

1 **A program to analyse optical coherence tomography images of the ciliary muscle**

2 Deborah S. Laughton¹, Benjamin J. Coldrick^{1,2}, Amy L. Sheppard¹ & Leon N. Davies¹

3 ¹Ophthalmic Research Group, Life & Health Sciences, Aston University, Birmingham, UK

4 ²Biomedical Engineering, Life & Health Sciences, Aston University, Birmingham, UK

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6 **Corresponding author**

7 Leon N Davies PhD, Ophthalmic Research Group, Life & Health Sciences, Aston University,

8 Birmingham, B4 7ET, UK

9 Tel: +44(0)121 204 4152

10 Fax: +44 (0) 121 204 4048

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20

21 **Abstract**

22 **Purpose**

23 To describe and validate bespoke software designed to extract morphometric data from ciliary
24 muscle Visante Anterior Segment Optical Coherence Tomography (AS-OCT) images.

25 **Method**

26 Initially, to ensure the software was capable of appropriately applying tiered refractive index
27 corrections and accurately measuring orthogonal and oblique parameters, 5 sets of custom-made
28 rigid gas-permeable lenses aligned to simulate the sclera and ciliary muscle were imaged by the
29 Visante AS-OCT and were analysed by the software. Human temporal ciliary muscle data from 50
30 participants extracted via the internal Visante AS-OCT caliper method and the software were
31 compared. The repeatability of the software was also investigated by imaging the temporal ciliary
32 muscle of 10 participants on 2 occasions.

33 **Results**

34 The mean difference between the software and the absolute thickness measurements of the rigid
35 gas-permeable lenses were not statistically significantly different from 0 ($t=-1.458$, $p=0.151$). Good
36 correspondence was observed between human ciliary muscle measurements obtained by the
37 software and the internal Visante AS-OCT calipers (maximum thickness $t=-0.864$, $p=0.392$, total
38 length $t=0.860$, $p=0.394$). The software extracted highly repeatable ciliary muscle measurements
39 (variability $\leq 6\%$ of mean value).

40 **Conclusion**

41 The bespoke software is capable of extracting accurate and repeatable ciliary muscle measurements
42 and is suitable for analysing large data sets.

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46 **Introduction**

47 Despite the involvement of the ciliary muscle in accommodation [1-3], presbyopia [4-5], and possibly
48 myopia development [6-9], there is a relative paucity of *in vivo* ciliary muscle research. Indeed,
49 imaging the ciliary muscle *in vivo* represents a significant challenge due to the obscured position of
50 the ciliary muscle behind the highly pigmented iris.

51 Traditionally, ultrasound biomicroscopy (UBM) has been utilised to acquire *in vivo* images of the
52 ciliary muscle [10-13]. However, sharper image definition obtained with Anterior Segment Optical
53 Coherence Tomography (AS-OCT) permits superior localisation of the scleral spur (a key reference
54 point for ciliary muscle measurements), compared to UBM images [13]. The axial resolution is 8 μm
55 and 25 μm for the Visante AS-OCT (Carl Zeiss Meditec, California, USA) in high resolution corneal
56 mode and the P40 UBM (Paradigm Medical Industries, Utah, USA) at 50 MHz [13], respectively.
57 Additionally, UBM necessitates supine posture, topical anaesthetic, coupling agents and
58 contralateral eye fixation, whereas the Visante AS-OCT permits non-contact ipsilateral imaging of the
59 fixating eye whilst the patient is sitting up-right, which affords enhanced patient comfort and
60 feasibly permits paediatric assessment [2,6]. Therefore more recent research has progressed to use
61 AS-OCT devices rather than UBM to image the ciliary muscle *in vivo* [1-6, 8-9].

62 Similarly to UBM devices, in-built Visante AS-OCT software allows calipers to be super-imposed onto
63 acquired images to extract measurements. During image analysis, the Visante AS-OCT internal
64 software outlines the boundaries of the ocular media and applies corrective refractive indices (n) to
65 improve measurement accuracy ($n=1.000$ anterior to the cornea, $n=1.338$ to the cornea, $n=1.343$
66 posterior to the cornea). However, the Visante AS-OCT also fits the same refractive index
67 adjustments to ciliary muscle images, with no option to alter the magnitude of the tiered refractive
68 index corrections. Therefore, previous authors have applied a refractive index of 1.000 to the entire
69 ciliary muscle image [1,4,6]. To provide data more closely associated with physiological *in vivo* ciliary
70 muscle parameters, Sheppard and Davies [1,4] adjusted their ciliary muscle caliper measurements to

71 account for a refractive index of 1.382, which is the best estimate of the refractive index of the
72 ciliary muscle based on *in vitro* bovine muscle tissue studies using confocal microscopy [14] and *in*
73 *vitro* human ventricular muscle studies using OCT [15]. However, the refractive indices of the
74 overlying sclera, as well as the ciliary muscle itself, need to be compensated for to ensure the
75 magnitude of the measured ciliary muscle parameters are as accurate as possible. Furthermore, the
76 ciliary muscle tissue is not accurately represented by the straight lines of the calipers because the
77 scleral and ciliary muscle tissues are curved, to varying degrees in different patients [16]. Therefore,
78 to improve the accuracy of morphological assessment, data have been exported for analysis with
79 external software [16].

