

1 **Title Page**

2 **Title**

3 Quantifying retinal area in ultra-widefield imaging using a 3-dimensional (3-D) printed
4 eye model

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37 **Abstract**

38

39 **Purpose:**

40 We aim to study the effects of different axial lengths on ultra-widefield imaging to
41 determine the presence of distortion in images despite software correction and
42 calculate an enlargement factor based on angular location.

43 **Design:**

44 Experimental image analysis study.

45 **Study objects:**

46 Three 3-dimensional printed model eyes simulating eyes with axial lengths of 22mm,
47 24mm and 26mm. Each model has a grid of rings 9 degrees apart centered at the
48 posterior pole

49 **Methods:**

50 Single centre study performed at the National Institute for Health Research Moorfields
51 Biomedical Research Centre, London, United Kingdom. Each model was imaged using
52 Optos 200TX (Optos, Dunfermline, United Kingdom). Two images for each model
53 eye that were corrected using V2 Vantage Pro software (Optos, United Kingdom)
54 were used for analysis and the average values obtained. Each image inter-ring area
55 was measured using ImageJ to obtain a measured image area in pixel and mm².
56 This was compared with the true calculated object inter-ring area and an
57 enlargement factor was determined.

58 **Main outcome measures:** Measured image inter-ring area in pixels and mm². True
59 calculated object inter-ring area in mm².

60 **Results:**

61 The enlargement factor of the rings gradually increases towards the periphery with
62 factors of 1.4 at 45 degrees and 1.9 at the equator. The axial lengths did not affect
63 the enlargement factor of the rings imaged in three different model eyes, p=0.9512.
64 The anterior equator exhibits a significant distortion despite the software correction.

65 **Conclusion:**

66 The enlargement factor is dependent on angular location and not axial length. The
67 enlargement factors can be used in clinical practice to more accurately measure
68 area in ultra-widefield imaging.

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75 **Text**

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77 **Introduction**

78 The evolution of ultra-widefield imaging over the past decade has redefined the
79 evaluation and management of retinal diseases. The implications of visualising a
80 wider view of the retina on our understanding of disease mechanisms is significant.¹
81 We have acquired new knowledge on the impact of peripherally located lesions on
82 the severity of diabetic retinopathy as well as the increasing importance of ultra-
83 widefield imaging in assessing diabetic retinopathy.^{2,3} Wide-angled retinal imaging
84 has also enabled us to ascertain the extent of peripheral retinal ischemia in retinal
85 vein occlusions and it was also instrumental in identifying peripheral changes in
86 uveitis and age related macular degeneration.⁴⁻⁶

87 It is believed that using an elliptical mirror with a focal point at the plane of the iris,
88 the Optos ultra-widefield system (Optos 200TX; Optos, Dunfermline, United
89 Kingdom) can potentially view 200 degrees of the retina in a single capture, at least
90 three times more than the view obtained with montaged 7-field standard fundus
91 images.⁷

92 Despite marked progress in the field, care needs to be taken to assess the quality
93 and reliability of the images obtained. So far, three unique observations have been
94 made. Firstly, obtaining a wide view of the three-dimensional retina and displaying
95 the image obtained in a flat two dimensional image causes a projection distortion of
96 the ultra-widefield image produced.⁴ Secondly, a horizontal stretch over the entire
97 image that magnifies into the periphery has also been reported in uncorrected
98 images.^{8,9} Finally, the impact of different axial lengths on the images produced can
99 vary the imaged size by almost 10%.¹⁰

100 The ultra-widefield system is unique and still evolving, and it is important to rectify
101 these flaws to realise the full potential of this system. Significant efforts have been
102 made to measure and quantify area in ultra-widefield images. Precise quantification
103 of area is challenging and the concept of a pixel ratio was used for the ischemic
104 index while comparisons to disc area used in the concentric rings method.^{11,12}
105 Spaide et al suggested an azimuthal projection technique and the Optos software
106 now incorporates its own stereographic projection software to correct the peripheral
107 distortion and the horizontal stretch.^{4,13} The stereographic projection software has
108 been studied and although the ischemic index in the corrected images are
109 comparable with uncorrected images, the variation can be as high as 14.8%.¹⁴

