

# 1      **Interplay between intraocular and intracranial pressure effects** 2      **on the optic nerve head in vivo**

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18     Short Title: In vivo IOP and ICP effects on the ONH

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## 46 **Abstract**

47        Intracranial pressure (ICP) has been proposed to play an important role in the sensitivity  
48 to intraocular pressure (IOP) and susceptibility to glaucoma. However, the *in vivo* effects of  
49 simultaneous, controlled, acute variations in ICP and IOP have not been directly measured. We  
50 quantified the deformations of the anterior lamina cribrosa (ALC) and scleral canal at Bruch's  
51 membrane opening (BMO) under acute elevation of IOP and/or ICP.

52        Four eyes of three monkeys were imaged *in vivo* with OCT under four pressure conditions:  
53 IOP and ICP either at baseline or elevated. The BMO and ALC were reconstructed from manual  
54 delineations. From these, we determined canal area at the BMO (BMO area), BMO aspect ratio  
55 and planarity, and ALC median depth relative to the BMO plane. To better account for the  
56 pressure effects on the imaging, we also measured ALC visibility as a percent of the BMO area.  
57 Further, ALC depths were analyzed only in regions where the ALC was visible in all pressure  
58 conditions. Bootstrap sampling was used to obtain mean estimates and confidence intervals,  
59 which were then used to test for significant effects of IOP and ICP, independently and in  
60 interaction.

61        Response to pressure manipulation was highly individualized between eyes, with  
62 significant changes detected in a majority of the parameters. Significant interactions between  
63 ICP and IOP occurred in all measures, except ALC visibility. On average, ICP elevation expanded  
64 BMO area by  $0.17\text{mm}^2$  at baseline IOP, and contracted BMO area by  $0.02\text{ mm}^2$  at high IOP. ICP  
65 elevation decreased ALC depth by  $10\mu\text{m}$  at baseline IOP, but increased depth by  $7\text{ }\mu\text{m}$  at high  
66 IOP. ALC visibility decreased as ICP increased, both at baseline (-10%) and high IOP (-17%).  
67 IOP elevation expanded BMO area by  $0.04\text{ mm}^2$  at baseline ICP, and contracted BMO area by  
68  $0.09\text{ mm}^2$  at high ICP. On average, IOP elevation caused the ALC to displace  $3.3\text{ }\mu\text{m}$  anteriorly  
69 at baseline ICP, and  $22\text{ }\mu\text{m}$  posteriorly at high ICP. ALC visibility improved as IOP increased, both  
70 at baseline (5%) and high ICP (8%).

71        In summary, changing IOP or ICP significantly deformed both the scleral canal and the  
72 lamina of the monkey ONH, regardless of the other pressure level. There were significant  
73 interactions between the effects of IOP and those of ICP on LC depth, BMO area, aspect ratio  
74 and planarity. On most eyes, elevating both pressures by the same amount did not cancel out the  
75 effects. Altogether our results show that ICP affects sensitivity to IOP, and thus that it can  
76 potentially also affect susceptibility to glaucoma.

77 **Research Highlights**

78 - In vivo ONH deformations caused by acute, controlled, simultaneous changes in IOP  
79 and/or ICP can be directly visualized and measured in the monkey eye using OCT.

80 - Acute changes of either IOP or ICP significantly deformed both the scleral canal and the  
81 lamina cribrosa, regardless of the other pressure level.

82 - Pressures interacted, meaning that the effects of one pressure depended significantly on  
83 the level of the other pressure.

84 - Elevating both pressures did not cancel out the effects of one of them being elevated.

85 - Our results show that ICP affects sensitivity to IOP, and thus that it can potentially also  
86 affect susceptibility to glaucoma.

## 87 1. INTRODUCTION

88 Glaucoma is a progressive and irreversible optic neuropathy and the second-leading  
89 cause of vision loss in the world <sup>1,2</sup>. While the mechanisms of neural tissue loss in glaucoma  
90 remain unclear, studies suggest that mechanical insult to the optic nerve head (ONH) contributes  
91 to the cascade of events that eventually result in neural tissue damage <sup>3–8</sup>. Much attention has  
92 been given to the mechanical insult associated with elevated intraocular pressure (IOP) <sup>6,9–11</sup>, but  
93 the role of intracranial pressure (ICP), which acts on the ONH from outside the eye, is relatively  
94 unexplored. Evidence from epidemiological and animal models suggests that ICP could also have  
95 an important influence on the ONH, and that it may be a missing factor needed to understand why  
96 subjects vary so widely in their sensitivities to elevated IOP <sup>12–16</sup>.

