SS) in
710 each image. D) Data obtained via the segmentation of the 5 central line scans was then
711 used to derive scleral thickness volume maps for each subject at each accommodation
712 demand. These thickness volume maps were then rotated to align precisely with the
713 baseline (0 D) map, and a thickness profile from the corresponding location of the 0 D
714 central line, in each of the final rotated thickness volumes was extracted and analysed.

715 Scleral thickness measurements were then taken at discrete points 1, 2 and 3 mm posterior
716 to the scleral spur (white dotted lines).

717

718 **Figure 4.** Change in average scleral thickness and total wall thickness (mean \pm SEM, μm)
719 from baseline for all subjects to the 3 D and 6 D stimulus. Data points marked with a cross
720 (\dagger) indicate a significant change from baseline ($p < 0.05$). Negative values indicate a
721 thinning with accommodation.

722

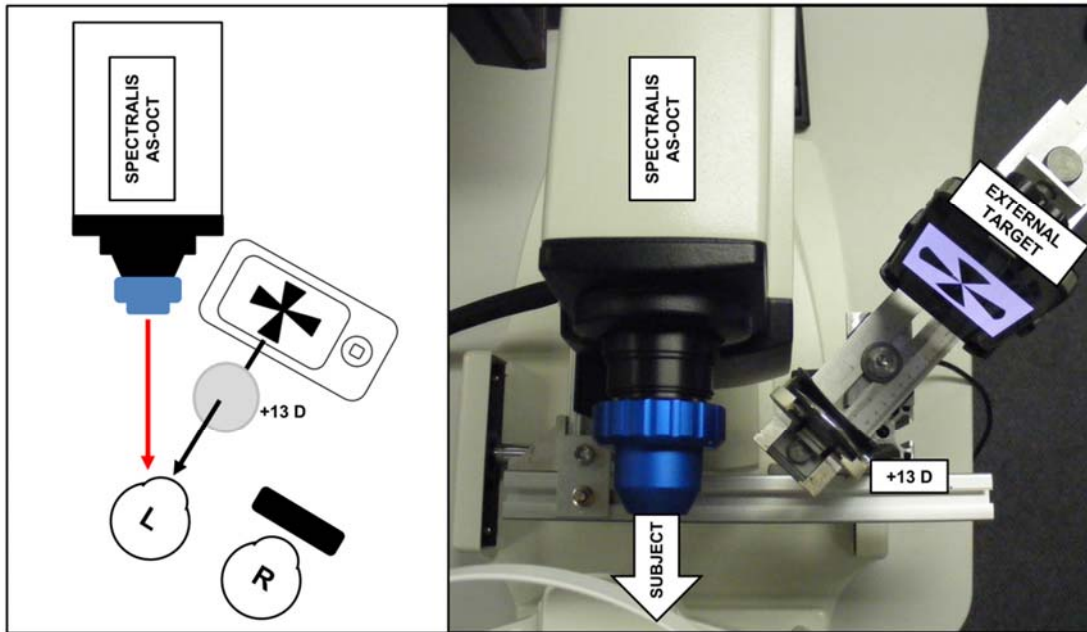
723 **Figure 5.** Change in scleral thickness (A and B) (mean \pm SEM, μm) and change in total wall
724 thickness (C and D) (mean \pm SEM, μm) from baseline at points 1, 2 and 3 mm posterior to
725 the scleral spur in the myopic ($n = 18$) and emmetropic ($n = 15$) subjects, to the 3 D (A and
726 C) and 6 D (B and D) stimulus. Data points marked with a cross (\dagger) indicate a significant
727 change from baseline ($p < 0.05$), and those marked with an asterisk (*) indicate a highly
728 significant change from baseline ($p < 0.001$). Negative values indicate a thinning with
729 accommodation, and positive values indicate a thickening.