110 Acknowledging that the Optos system can view up to 200 degrees in a single image,
111 20 degrees of the anterior equatorial retina will also be included in the image. It is
112 perfectly reasonable to assume that a hemisphere will have predictable projection
113 errors. However, if we place a set of annular rings in a sphere, the equator will have
114 the largest area and the area of the annulus anterior and posterior to the equator will
115 be smaller. This can be explained using the spherical cap formula on a sphere with a
116 radius of 11mm. The area for three annulus subtending 10° such as 40-50°, 80-90°
117 and 130-140°, which represents an annulus straddling 45°, 90° and 135° can be
118 calculated. The area for these are 93.7mm², 132.5mm² and 93.7mm². The area
119 increases from the posterior pole towards the equator and subsequently decreases
120 towards to anterior pole. This is particularly important when measuring area beyond

121 the equator as the size decreases although projection artefacts are likely to
122 increasingly distort the images.

123 The primary aim of this study was to utilise a 3-D printed eye model to study if there
124 remains a distortion in the image produced and if so, suggest ways to rectify them to
125 enable quantification of lesion dimensions accurately. We also intend to study the
126 effects of axial length on the image produced and the concept of an anterior equator
127 distortion by studying the enlargement factor based on the angular location in the
128 image.

129

130 **Methods**

131 This image analysis study was performed in the National Institute for Health
132 Research Moorfields Biomedical Research Centre and University College London
133 Institute of Ophthalmology, London, United Kingdom.

134 *Model eye*

135 Three model eyes of different axial lengths were developed and 3D printed by
136 3DPrintUK, London using an EOS P100 (EOS Ltd, Germany) with material from
137 Nylon PA2200. The models were spheres with an 8mm aperture simulating the pupil.
138 The thickness of the model eye wall is 2mm. A sulcus was created to accommodate
139 a three piece +21.0 Dioptre intraocular lens. Therefore, the position of the lens would
140 simulate a lens positioned at the sulcus. The lens used was the Acrysof multi-piece
141 MA60AC (Alcon, Texas, USA) with a 6.0mm optic and a reported spherical
142 aberration of +0.14 +/- 0.09 μ m.¹⁵ A grid composed of multiple concentric rings
143 centred at the posterior pole were made for each model eye. The grooves are
144 0.4mm in width. Each ring is 9 degrees apart, beginning in the posterior pole and
145 extending to the 'pupil' or aperture. These model eyes consist of three different
146 internal diameters and thus simulating three different axial lengths, 22mm, 24mm
147 and 26mm. Figure 1 is an example of the design for a model eye with an axial length
148 of 24mm. The true object area of each ring which is the inter-ring area including the
149 grooves can be calculated using the known dimensions by applying the spherical
150 cap formula. As the rings are positioned 9 degrees apart, the area of ring 5 for
151 example, which is located between 36 and 45 degrees from the posterior pole, has a
152 larger true object area in the 26mm model eye than the 22mm model eye

153 *Image Acquisition*

154 Each model eye was imaged using the Optos 200TX (Optos, Dunfermline, United
155 Kingdom). The model eyes were positioned at the imaging area and the 'green in-
156 focus' light was obtained prior to obtaining a central image. Each image output is
157 automatically corrected for three-dimension to two-dimension projection errors by the
158 V2 Vantage Pro software (Optos, Dunfermline, United Kingdom) which utilises
159 stereographic projection techniques.

160 *Image Analysis*

161 Two central images of each model eye resulting in a total of six images were used
162 for analysis. The grid was traced using Photoshop CS2 (Adobe, San Jose, USA).
163 The measurements were made at the outer boundary of each groove as this is better
164 delineated. The pixel area of each ring area were measured using the magic wand

165 tool in Image J.¹³ This is exhibited in Figure 2. The true calculated area for the
166 central circle is divided by the measured pixel area to obtain the equivalent area for
167 each pixel. The central circle at the posterior pole was used as a reference as the
168 distortion at zero degrees is minimal.^{8,16} The measured image area was then
169 determined by multiplying the measured pixel area with the equivalent area for each
170 pixel. The images obtained were divided into four quadrants, superior, right, inferior
171 and left. The superior and inferior quadrants represent the vertical component and
172 the right and left quadrants represent the horizontal component. The average
173 measurements of the quadrants were obtained from two images of each model eye
174 using ImageJ.