97 The *in vivo* effects on the ONH of acute variations in ICP remain poorly understood. In  
98 particular, it remains unclear whether ICP variations can cause deformations of the lamina  
99 cribrosa (LC) or the scleral canal. It is also unclear whether the effects of IOP and ICP are  
100 independent or if they interact with each other. It has been suggested that the two pressures might  
101 counterbalance <sup>13,15,17–19</sup>. This implies that ONH deformations caused by an elevated IOP might  
102 be removed or “cancelled out” by an elevated ICP. Simulations <sup>14,20</sup> and experiments <sup>21</sup> suggest  
103 that the effects of IOP and ICP on the LC can be substantial and do not balance out. However, to  
104 the best of our knowledge, the effects of ICP on the ONH, independently and in conjunction with  
105 elevated IOP, have not been directly measured in a primate *in vivo*. To understand the effects of  
106 chronically altered pressures on the ONH in glaucoma, we can first work to understand the  
107 biomechanics of the ONH under acute changes in pressures.

108 Our goal was to test if acute changes in ICP or IOP can affect ONH structure and if these  
109 variables interact. Interactions would indicate that to understand the effects of one pressure it is  
110 necessary to also understand the effects of the other pressure. Specifically, we aimed to quantify  
111 *in vivo* the deformations of the anterior lamina cribrosa (ALC) and scleral canal at Bruch’s  
112 membrane opening (BMO) of monkey eyes under acute, controlled variations of IOP and/or ICP.

113 **2. METHODS**

114 We used a previously reported <sup>21</sup> in vivo monkey model, rhesus macaque, in which IOP  
115 and ICP were acutely controlled, independently and simultaneously. Four eyes of three monkeys  
116 (M1, M2, M3L, M3R) were imaged in vivo, with optical coherence tomography (OCT). ONH  
117 structures were manually delineated in these images. From each scan, five parameters of interest  
118 were measured: BMO area, aspect ratio, and planarity as well as ALC median depth and visibility.  
119 The parameters were analyzed to test for significant effects of IOP and ICP, independently and  
120 in interaction using a bootstrapping approach. Note that the bootstrapping and statistical analysis  
121 were designed to evaluate the reliability in the parameters measured, and in detecting their  
122 changes upon IOP and ICP changes. The study was not designed to determine whether the  
123 measurements in the four eyes studied are representative of a larger population. The details of  
124 each of these steps are provided below.

125 **Animal Handling**

126 Animal handling, pressure control, and imaging were conducted as described elsewhere  
127 <sup>21</sup>. Animal handling followed National Institute of Health (NIH) Guide for the Care and Use of  
128 Laboratory Animals, adhered to the Association of Research in Vision and Ophthalmology  
129 (ARVO) statement for the Use of Animals in Ophthalmic and Vision Research, and the protocol  
130 was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of  
131 Pittsburgh. Before the experiment, a clinical examination was conducted to exclude eyes with  
132 gross abnormality. For these experiments, animals were initially sedated with 20 mg/kg ketamine,  
133 1 mg/kg diazepam, and 0.04 mg/kg atropine. They were maintained on 1-3% isoflurane for the  
134 remainder of the experiment. Animals were put on a ventilator and given vecuronium bromide, a  
135 paralytic, intravenously at 0.04-0.1 mg/kg/hr to reduce eye shifting throughout the experiment.  
136 Eyes were scanned while animals were in the prone position, with the head held upright and  
137 facing the OCT device. The pupils were dilated using tropicamide ophthalmic solution 0.5%  
138 (Bausch & Lomb, Rochester, NY). The corneal surface of each eye was covered with a rigid, gas  
139 permeable contact lens (Boston EO, Boston, MA) to preserve corneal hydration and improve  
140 image quality. The eyes were kept open using a wire speculum and the corneas were hydrated  
141 with saline between scans. The animals' blood pressures and heart rates were monitored  
142 throughout the study.

143 **Pressure Manipulation**

144 For the pressure manipulation we followed the same general approach described  
145 elsewhere<sup>21</sup>. After thorough irrigation of the cannula to remove all air bubbles, IOP was controlled