80 Due to the lack of uniformity of the ciliary muscle outline in Visante AS-OCT images, Kao and
81 colleagues' [16] software required manual localisation of the scleral spur before automated image
82 analysis commenced. Once the sclera and ciliary muscle had been outlined, refractive indices of 1.41
83 and 1.38 were applied across the y-axis of the scleral and ciliary muscle image sections, respectively.
84 The software produced vertical thickness measures at 1, 2 and 3 mm behind the scleral spur,
85 maximum thickness and measured the cross-sectional area of the anterior ciliary body. However, the
86 edge detection algorithms appeared to incorporate both the ciliary muscle and the pigmented ciliary
87 epithelium, which may overestimate ciliary muscle measurements. Furthermore, measurements of
88 ciliary muscle length were not obtained.

89 Despite the Visante AS-OCT's use in previous morphometric studies of the ciliary muscle, the
90 instrument remains susceptible to optical and instrument distortions, and has limited inbuilt
91 capabilities to quantify ciliary muscle parameters. Consequently, to overcome the limitations of
92 previously designed software [16] and to address concerns of the subjectivity of identifying the
93 posterior end point of the ciliary muscle [17], bespoke software was developed. The aim of this
94 study was to describe and validate the bespoke software and to compare data extracted by the
95 software and the internal Visante AS-OCT calipers.

96 **Method**

97 The study was approved by the Aston University Research Ethics Committee and was conducted in
98 accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all
99 participants after explanation of the nature and possible consequences of the study. One UK
100 registered optometrist (DL) acquired and extracted all the human data.

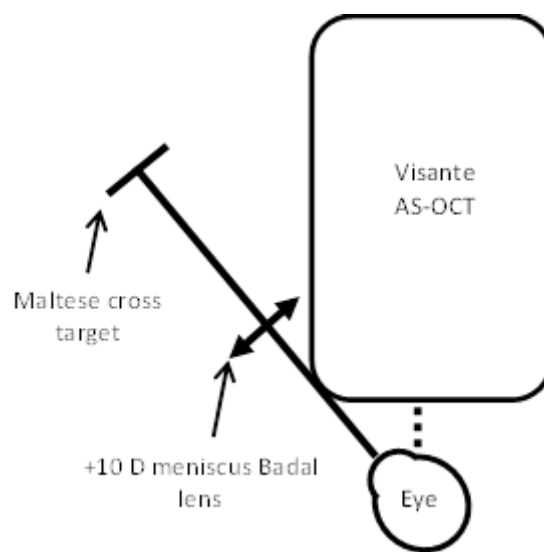
101 ***Ciliary muscle image acquisition***

102 It is likely that the repeatability of nasal ciliary muscle image analysis is superior to temporal ciliary
103 muscle image analysis because the scleral spur is more easily discernible nasally [18], therefore only
104 temporal ciliary muscle images were analysed in the present study.

105 Each participant wore an eye patch over their left eye throughout data collection. Participants were
106 asked to place their chin and forehead against the Visante AS-OCT supports and fixate straight-
107 ahead at the centre of the internal star target. The chin rest was adjusted to align the participant's
108 right eye to allow visualisation of the anterior crystalline lens surface, which was guided by the real-
109 time Visante AS-OCT video stream of the external eye. High resolution corneal mode was selected
110 (scanning an area of 10 mm in width and 3 mm in depth) and participants were aligned to ensure the
111 vertical white fixation line was visible through the centre of the image, which indicated the
112 measurement beam was coincident with the optical axis of the eye [19].

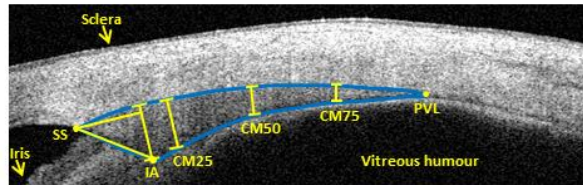
113 In order to image the full length of the ciliary muscle using the Visante AS-OCT, patients must avert
114 their gaze to a point external to the central viewing window because the iris blocks visualisation of
115 the ciliary muscle in primary gaze. Fig. 1 illustrates the bespoke Badal lens system with a moveable
116 Maltese cross target, attached to the forehead rest of the Visante AS-OCT to provide a steady
117 peripheral fixation target and to correct for ametropia. The minimum level of horizontal eye
118 movement required to ensure the peripheral target is unobstructed by the instrument casing is 40°
119 from the internal Visante AS-OCT star target. Fixating externally causes the Visante AS-OCT beam to

120 be directed through the sclera, rather than the cornea, reducing optical distortion due to the flatter
121 scleral plane. Since all participants were aligned to the optical axis of the Visante AS-OCT in primary
122 position, only minor vertical alignment adjustments were required once the participant adducted
123 their right eye to view the centre of the external Maltese cross. Horizontal alignment was
124 determined by the real-time Visante AS-OCT video stream of the OCT image, which was adjusted to
125 ensure simultaneous visualisation of the scleral spur and ciliary muscle posterior visible limit, as
126 depicted in Fig. 2.