175

176 ***Image Enlargement Factor***

177 The average measured image area of each ring from the two images for each axial
178 length were divided by the true calculated area to obtain an enlargement factor. This
179 was performed for each respective model eye. The enlargement factor obtained for
180 each ring in each model eye was used to assess if distortion is still present in
181 corrected ultra-widefield images. The enlargement factor was also calculated using
182 the same method for the vertical and horizontal component of each inter-ring area for
183 the three different model eyes.

184 ***Influence of axial lengths***

185 The measured image pixel area of each ring for each model eye were plotted against
186 the degrees from the posterior pole. This was repeated with the true calculated area
187 for each ring for each model eye of different axial lengths. This was done to
188 understand the effects axial length and angular location has on peripheral distortion.

189 ***Anterior equator distortion***

190 To determine if the anterior equator distortion is present, the measured image pixel
191 area of ring 10 (pre-equator) in all six images used from three different axial lengths
192 were compared with the area of ring 11 (anterior equator) in each respective image.
193 The inter-ring area for each annulus between 81-90° and 90-99° which are
194 represented by rings 10 and 11 are calculated to be 118.9mm².

195 ***Statistical analysis***

196 Kruskal-Wallis test was used to assess statistical significance between the
197 enlargement factors between the three different axial lengths. Linear regression was
198 used to assess the relationship between the enlargement factor of each inter-ring
199 area and the location of the rings. Mann-Whitney U test was used to assess the
200 differences in pre-equatorial and post equatorial rings. A significance level was set at
201 0.05.

202

203 **Results**

204 ***1. Image enlargement factor***

205 The inter-ring area enlargement factors for each model eye of simulated axial
206 lengths of 22mm, 24mm and 26mm are detailed in table 1. A graphical
207 representation of this is provided in figure 3. There is still a graduated increase in

208 distortion which is related to the angular position from the posterior pole, $R^2=0.9739$,
209 $p=<0.0001$. There were no significant difference between the enlargement factors for
210 different axial lengths, $p=0.9512$. By using the 24mm model, the percentage of the
211 area of each ring over the entire image was identified and the enlargement factor
212 weighted to the percentage covered by each ring was determined to obtain a global
213 enlargement factor. This was found to be 1.62, and thus a conversion factor
214 (1/enlargement factor) of 0.62. The mean enlargement factor for the vertical
215 component was 1.54 while that of the horizontal component was 1.37, $p=0.0629$.

216 **2. Influence of axial length**

217 The enlargement ratio followed a similar pattern with no statistically significant
218 difference between the three different axial lengths, $p=0.9512$. The exact
219 measurements are tabulated in Table 1 and this is further presented in Figure 3.
220 Figure 4 details the measured image area in pixels and the true calculated object
221 inter-ring area for each ring in each of the three models.

222 **3. Anterior equator distortion**

223 The mean area of ring 10 (pre-equator) was 14915 pixels 95% CI [12916, 16915].
224 The mean area of ring 11 (anterior equator) was 15827 pixels 95% CI [13454,
225 18201]. The difference between the two was statistically significant, $p=0.0025$.