146 by inserting a 27-gauge needle into the anterior chamber and connecting it to a saline reservoir  
147 (**Figure 1a**). ICP was controlled by inserting a lumbar drain catheter (Medtronic, Minneapolis, MN)  
148 2.5 cm into the lateral ventricle of the brain and connecting it to a separate saline reservoir. IOP  
149 and ICP were thus controlled by adjusting the height of the corresponding reservoir. ICP was also  
150 monitored by an ICP pressure also placed into the brain, at least 5mm from the catheter (ICP  
151 Express monitoring system, DePuy Synthes, Raynham, MA). Before using the pressure  
152 transducer, it was calibrated while submerged in saline solution. IOP and ICP values were  
153 controlled within 1 mmHg. Target ICPs were 10, 25, 40 and 5 mmHg. IOPs were set to 15, 30, 50  
154 and 5 mmHg. Based on our experience and the literature, baseline pressures were defined as  
155 an IOP of 15 mmHg and an ICP of 10 mmHg<sup>22,23</sup>. Four pressure conditions were included in this  
156 study, one baseline and three experimental with one or both pressures elevated: (IOP/ICP);  
157 15/10 mmHg, 15/25 mmHg, 30/10 mmHg, and 30/25 mmHg.

158 **Imaging**

159 Eyes were imaged with spectral domain optical coherence tomography (OCT) (Bioptigen,  
160 Research Triangle, NC) with a scan rate of 20,000 A-scans/second, modified with a broadband  
161 superluminescent diode (Superlum, Dublin, Ireland,  $\lambda = 870$  nm,  $\Delta\lambda = 200$  nm). OCT volume  
162 scans were acquired with a 5mm x 5mm x 2mm (512 x 512 x 1024 pixels sampling, with 1 Frame  
163 per B-scan, i.e. no repetitions) setting, centered on the ONH region (**Figure 1a**). Multiple scans  
164 were obtained at each pressure condition, and the best quality scan at each pressure condition  
165 was chosen for manual delineation. Image quality criteria are detailed elsewhere<sup>21</sup>. Image quality  
166 tended to decrease with increasing anesthesia time. To ensure that image quality remained high  
167 and to minimize the amount of time each animal spent under anesthesia, in the early experiments  
168 we imaged only one eye. After becoming more comfortable and quicker with our animal protocols,  
169 imaging could be performed fast enough to capture images from both eyes in the third monkey.  
170 After each IOP and/or ICP change we stepped back for 5 minutes, waiting for the eyes to stabilize.  
171 In addition, at each pressure we spent 20-30 minutes adjusting equipment and conducting the  
172 imaging. Exact times varied slightly depending on how quickly we were able to get the imaging  
173 setup, keeping the cornea hydrated, etc. Since only a subset of images were analyzed for this  
174 work, the actual times between pressures were always at least 60min for Monkeys 1 and 2, and  
175 45 min for Monkey 3. Based on our experience and the literature, we believe that these times are  
176 sufficient to minimize viscoelastic effects.<sup>24-27</sup>.

177 All scans were re-sampled at 1 x 1 x 1 scale for analysis<sup>28</sup> Eyes vary in optical power and  
178 OCT systems are optimized for imaging human eyes. Hence, OCT images of monkey ONHs must

179 be rescaled in the transverse dimensions. To set the dimensions, we followed the process  
180 described previously <sup>21</sup>. Briefly, after the experiment, eyes were enucleated, processed for  
181 histology, and sections were imaged with polarized light microscopy. The images were  
182 reconstructed into 3D stacks and used to obtain eye-specific transverse scaling factors based on  
183 the dimensions of the scleral canal at BMO. Elsewhere we have shown that histological  
184 processing does not alter the scale of eye tissues <sup>29</sup>.

## 185 **Delineation**

186 Delineations were done by an experienced observer, masked to both IOP and ICP  
187 conditions, in an open-source imaging processing package, Fiji <sup>30</sup>. Two ONH landmarks, the  
188 scleral canal at BMO and the anterior boundary of the LC (ALC), were delineated in equally  
189 spaced OCT B-scans (**Figure 1**). The ALC was sampled at a higher transverse resolution, every  
190 31  $\mu\text{m}$ , than the BMO, every 62  $\mu\text{m}$ , to best resolve its comparatively non-uniform structure. The  
191 BMO best-fit plane was used as a reference for measurements of BMO planarity and ALC depth.  
192 The ALC and scleral canal at BMO were selected for analysis because they have often been used  
193 in studies of monkey ONH biomechanics <sup>31</sup>, and because simulation analyses have shown that  
194 they are useful to capture essential elements of ONH biomechanics <sup>32</sup>.