730 **Tables**

	Scleral Thickness			Total Wall Thickness		
	(mean ± SD, μm)			(mean ± SD, μm)		
	1 mm	2 mm	3 mm	1 mm	2 mm	3 mm
All subjects	512 ± 52	504 ± 60	543 ± 67	751 ± 81	730 ± 87	752 ± 91
Myopes	513 ± 62	505 ± 72	544 ± 73	751 ± 86	726 ± 89	743 ± 91
Emmetropes	511 ± 39	503 ± 43	542 ± 62	751 ± 79	735 ± 87	763 ± 92

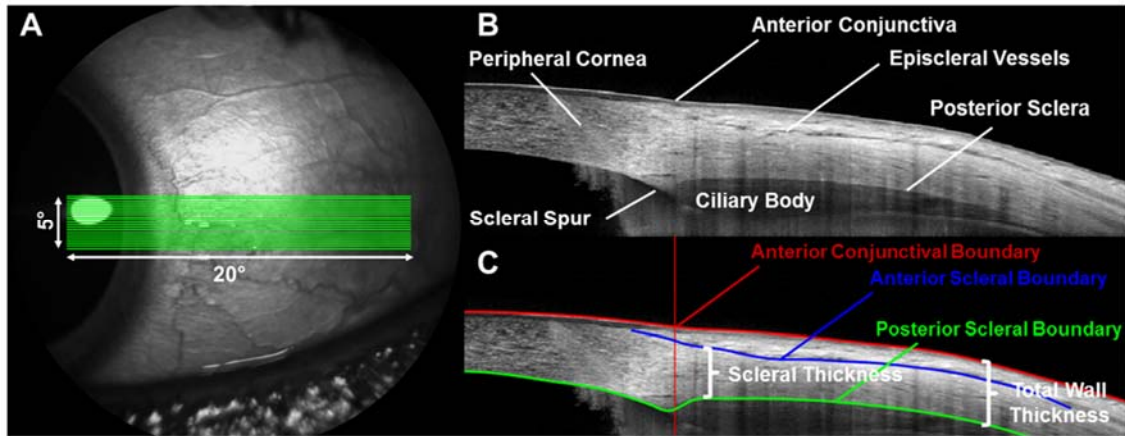
731

732 Table 1. Baseline scleral thickness (mean ± SD, μm) and total wall thickness (mean ± SD,
 733 μm) at points 1, 2 and 3 mm posterior to the scleral spur in all subjects (*n* = 33), myopes (*n* =
 734 18) and emmetropes (*n* = 15).