226

227 **Discussion**

228 Numerous methods have been utilised to study image distortion in ultra-widfield
229 imaging but this is the first reported study whereby 3-D printed model eyes have
230 been used with the analysis of the influence of varying axial lengths on the image
231 produced.⁸⁻¹⁰ Previous reports have used known sizes such as the Argus implant or
232 the optic disc but using a 3-D printed model eye, more detailed analyses can be
233 performed.^{9,10,16} From our study, we have identified several key findings. Firstly,
234 despite software correction, there is still an increasing distortion towards the
235 periphery. The average enlargement factor at 9-18 degrees from posterior pole is
236 1.13 and 1.90 at 81-90 degrees from the posterior pole. We have also identified that
237 the enlargement factors for the three different axial lengths follows a similar curve as
238 seen in figure 3. Therefore, these enlargement factors may be used in all eyes
239 independent of their axial lengths. This finding is due to the fact that angles were
240 used to delineate the rings in the model eye, i.e. each ring is 9 degrees from the
241 next. Therefore, the enlargement factor is dependent on the angular location.
242 Although, the size is different between model eyes, the angular position of each
243 segment is similar between different axial lengths and so the enlargement ratio is
244 similar. Secondly, although the true calculated object area for each ring in the three
245 different models are different, the measured image pixel size of each ring for the
246 three different axial lengths are almost identical as depicted in figure 4. This helps
247 explain the finding by Sagong et al that reported the size of objects can vary as
248 much as 10% depending on axial lengths.¹⁰ The larger the axial length, there is more
249 'shrinkage' of a similar sized object and vice versa. We propose that this is related to
250 the mechanism by which the ultra-widfield system obtains images and therefore
251 theoretically the inside of a football and a ping pong ball will look rather similar in the
252 image produced despite obvious differences in size.

253 We have also shown that the distortion is still present and larger towards the
254 periphery which has an implication towards the ischemic index measurements
255 utilised in previous studies. As the ischemic index takes the percentage of non-
256 perfused retina as a whole, variability in the distribution of retinal non-perfusion will
257 affect the corrected ischemic index as described by Tan et al, whereby the difference
258 ranged from -5.9% to 14.8%.¹⁴ This is due to the variability in the enlargement factor
259 which is based on the angle from the posterior pole.

260 This study also confirms the presence of an anterior equator enlargement and that it
261 contributes to the distortion obtained. This anterior equator phenomenon is an
262 interesting concept especially when imaging technology improves and allows more
263 peripheral imaging. Our study suggests that the anterior equator appears to follow
264 the same projection curve irrespective of axial lengths with no reduction in size. We
265 acknowledge that the numbers are small, six sets of measurements from three
266 different models.

267 Interestingly, in the uncorrected images, a horizontal stretch was identified using
268 different models.^{8,9} The new software (V2 Vantage Pro, Optos) corrects for this
269 distortion. Although, there appears to be a trend for the vertical component of images
270 to be stretched more than the horizontal in the corrected images but this was not
271 found to be significant in our study.

272 There are several limitations in our study and this includes the assumption that in
273 practice, the eye is a perfect sphere like the model eyes used. In reality, variations in
274 ocular shape and deviation from a perfect sphere will affect the accuracy in
275 translating our findings into practice. Secondly, only three different axial lengths were
276 studied.

277 For clinical use, using our data, we have produced an enlargement factor based on
278 the position in the image. This can be helpful in clinical practice to obtain an
279 approximate size of lesions in varying positions of an ultra-widefield image. We
280 acknowledge that for a more precise quantification of area, Croft et al have proposed
281 and proven that projecting the image into a three-dimensional model and using
282 spherical trigonometry, accurate measurements can be made.¹⁶ We acknowledge
283 that using this method, it may be more accurate however from a practical point in
284 clinical practice, it will be difficult.

285 We appreciate that in digital imaging, the sizes in ultra-widefield imaging are in pixels
286 and therefore any object in the posterior pole whereby the distortion is less can be
287 used as a reference.^{8,16} For example, in an image with an optic disc area of 2.54mm²
288 that measures 800 pixels, a lesion at 85-90 degrees from the centre measuring 6000
289 pixels, the actual size of the lesion should be approximately 10.10mm² instead of
290 19.05mm², using a conversion factor of 0.53.

291 The concentric rings method has been reported as a reliable method in determining
292 retinal non-perfusion.¹² By superimposing the rings, an ultra-widefield image of the
293 retina and the image of a 24mm model eye, we have summarised the enlargement
294 factor and angle imaged for each of the concentric rings. This is further detailed in
295 Table 2.