127

128 **Fig. 1.** Schematic diagram of the Visante AS-OCT with bespoke Badal lens system attachment. The
129 dashed line represents the path of the OCT beam through the sclera. The Maltese cross target is
130 positioned 10 cm from the Badal lens in order to stimulate 0.00 D of accommodation in an
131 emmetropic patient.



132

133 **Fig. 2.** Visante AS-OCT image of a human ciliary muscle section. The ciliary muscle is outlined in blue
 134 with superimposed yellow caliper measurements. PVL = posterior visible limit; SS = scleral spur; IA =
 135 inner apex; CM25, CM50, CM75 = thickness at 25%, 50% and 75% of curved total length (SS to PVL);
 136 maximum thickness = perpendicular distance from IA to sclera; anterior length = perpendicular
 137 distance from line of maximum thickness to SS. The pigmented epithelium is visible underneath the
 138 inner apex and the inferior ciliary muscle border.

139 Once accurately aligned, the Maltese cross target was moved to provide a 0.00 D accommodative
 140 stimulus for each participant. Participants were asked to focus on the centre of the Maltese cross
 141 target and keep it as clear as possible throughout data collection, whilst also keeping their head and
 142 eyes as still as possible. Three consecutive images of the right eye temporal ciliary muscle were
 143 acquired and saved.

144 **Software design**

145 The preparation of ciliary muscle images for analysis was similar to the process used by Kao *et al.*
146 [16]; all images acquired in high resolution corneal mode were exported in raw DICOM (Digital
147 Imaging and Communications in Medicine) form (n=1.00) and were imported to Matlab R2012b (The
148 MathWorks Inc., Massachusetts, USA) and subsequently resized to 512 x 1280 pixels, matching the
149 correct aspect ratio. The images were not cropped or reduced in size for processing.

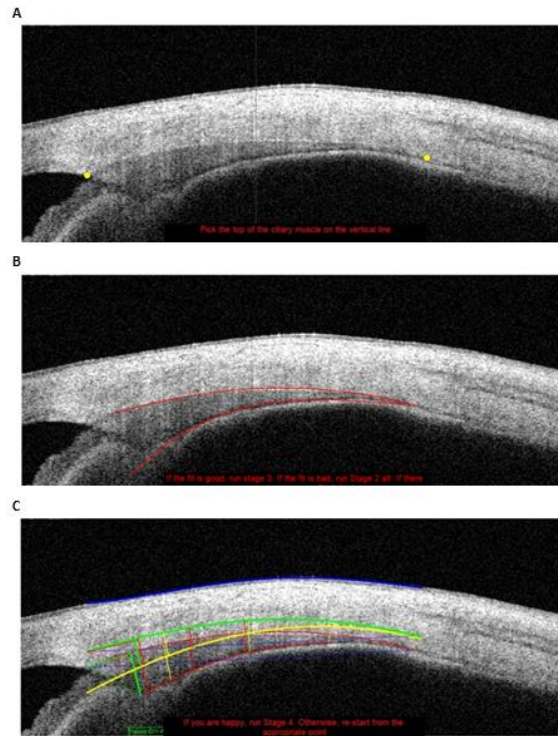
150 Due to the difficulties in localising the outline of the ciliary muscle, the bespoke software required
151 multiple landmarks to be manually selected before extracting data. Initially, the scleral spur and a
152 point beyond the posterior visible limit were selected manually (highlighted by yellow dots in Fig.
153 3A). The software then calculates the distance between these two markers and superimposes a
154 vertical line midway, prompting the user to pick the points where the scleral/ciliary muscle and
155 ciliary muscle/pigmented ciliary epithelium boundaries bisect the line. These initial steps identify the
156 area of interest to the software, which then superimposes a block of 10 vertical lines spaced at 1
157 pixel intervals every 0.5 mm between the scleral spur and posterior point chosen. The change in
158 pixel intensity along each line is determined. To define the ciliary muscle border, a 2nd order Fourier
159 series is fitted to the intensity profile and differentiated. The location of the largest peak formed in
160 the differentiated intensity profile corresponds to the point where the line bisects the ciliary muscle
161 boundary (the crossing point). This process is repeated for the top and bottom of each line
162 separately in order to determine crossing points of both the superior and inferior ciliary muscle
163 boundaries on the OCT image. A 2nd order polynomial curve is fitted to the crossing points of each
164 boundary.