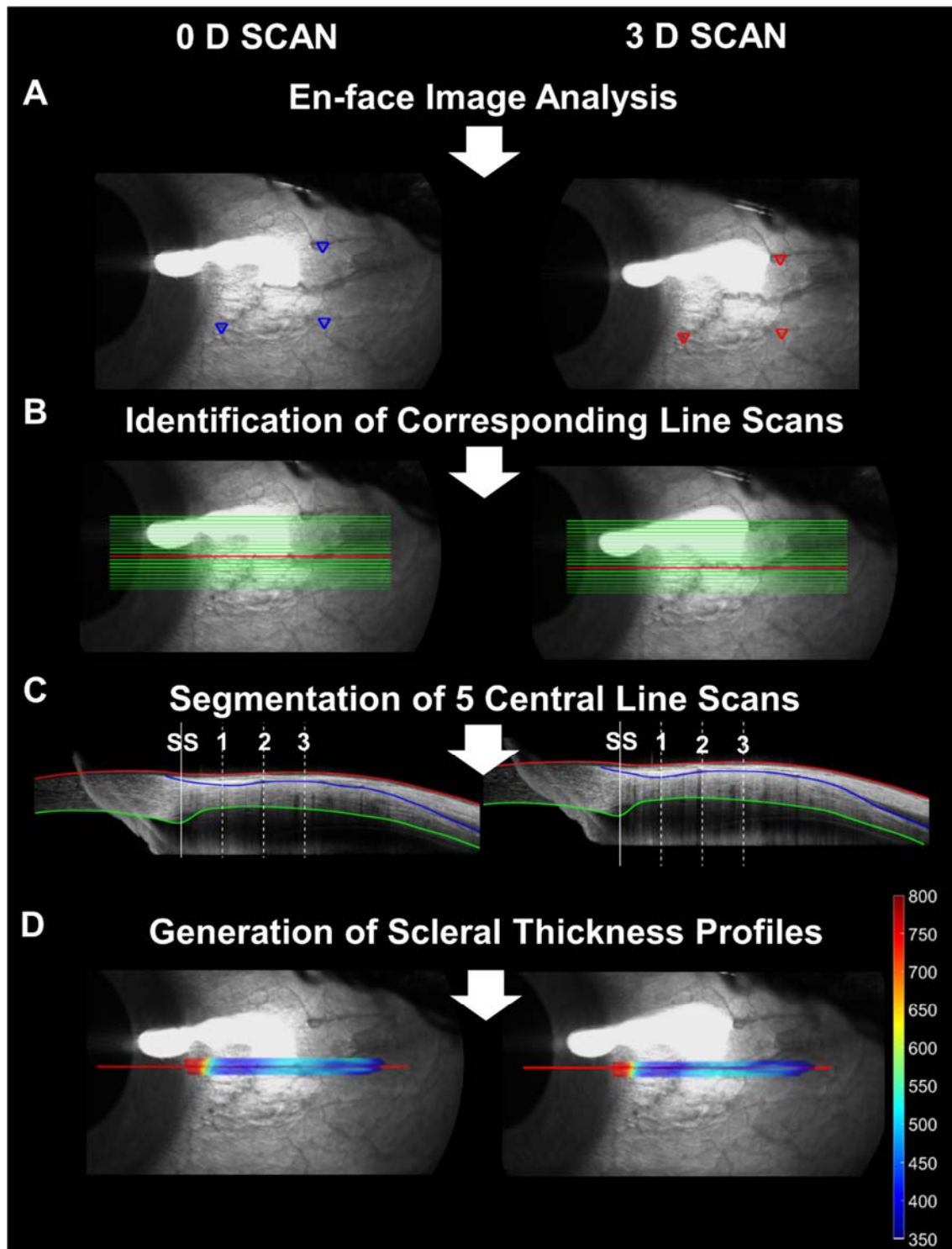
735

736 Figure 1. Aerial view of the Badal optometer which was mounted in front of the anterior
 737 segment objective lens of the Spectralis AS-OCT. The temporal sclera of the participant's
 738 left eye was measured while they viewed a Maltese cross displayed on an LCD screen
 739 imaged through a +13 D Badal optometer which allowed for the correction of each
 740 participant's ametropia, and to provide accommodation stimuli at 0, 3 and 6 D. The right eye
 741 was occluded for the duration of the task.