296 This revelation of a significant magnification in the periphery has also been
297 suggested by Oishi et al⁸ however by identifying a specific enlargement factor and
298 thus a conversion factor for images, we are now able to better quantify area in ultra-

299 widefield imaging. In previous studies, the maximum area identified in ultra-widefield
300 imaging were 1148mm² and 1856mm² by using a standard disc area of
301 2.54mm².^{12,17} This is a unlikely to be accurate and mirrors a peripheral distortion as
302 the predicted size of the retina including the optic disc has been mathematically
303 determined to be 1133.8mm² and the area of perfused area in normal retina in ultra-
304 widefield angiography was found to be 977.0mm².^{18,19} By using the global
305 conversion factor of 0.62, these values from previous studies would be converted to
306 711.8mm² and 1150.7mm² which is more realistic. Furthermore, previous

307 In conclusion, ultra-widefield imaging is used frequently in clinical research to assess
308 the peripheral retina and an accurate quantification of area is required to further
309 validate the results obtained. The enlargement factor is based on angular location
310 despite varying axial lengths. We propose a conversion factor that can be used to
311 improve the accuracy in quantifying area in ultra-widefield images after incorporating
312 corrections for peripheral and anterior equator distortion.

313

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377 **Figure Captions**

378 Figure 1: The design of the model eye with an axial length of 24mm with section A-A
379 representing the coronal plane and section B-B, the sagittal plane (Top left). The
380 radius of the model is 13mm, R13 (top right). The walls of the model eye have a
381 thickness of 2mm. Each model is made up of multiple rings centered at the posterior
382 pole with each ring separated by nine degrees as in the image. Top right image
383 represents the sagittal plane and bottom left image represents the coronal plane.
384 Bottom right image represents the model eye viewed externally.

385 Figure 2: The grids in the original image (left) is traced using Photoshop CS2
386 (Adobe, San Jose, USA) (middle). In this example, the line thickness is set at 5
387 pixels for ease of the reader however, in determining the area, this was set at 1 pixel
388 for increased accuracy. The traced image which was used to determine the area of
389 each ring in pixels using ImageJ (right).

390 Figure 3: Graph representing the enlargement factors of model eyes with simulated
391 axial lengths of 22mm, 24mm and 26mm. The results were plotted against an x-axis
392 of the angles (in degrees) from the posterior pole of the model eye.

393 Figure 4: The measured pixel area of each ring (Left) and the true calculated area in
394 mm² of each ring (Right) for the model eyes with axial lengths of 22mm, 24mm and
395 26mm.

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415 board and receives speaker fees from Novartis, Allergan and Bayer.