165 The ciliary muscle/pigmented ciliary epithelium border is not as easily discriminated as the
166 scleral/ciliary muscle border, therefore the software provides an option to manually pick three
167 points along the boundary to improve the fit of the curve, if the automated fit is not satisfactory (Fig.
168 3B). Due to relatively poor image clarity around the inner apex of the ciliary muscle, the inner apex

169 must also be selected manually. Once the fit of the curves to the ciliary muscle borders has been
170 finalised, the OCT image is converted to a binary image in order for internal MatLab edge detection
171 algorithms to identify the air/scleral boundary and fit a 2nd order polynomial curve to it. During
172 software development and testing, it was determined that 2nd order curves accurately and
173 satisfactorily fitted the contour of the ciliary muscle and sclera in all patients tested.

174 Subsequently, the software applies a tiered refractive index correction to the scleral and ciliary
175 muscle tissue (1.41 and 1.38, respectively), as shown in Fig. 3C by the higher yellow and green
176 curves. The posterior visible limit of the ciliary muscle is identified as the point where the curves
177 fitted to the ciliary muscle borders reach minimum separation posteriorly. The software exports the
178 Straight-line TL (straight-line distance between the scleral spur and posterior visible limit), Curved TL
179 (ciliary muscle total length measured along the scleral/ciliary muscle boundary), Max T (maximum
180 thickness; see Fig. 2), Ant L (anterior length measured perpendicularly from the line of maximum
181 thickness to the scleral spur), SS-IA (distance between scleral spur to the inner apex), CM2 (thickness
182 measured 2 mm from the scleral spur along the scleral curve), CM25 (thickness measured at 25% of
183 the total curved length of the ciliary muscle), CM50 (thickness measured at 50% of the total curved
184 length of the ciliary muscle) and CM75 (thickness measured at 75% of the total curved length of the
185 ciliary muscle), directly to an Excel spreadsheet, allowing the examiner to be masked to the results.

186 Due to the uncertainties of measuring thickness at fixed distances from the scleral spur, which is
187 likely to represent a different anatomical area of the ciliary muscle between subjects, CM1
188 (thickness measured 1 mm behind the scleral spur along the scleral curve) and CM3 (thickness
189 measured 3 mm behind the scleral spur along the scleral curve) were not quantified. However, CM2
190 was included due to the hypothesis this area may act as a fulcrum point during accommodation,
191 where the net change in thickness is negligible [2].



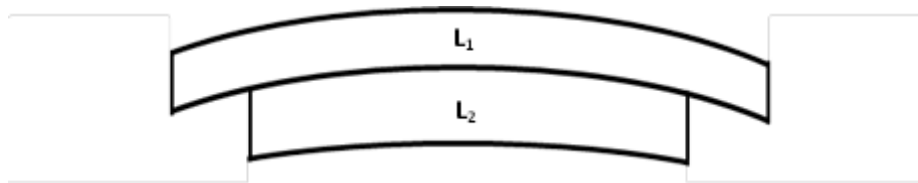
192

193 **Fig 3.** A) Screenshot from the software after the scleral spur and a point beyond the posterior visible
 194 limit (yellow dots) have been clicked on. The user is required to select where the top and bottom
 195 ciliary muscle boundaries are bisected by the superimposed vertical line. B) The software outlines
 196 the boundaries of the ciliary muscle and gives the option to manually redefine the lower curve. C)
 197 After manually selecting the inner apex the software extracts the ciliary muscle data, correcting for
 198 the refractive indices of the sclera and ciliary muscle (higher yellow and green curves) and transfers
 199 the data to an Excel document.

200 ***Software analysis of artificial ciliary muscle sections***

201 To ensure the software was capable of appropriately applying refractive index corrections and
 202 accurately measuring orthogonal and oblique parameters, a series of custom-made rigid gas-
 203 permeable lenses (No. 7 Contact Lens Laboratory Ltd, Hastings, UK) of known dimensions were
 204 imaged by the Visante AS-OCT. One silicon-acrylate lens ($n = 1.48$) simulated the sclera (L_1 in Fig. 4)

205 and 5 fluoro-polymer lenses ($n=1.44$) of varying thickness (0.3, 0.45, 0.6, 0.75, 0.9 mm) each
206 simulated the ciliary muscle (L_2 in Fig. 4). Each of the ciliary muscle lenses were of constant
207 thickness. The total diameter of the sclera lens was 10 mm and each ciliary muscle lens was 6 mm.
208 The radius of curvature of the lenses was 12 mm. A ciliary muscle lens and the sclera lens were
209 positioned together, as shown in Fig. 4, for image acquisition. For each of the 5 ciliary muscle and
210 sclera lens combinations, 10 OCT images were acquired and exported as raw data.



211

212 **Fig. 4.** Schematic diagram of two rigid gas-permeable lenses designed to simulate the sclera (L_1) and
213 the ciliary muscle (L_2).