742

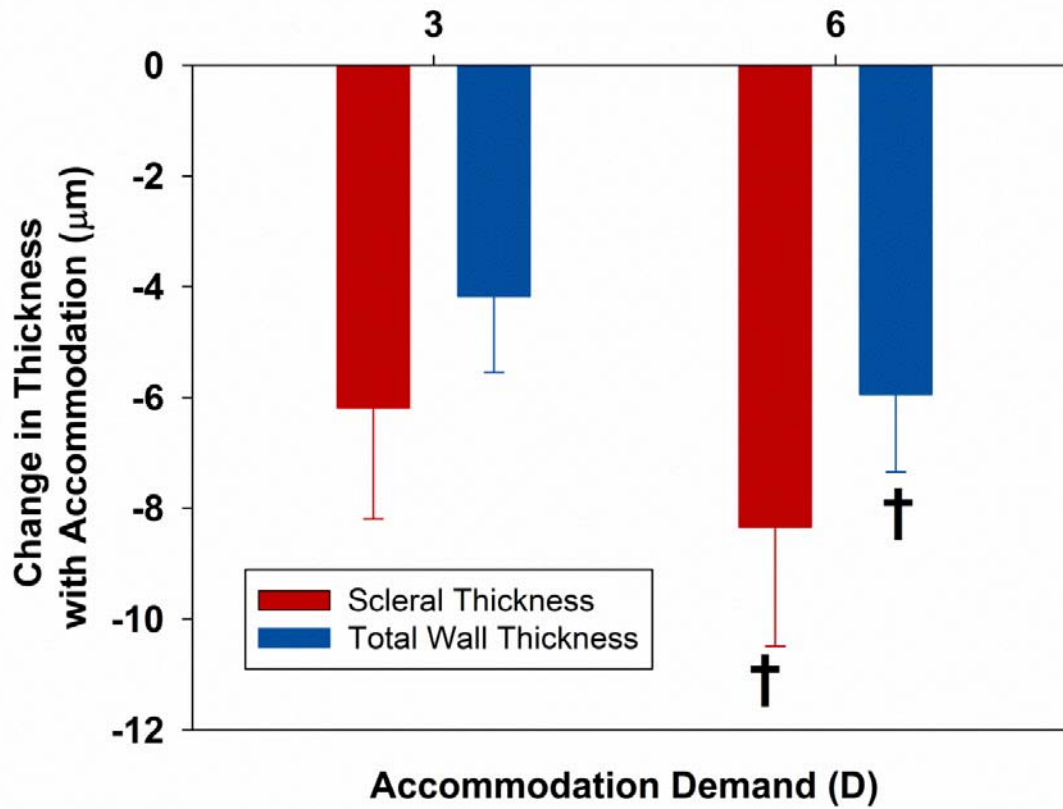
743 Figure 2. A) En-face image showing the scanning protocol for obtaining anterior scleral
 744 images with the AS-OCT. A 20° x 5° volume scan with 21 lines of 30 averaged B-scans was
 745 used to obtain images of the anterior temporal sclera utilising the instrument's EDI
 746 technique. B) A typical B-scan of the anterior temporal sclera with anatomical landmarks
 747 labelled. C) B-scan with automated segmentation, showing the anterior conjunctiva marked
 748 in red and the posterior sclera in green. The blue line represents the anterior scleral
 749 boundary, which required manual segmentation. The vertical red line marks the position of
 750 the scleral spur. Scleral thickness was measured between the anterior scleral and posterior
 751 scleral boundary, and total wall thickness was measured between the anterior conjunctival
 752 boundary and posterior scleral boundary.