416

417 Table 1: The enlargement factors for each model eye of axial lengths 22mm, 24mm,
 418 and 26mm

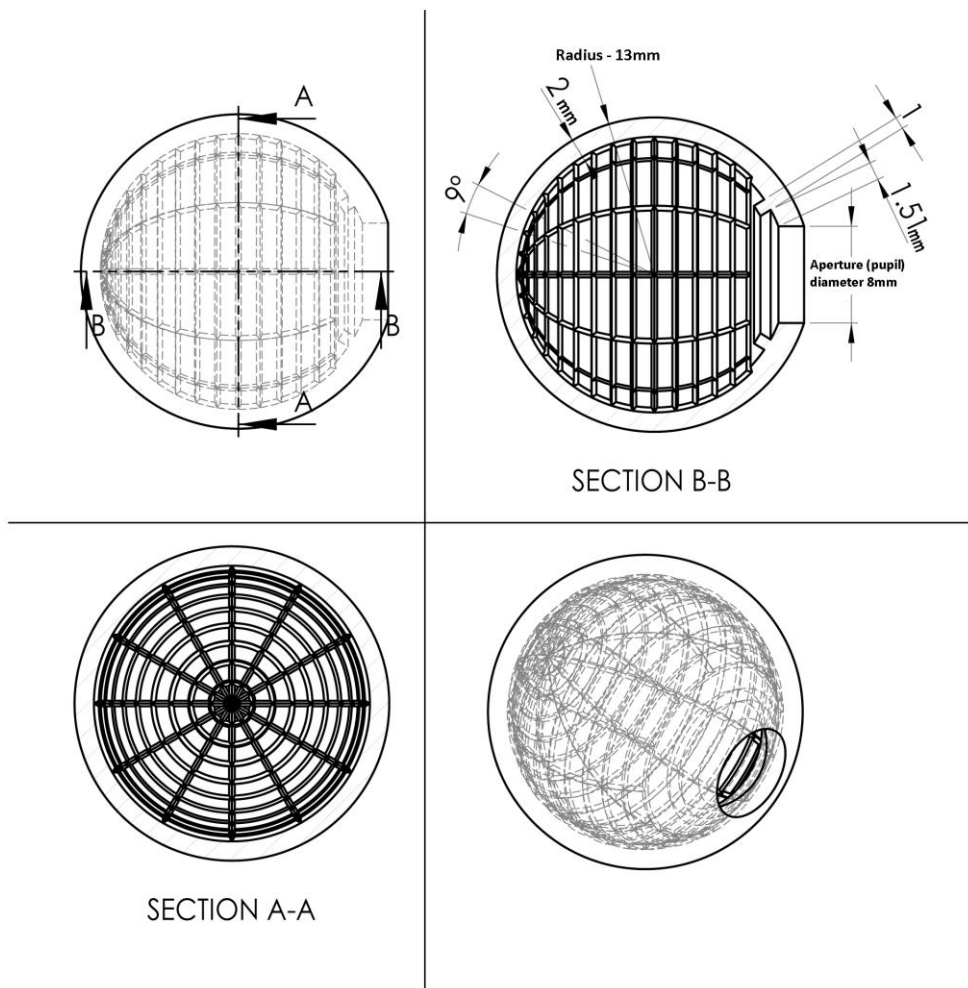
419

Angle (degrees)	Ring number	Axial length of 22mm	Axial length of 24mm	Axial length of 26mm
0-9	1	1.00	1.00	1.00
9-18	2	1.15	1.10	1.12
18-27	3	1.19	1.15	1.15
27-36	4	1.25	1.23	1.23
36-45	5	1.33	1.30	1.31
45-54	6	1.40	1.37	1.36
54-63	7	1.51	1.47	1.47
63-72	8	1.62	1.61	1.62
72-81	9	1.74	1.76	1.73
81-90	10	1.90	1.87	1.93
90-99	11	2.06	1.97	2.04

429

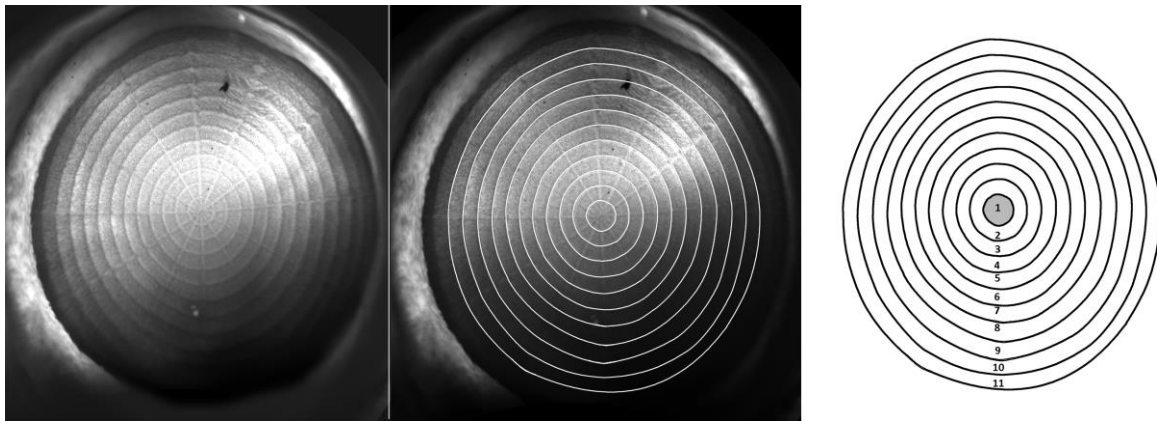
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431 Figure 1