214 For this exercise only, the ciliary muscle software was adapted to allow 3 points across each of the
215 lens boundaries to be manually selected for subsequent automated polynomial curve (order 2)
216 fitting. The software determines the length of the ciliary muscle lens as the straight-line distance
217 between the first and third points plotted across the scleral/ciliary muscle boundary, therefore the
218 edges of the lens were manually selected. The thicknesses of the ciliary muscle lenses were
219 measured at 25 (CM25), 50 (CM50) and 75% (CM75) across the lens diameter. Each thickness
220 measurement was measured perpendicular to the scleral/ciliary muscle boundary curve. The
221 refractive indices of the scleral and ciliary muscle lenses (1.48 and 1.44, respectively) were inputted
222 to allow the program to compensate the measurements. All measurements were exported directly
223 to an Excel spreadsheet, masking the examiner to the results during data extraction. The diameter
224 and thickness measurements of the ciliary muscle lenses were also measured 10 times by Vernier
225 calipers and compared to the results produced by the software.

226 The bias of the measurements was calculated from the mean difference between the two
227 techniques. A paired t-test was used to determine whether the bias was significantly different from

228 0. The spread over which 95% of the data lie (limits of agreement, LoA) was calculated and the
229 agreement of the software and Vernier calipers was analysed using a Bland-Altman plot [20].

230 ***Software and Visante AS-OCT caliper agreement***

231 Human ciliary muscle parameters acquired by the software were compared to those acquired via the
232 traditional method of ciliary muscle Visante AS-OCT image analysis: internal Visante AS-OCT calipers.
233 Disaccommodated temporal ciliary muscle images of 50 patients (mean age 39.11 ± 3.18 years;
234 mean spherical equivalent -1.17 ± 2.09 D, range -7.07 to $+0.49$ D; mean astigmatism -0.59 ± 0.57 D)
235 were acquired and data were subsequently extracted by the software and the Visante AS-OCT
236 calipers on separate occasions.

237 The software was designed to measure the ciliary muscle thickness with reference to the curved
238 ciliary muscle length (following the contour of the scleral/ciliary muscle border), whereas the Visante
239 AS-OCT calipers cut across the ciliary muscle to measure its thickness at horizontal distances from
240 the scleral spur. Therefore, the calipers are likely to underestimate the thickness measurements in a
241 more curved ciliary muscle [6,16]. Due to the aforementioned differences in the origin of thickness
242 measurements from the scleral spur, only the Straight-line TL and Max T measurements were
243 compared between the two methods. The software Straight-line TL and the software Curved TL
244 values were also compared.

245 Internal Visante AS-OCT caliper measurements were acquired by applying a refractive index of 1.00
246 to the entire image and superimposing calipers on to the ciliary muscle image to extract Straight-line
247 TL and Max T measurements. For comparison with the internal Visante AS-OCT caliper
248 measurements, the software was adapted to export the raw ciliary muscle measurements with a
249 refractive index of 1.00 applied to the entire image.

250 The bias was calculated from the mean difference between the techniques and a paired t-test was
251 used to determine whether the bias was significantly different from 0. The agreement of the

252 techniques was also analysed using Bland-Altman plots. The difference between the software
253 Straight-line TL and the software Curved TL values was analysed using a paired t-test.

254 ***Repeatability of software analysis of human ciliary muscle***

255 Additionally, the repeatability of human ciliary muscle OCT imaging and software interpretation was
256 determined by inviting 10 participants to return for ciliary muscle imaging on a separate occasion,
257 less than 1 week after their first appointment. A further three ciliary muscle images were acquired
258 and analysed.

259 In order to determine the errors arising from patient alignment and software interpretation, the
260 ciliary muscle of a single patient's right eye was imaged 10 times at 0.00 D accommodative stimulus
261 during one appointment. The patient was asked to remove and reposition their chin and forehead
262 between the acquisition of each image. In order to isolate the repeatability of the software
263 interpretation and analysis of a ciliary muscle image, 1 image was analysed 10 times.

264 The bias of each parameter was calculated from the mean difference between visits. A paired t-test
265 was used to determine whether the bias was significantly different from 0.