753

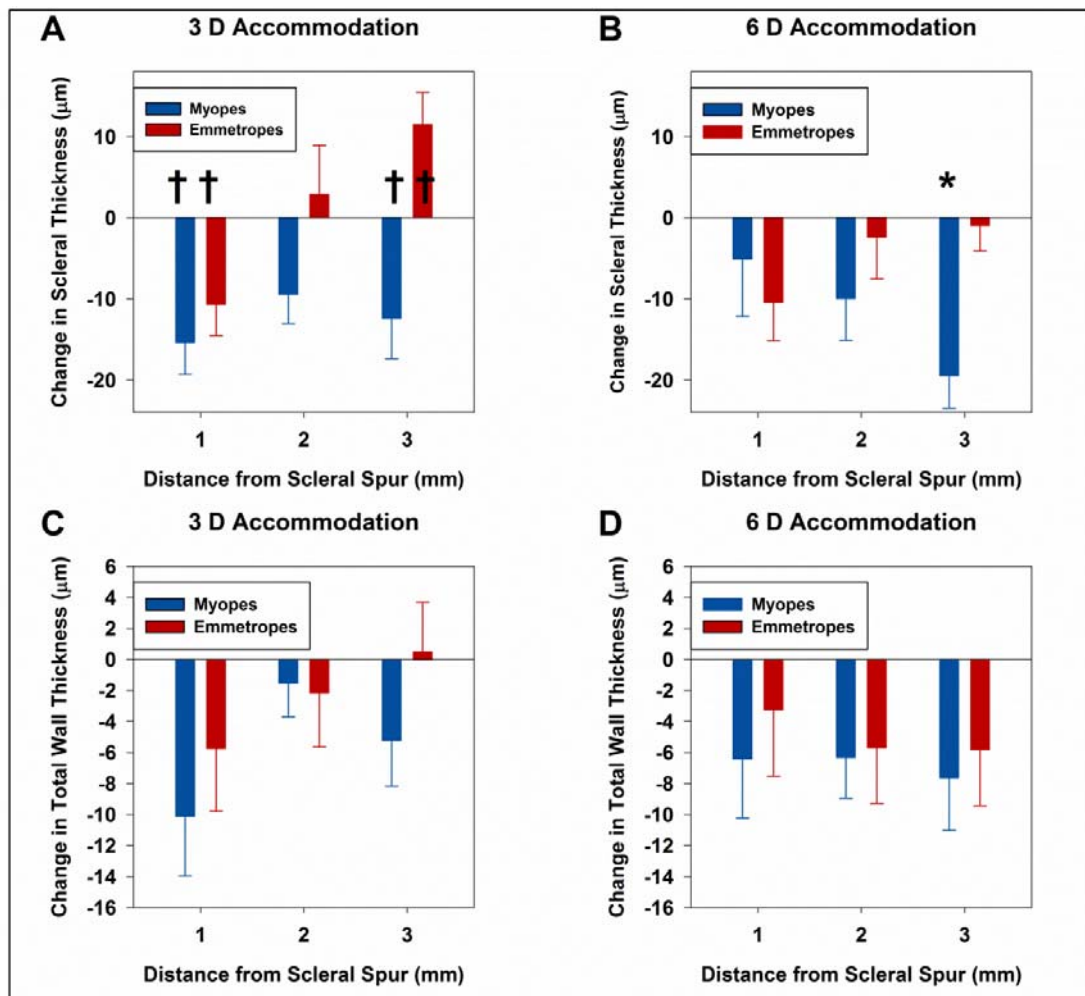
754 Figure 3. Overview of the OCT image analysis procedures used to determine scleral
 755 thickness and total wall thickness. For simplicity, the 6 D scan analysis procedure has been
 756 omitted. A) Three common anatomical landmarks were chosen on the 0 D (baseline) en-
 757 face image and subsequent 3 D and 6 D scans in order to rotationally and translationally
 758 align the scans with the baseline image. B) The scan line corresponding to the position of

759 the central line scan of the baseline (0 D) en-face image was identified in the 3 D scan (red
760 line). C) The 5 central line scans in the 0 D scan (and corresponding line scans in the 3 D
761 scan) were then analysed to segment the anterior conjunctival boundary (red), anterior
762 scleral boundary (blue), and posterior scleral boundary (green). A vertical reference line
763 (shown by the solid white line) was placed to mark the position of the scleral spur (SS) in
764 each image. D) Data obtained via the segmentation of the 5 central line scans was then
765 used to derive scleral thickness volume maps for each subject at each accommodation
766 demand. These thickness volume maps were then rotated to align precisely with the
767 baseline (0 D) map, and a thickness profile from the corresponding location of the 0 D
768 central line, in each of the final rotated thickness volumes was extracted and analysed.
769 Scleral thickness measurements were then taken at discrete points 1, 2 and 3 mm posterior
770 to the scleral spur (white dotted lines).



771

772 Figure 4. Change in average scleral thickness and total wall thickness (mean \pm SEM, μm)
 773 from baseline for all subjects to the 3 D and 6 D stimulus. Data points marked with a cross
 774 (\dagger) indicate a significant change from baseline ($p < 0.05$). Negative values indicate a
 775 thinning with accommodation.



777

778 Figure 5. Change in scleral thickness (A and B) (mean \pm SEM, μm) and change in total wall
 779 thickness (C and D) (mean \pm SEM, μm) from baseline at points 1, 2 and 3 mm posterior to
 780 the scleral spur in the myopic ($n = 18$) and emmetropic ($n = 15$) subjects, to the 3 D (A and
 781 C) and 6 D (B and D) stimulus. Data points marked with a cross (†) indicate a significant
 782 change from baseline ($p < 0.05$), and those marked with an asterisk (*) indicate a highly
 783 significant change from baseline ($p < 0.001$). Negative values indicate a thinning with
 784 accommodation, and positive values indicate a thickening.

785