432

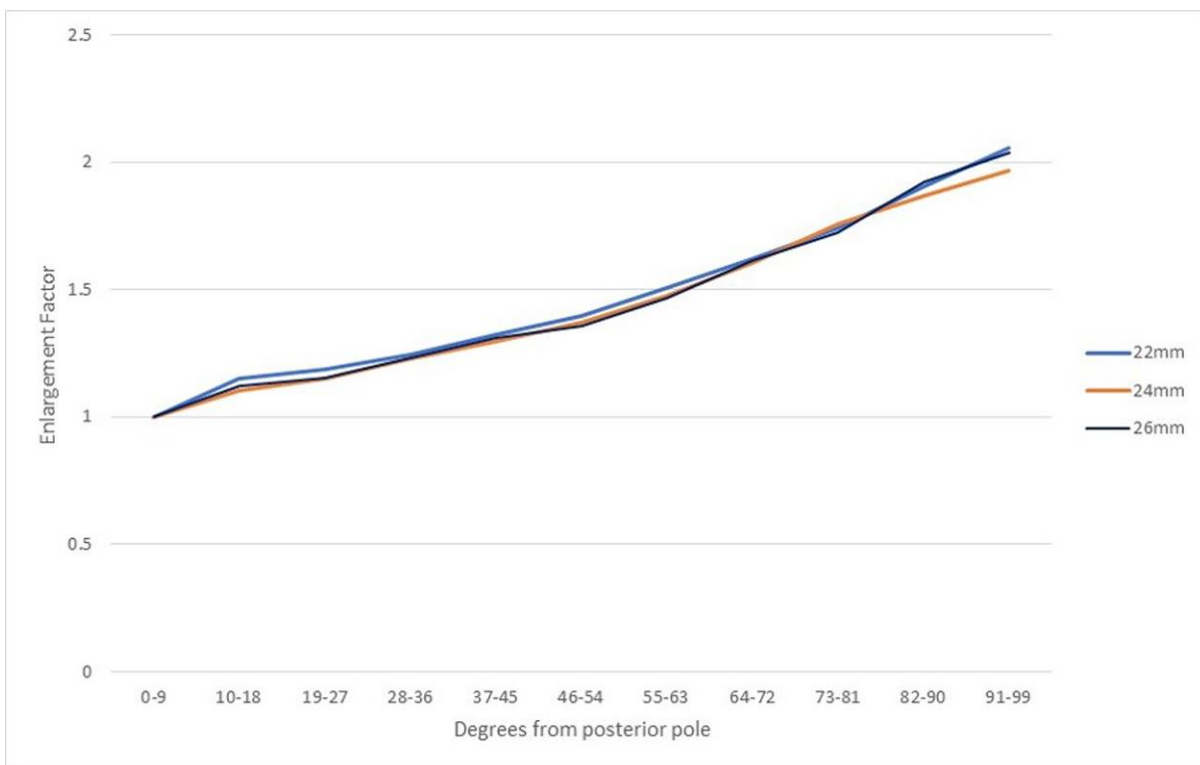
433 Figure 2



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436 Figure 3



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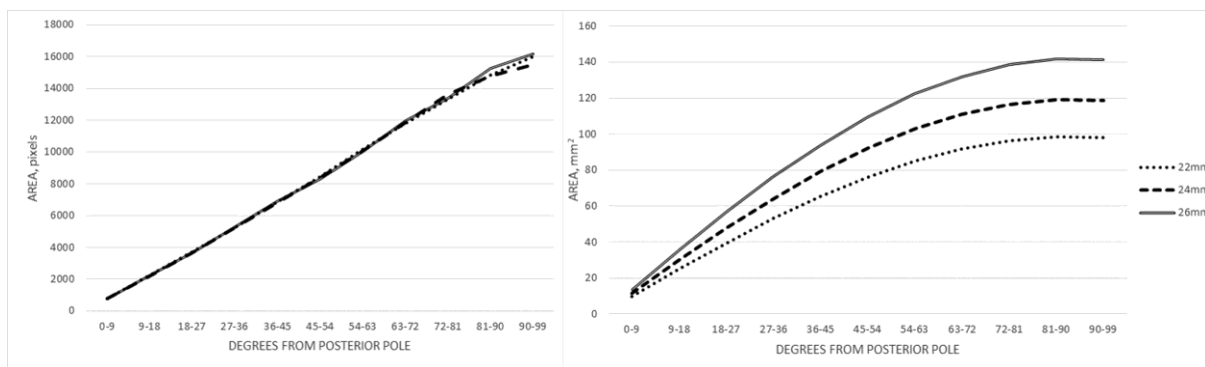
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446 Figure 4



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