266 **Results**

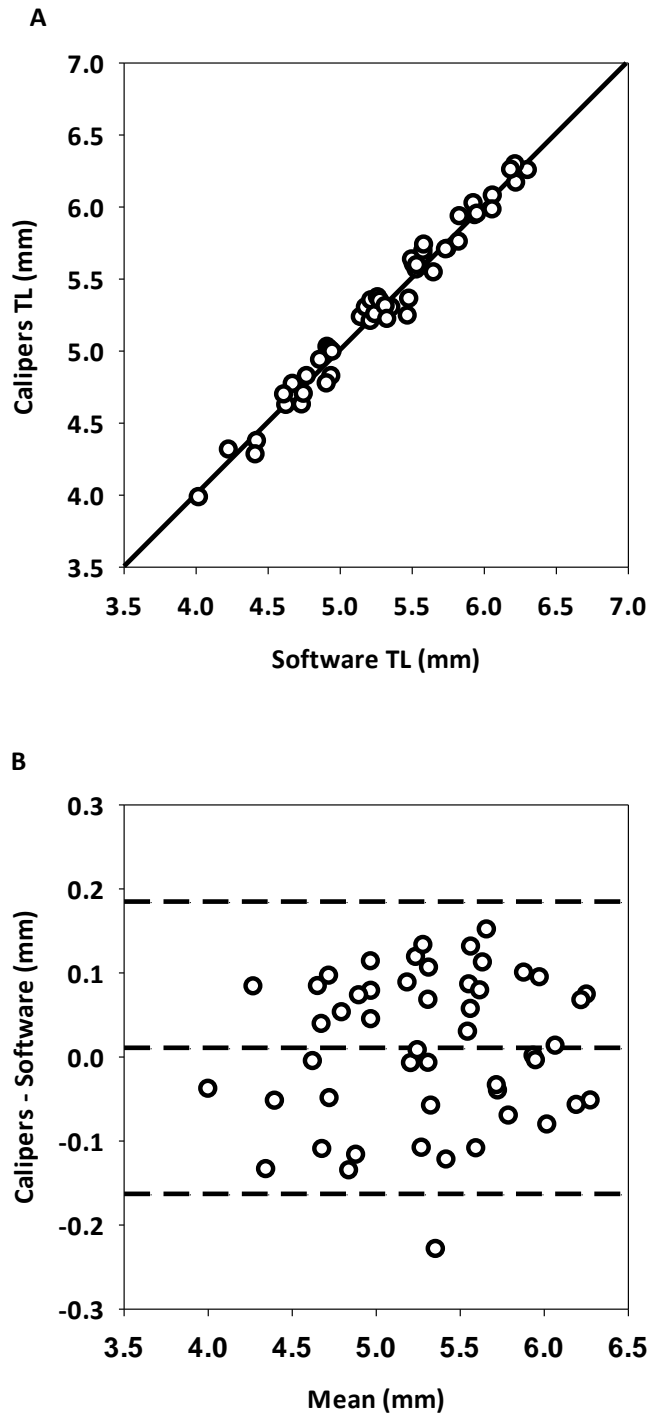
267 ***Artificial ciliary muscle sections***

268 The mean difference between the software and the Vernier caliper measurements are displayed in
269 Table 1. The bias of CM25, CM50 and CM75 were not significantly different from 0. The bias was not
270 correlated with the magnitude of the measurement. However the total diameter was significantly
271 underestimated by the software ($p=0.001$).

272 ***Software and caliper agreement***

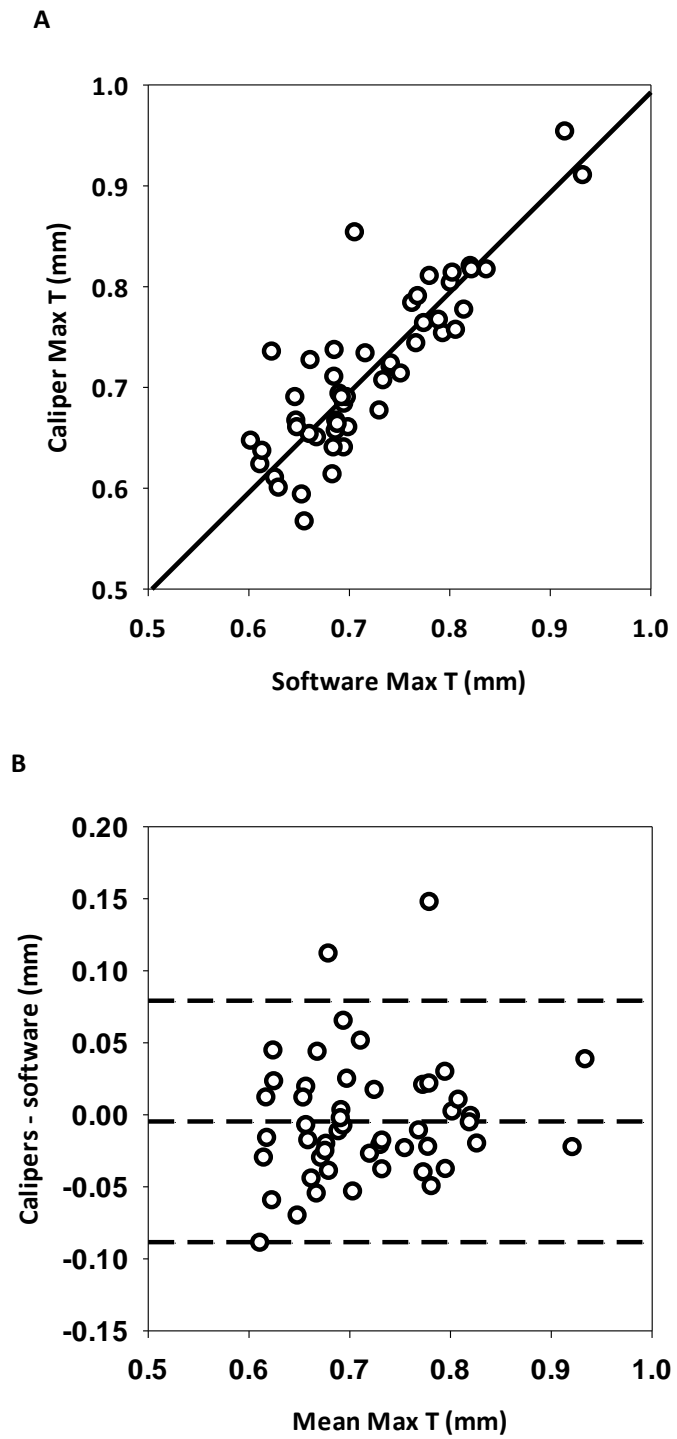
273 The mean difference between the software and the Visante AS-OCT internal caliper total Straight-
274 line TL and Max T measurements are displayed in Table 2. The bias of each parameter was not
275 significantly different from 0. The data are displayed graphically with Bland-Altman plots in Figs 5