## 195 **3D Reconstruction & Registration**

196 Motion artifacts, from breathing, heartbeat, or surgical table vibrations were discernible in  
197 the slow-scan (superior-inferior) direction as a wavy pattern in the otherwise smooth structure of  
198 Bruch's membrane. These were removed by translating B-scan images in the anterior-posterior  
199 direction. Custom scripts were used to import manual delineations of BMOs made on virtual  
200 superior-inferior volume cross-sections from Fiji into Matlab (Mathworks, Natick, MA, USA).  
201 Custom scripts were also used to interpolate between the scattered manual markings of the BMO  
202 and ALC. This allowed us to obtain 3D reconstructions for analysis (**Figure 2**). When mitigating  
203 motion artifacts, we used positions as far as possible from the canal at BMO as landmarks for  
204 alignment. This was done to minimize alignment-based changes impacted by changes of BMO  
205 planarity themselves. Additionally, we filtered motion artifacts with frequencies corresponding to  
206 heartbeat. Images of the ALC across different pressure conditions within an eye were registered  
207 by aligning the center and principal axes of the BMO best-fit plane.

## 208 **BMO Area, Aspect Ratio, Planarity**

209 BMO area was computed as the projected area of BMO on the best-fit plane. BMO aspect  
210 ratio was computed from the ratio between the major and minor principal axes of this plane  
211 (**Figure 2a-c**). The planarity of the BMO was defined as the average of the distances from BMO

212 points to the best-fit plane, measuring the extent to which the BMO deviated from a flat plane <sup>8,33</sup>.  
213 Note that with this definition, a perfectly planar canal opening has a planarity of zero, with planarity  
214 increasing as the shape deviates from the perfect plane.

## 215 **Lamina-Visibility, Median Depth**

216 The 3D ALC surface was projected onto the BMO best-fit plane and ALC visibility, or  
217 analyzable ALC, was computed as percent of projected ALC area normalized to baseline BMO  
218 area (**Figure 2e-d**). To avoid potential biases due to variable LC visibility with IOP, ALC median  
219 depth, relative to BMO best-fit plane, was measured in regions where ALC was visible in all  
220 pressure conditions within each eye. We defined the sign of the depth with positive direction being  
221 anterior to the BMO and vice versa.

## 222 **Repeatability**

223 Repeatability of measurements was evaluated as we have done previously <sup>34</sup>. Briefly, an  
224 OCT volume was processed and marked three times for each of five parameters and standard  
225 deviations over the three markings used as a measure of repeatability.

## 226 **Bootstrap Sampling**

227 Bootstrap sampling was used to assess the reliability of observed structural  
228 deformations for each eye and make the best possible use of the limited number of monkeys  
229 and eyes. A custom Matlab script was used to randomly select a subset of 75% of the B-scan  
230 markings in each volume, for each ONH. These sampled markings were then used to  
231 reconstruct and compute the five canal and ALC parameters, as described above. The  
232 procedure was repeated 10 times to obtain sampling distributions for each parameter within  
233 each eye at each pressure condition. **Figure 3** illustrates the process of bootstrap sampling and  
234 subsequent statistical analyses.

## 235 **Estimates & Percentage changes**

236 Within each eye, the estimates of each ONH parameter at each pressure condition were  
237 computed as the mean of the bootstrap distribution. The confidence intervals (CI) of the estimates  
238 were defined as the 95<sup>th</sup> percentiles (between the 2.5% and 97.5% percentiles). To compute these  
239 percentiles, the bootstrap distributions were fit by a normal distribution and the percentiles  
240 estimated from the normal. A small CI indicates that the bootstrap distribution is tight, indicating  
241 that selecting different subsets of the markings leads to similar measures. Conversely, a wide CI  
242 indicates that the bootstrap distribution is wide, indicating that the measure is sensitive to the  
243 specific set of markings from which it was derived.

244 The percentage change of the estimate at each pressure condition was calculated with  
245 respect to the baseline estimate. Positive percentage changes of ALC median depth  
246 corresponded to more anterior ALCs (and thus, negative changes to more posterior ALCs), and  
247 positive percentage changes of BMO area corresponded to scleral canal expansions (and thus  
248 negative changes to scleral canal contractions).

249 **Statistical analysis**

250 For each parameter in each eye, we computed the statistical significance of the  
251 independent (main effect) and interaction effects of IOP and ICP as described in **Figure 3**. A main  
252 effect was deemed significant when the range of slopes within the 95% confidence interval did  
253 not include 0. An interaction effect was deemed significant if the ranges of slopes within the 95%  
254 confidence interval did not overlap. Mean changes of significantly different cases were computed  
255 and reported.

256

257 **3. RESULTS**

258 We successfully acquired images and measurements on all eyes and conditions, except  
259 for two cases. No eye of the monkeys reported here had a gross abnormality. Images of the left  
260 eye of Monkey 3 at elevated IOP, at both baseline and elevated ICP, did not have good LC  
261 visibility and were therefore removed from analysis. When measuring repeatability, standard  
262 deviations over the three markings were 0.01, 0.01 mm<sup>2</sup>, 0.44 µm for BMO aspect ratio, area and  
263 planarity, respectively. For ALC depth and visibility, the standard deviations were 5.5 µm and 1%,  
264 respectively. For comparison, the voxel edge length of the OCT images was 3.125 µm.