276 and 6, which show the bias was not correlated to the magnitude of the measurement. The mean
277 software Curved TL measurements (5.391 ± 0.571 mm) were significantly longer than the mean
278 software Straight-line TL measurements (5.301 ± 0.560 mm; $t=-23.356$, $p<0.001$).



279
280 **Fig. 5. A.** Total straight-line length measured by the software and the internal Visante AS-OCT
281 calipers. Regression line $y=0.132+0.973x$, $R^2=0.876$, $p<0.001$. **B.** Bland-Altman plot of the agreement
282 between total straight-line length measured by the software and the internal Visante AS-OCT

283 calipers. Regression line $y=-0.068+0.015x$, $R^2=0.009$, $p=0.514$. The dashed lines represent the limits
284 of agreement.



285
286 **Fig. 6. A** Maximum thickness measured by the software and the internal Visante AS-OCT calipers.
287 Regression line $y=0.152+0.794x$, $R^2=0.732$, $p<0.001$. **B.** Bland-Altman difference versus mean plot of
288 the agreement between maximum thickness measured by the software and the internal Visante AS-

289 OCT calipers. Regression line $y=-0.063+0.081x$, $R^2=0.021$, $p=0.317$. The dashed lines represent the
290 limits of agreement.

291

292 **Repeatability**

293 The bias of ciliary muscle parameters measured in 10 patients across 2 visits is displayed in Table 3.
294 The bias represented $\leq 6\%$ of the mean value of each parameter and was not significantly different
295 from 0. Tables 4 and 5 obtained from 1 patient realigned 10 times and 1 image analysed 10 times,
296 respectively, suggest approximately 60% of the difference encountered between visits is likely to be
297 due to the inherent variability associated with manually selecting points for analysis with the
298 bespoke software.

299 **Discussion**

300 The software described here is capable of accurately outlining the ciliary muscle, applying
301 appropriate refractive index corrections and extracting a variety of repeatable orthogonal and
302 oblique ciliary muscle parameters, thus verifying its suitability for *in vivo* ciliary muscle analysis.
303 Compared to the Visante AS-OCT calipers, the software enables more accurate measurements of the
304 curved ciliary muscle tissue to be acquired by following the scleral/ciliary muscle contour, rather
305 than cutting horizontally across the ciliary muscle to measure thicknesses with respect to the
306 distance from scleral spur. Image analysis can also be performed remotely to the Visante AS-OCT
307 device on an external computer. As with the Visante AS-OCT's calipers, the bespoke software is not
308 fully automated and requires user input at various stages of the analysis.

309 The software raw parameters (n=1.00) compared favourably to internal Visante AS-OCT caliper
310 Straight-line TL and Max T measurements, suggesting the location of the posterior visible limit,
311 utilised in the total length measurement, is not only evident across a large sample of patients, but
312 can also be consistently identified subjectively and objectively. Nevertheless, the concerns of
313 previous authors over the visibility of the posterior end point of the ciliary muscle are not entirely

314 unfounded [17]; extensive analysis of ciliary muscle images during software development has shown
315 there is large intersubject variability in the visibility of the posterior limit of the ciliary muscle. In
316 order to simplify localisation for the software, the posterior visible limit was defined as the point
317 where the scleral/ ciliary muscle and ciliary muscle/ pigmented ciliary epithelium contours reached
318 minimum separation posteriorly, which produced highly repeatable results.

319 Orthogonal and oblique thickness measurement accuracy was evidenced by computation of the
320 distance between 2 polynomial curves (order 2) fitted to OCT images of 2 superimposed rigid gas-
321 permeable lenses. As expected, the total diameter measurements were significantly underestimated
322 by the software ($p=0.001$) due to difficulties ensuring the scleral and ciliary muscle lenses were
323 perfectly centred, and that the measurement beam scanned across the centre of both lenses.
324 Nonetheless, the validity of horizontal measurements has been confirmed by the good
325 correspondence between the internal Visante AS-OCT caliper and the software Straight-line TL
326 measurements. The thickness measurements obtained are unaffected by the aforementioned
327 alignment issues because all the ciliary muscle lenses were of constant thickness.

328 The agreement between the ciliary muscle lens thickness values measured by the software and
329 Vernier calipers demonstrates the software can appropriately compensate for tiered refractive index
330 levels and the geometric distortion of the exported image in raw DICOM form is negligible. These
331 findings also support the conclusions of Kao *et al.* [16], who reported images exported from the
332 Visante AS-OCT images are free from geometric distortions and only need to be adjusted for the
333 refractive index of the tissue(s) to be suitable for accurate morphological assessment.

334 The bias and variance of the difference in ciliary muscle parameters extracted from 10 patients on 2
335 separate visits was similar to internal Visante AS-OCT caliper measurement values reported
336 previously [1]. Furthermore, the limits of agreement reported by the software (-0.166 to 0.135 mm)
337 were narrower than for the calipers (-0.228 to 0.193 mm) for the measurement of total straight-line
338 length, suggesting superior repeatability of the localisation of the posterior visible limit by the

339 software. The intersession repeatability of the software developed by Kao and colleagues [16] was
340 not reported.

341 It is unlikely fully-automated software could be developed to analyse the current ciliary muscle
342 images produced by the Visante AS-OCT due to the non-uniformity of the acquired image. A custom-
343 made OCT instrument has been able to obtain sharper definition around the inner apex of the ciliary
344 muscle, however manual selection of key landmarks is still required to initiate image analysis [21].

345 The newly developed software described by the current study extracts valid and repeatable ciliary
346 muscle parameters and serves to reduce the subjectivity of ciliary muscle analysis. The image
347 examiner must be highly trained to extract repeatable results due to the ambiguity of ciliary muscle
348 landmarks in some patients. The software described here also has the capacity to extract a variety of
349 additional measurements to previous software [16], including ciliary muscle length, which is a vital
350 measurement for presbyopia research [16].

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400 **Table 1.** Comparison of parameters obtained from 5 artificial ciliary muscle sections by the software
401 and Vernier calipers. A negative mean difference indicates the software values are larger than the
402 Vernier caliper measurements.

Parameter	Mean difference (mm)	Standard deviation (mm)	Limits of agreement		t	p
			Lower	Upper		
			(mm)	(mm)		
Total diameter	0.046	0.092	-0.135	0.226	3.507	0.001
CM25	-0.001	0.017	-0.034	0.032	-0.427	0.671
CM50	-0.003	0.016	-0.034	0.028	-1.458	0.151
CM75	0.000	0.016	-0.031	0.031	-1.810	0.857

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405 **Table 2.** Comparison of ciliary muscle parameters obtained from 50 patients by the software and
 406 the internal Visante AS-OCT calipers. A negative mean difference indicates the software values are
 407 larger than the caliper measurements.

Parameter	Mean	Standard	Limits of agreement		<i>t</i>	<i>p</i>
	difference	deviation	Lower	Upper		
	(mm)	(mm)	(mm)	(mm)		
Straight-line TL	0.011	0.089	-0.163	0.185	0.860	0.394
Max T	-0.005	0.043	-0.089	0.079	-0.864	0.392

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423 **Table 3.** Intersession repeatability data of ciliary muscle parameters extracted by the software from
 424 2 visits of 10 patients. A negative mean difference indicates the measurement was larger at visit 1.

Parameter	Mean difference (mm)	Standard deviation (mm)	Limits of agreement		t	p
			Lower	Upper		
			(mm)	(mm)		
Straight-line TL	-0.016	0.077	-0.166	0.135	-0.569	0.583
Curved TL	-0.017	0.072	-0.157	0.124	-0.736	0.480
Max T	-0.003	0.022	-0.047	0.040	-0.281	0.785
Ant L	0.011	0.059	-0.104	0.126	0.577	0.578
SS-IA	0.007	0.054	-0.099	0.112	0.354	0.731
CM2	-0.007	0.030	-0.066	0.051	-0.761	0.466
CM25	-0.003	0.018	-0.038	0.033	-0.832	0.427
CM50	0.004	0.023	-0.041	0.050	0.535	0.606
CM75	0.009	0.020	-0.030	0.048	1.335	0.215

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433 **Table 4.** Ciliary muscle parameters extracted by the software from 10 images acquired from one
434 patient who removed and repositioned their head between acquisitions.

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Parameter	Mean (mm)	Standard deviation (mm)
Straight-line TL	5.164	0.068
Curved TL	5.228	0.075
Max T	0.482	0.014
Ant L	1.182	0.043
SS-IA	1.254	0.052
CM2	0.345	0.025
CM25	0.475	0.016
CM50	0.261	0.015
CM75	0.114	0.015

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455 **Table 5.** Ciliary muscle parameters extracted by the software from one image analysed 10 times.

Parameter	Mean (mm)	Standard deviation (mm)
Straight-line TL	5.153	0.043
Curved TL	5.218	0.038
Max T	0.454	0.011
Ant L	1.237	0.021
SS-IA	1.276	0.040
CM2	0.334	0.016
CM25	0.456	0.008
CM50	0.259	0.006
CM75	0.121	0.011