265 Deformations of ONH structures resulting from IOP and ICP variations were evident by  
266 overlaying delineations in corresponding B-scans (**Figure 4**). Baseline parameters are  
267 summarized in **Table 1**. The scleral canal at BMO was evaluated as an indicator of greater scleral  
268 canal morphology. The effects of ICP elevation on the shape of the scleral canal at BMO, at high  
269 and low IOP are illustrated in Figure 5. Overall, pressure changes had significant effects on the  
270 various morphological parameters. The effects are shown in **Figures 6 to 8** and summarized in  
271 **Tables 2 and 3**. Results of the statistical tests are summarized in **Figure 9**. The location and  
272 direction of force generated by IOP and ICP are illustrated in **Figure 10**.

273 On average, ICP elevation expanded canal area at BMO by 0.17mm<sup>2</sup> at baseline IOP and  
274 contracted the BMO area by 0.02 mm<sup>2</sup> at high IOP. ICP elevation decreased ALC depth by 10µm  
275 at baseline IOP, but increased depth by 7µm at high IOP. ALC visibility decreased as ICP  
276 increased, both at baseline (-10%) and high IOP (-17%). IOP elevation expanded BMO area by

277 0.04 mm<sup>2</sup> at baseline ICP, and contracted BMO area by 0.09 mm<sup>2</sup> at high ICP. On average, IOP  
278 elevation caused the ALC to displace 3.3 μm anteriorly at baseline ICP, and 22 μm posteriorly at  
279 high ICP. ALC visibility improved as IOP increased, both at baseline (5%) and high ICP (8%).

280

### 281 **BMO Area, Aspect Ratio, Planarity**

282 For BMO area, ICP elevation had a significant effect at both high and low IOP on 3 eyes  
283 (M1-M3R) and at low IOP on M3L (**Figures 5 and 6, Tables 2 and 3**). IOP elevation had significant  
284 effects at both high and low ICP on 3 eyes (M1-M3R). There were significant interactions of ICP  
285 and IOP effects on BMO area in 3 eyes (M1-M3R).

286 For BMO aspect ratio, ICP elevation had significant effects at low IOP on 2 eyes (M1,  
287 M3L) and at high IOP on M2 (**Figure 6, Tables 2 and 3**). IOP elevation had significant effects at  
288 both high and low ICP on 2 eyes (M1, M3R) and at low ICP on M2. There was 1 significant  
289 interaction of ICP and IOP effects on BMO aspect ratio in M2.

290 For BMO planarity, ICP elevation had significant effects at low IOP on 2 eyes (M3R, M3L)  
291 and at high IOP on 3 eyes (M1-M3R) (**Figure 6, Tables 2 and 3**). IOP elevation had significant  
292 effects at low ICP on M3R and at high ICP on M1. There were significant interactions of ICP and  
293 IOP effects on BMO planarity in 2 eyes (M1, M3R).

294 Considering all cases, pressure variations induced the largest changes on BMO planarity  
295 (-14% to 123%), followed by the BMO area (-6% to 15%), and finally by the BMO aspect ratio with  
296 very small changes (-4% to 2%) (**Figure 6**).

### 297 **Lamina Depth and Visibility**

298 For ALC median depth, ICP elevation had significant effects at low IOP on 3 eyes (M1,  
299 M2, M3L) and at high IOP on 2 eyes (M1, M3R) (**Figure 7, Tables 2 and 3**). IOP elevation had  
300 significant effects at low ICP on 3 eyes (M1-M3R) and at high ICP on M1. The effects of IOP and  
301 ICP on ALC depth had a significant interaction for M2.

302 Across the 4 eyes, baseline ALC visibility and median depth average were 57% and  
303 139μm, respectively (**Table 1**). The amount of ALC visible in scans at each pressure condition  
304 varied between eyes, ranging from 15-45%, with an average of 34%. Regardless of pressure  
305 conditions, ALC visibilities were higher in M2, M3R, and M3L. These monkeys had analyzable  
306 ALC in both nasal and temporal sides, compared to M1L, in which ALC was visible only on the  
307 temporal side (**Table 1, Figure 7**). ICP elevation had significant effects on ALC visibility at low  
308 IOP and at high IOP (**Figure 7, Tables 2 and 3**). No significant interaction between ICP and IOP  
309 effects on ALC visibility was detected.

310 Considering all pressure settings across the 4 eyes, changes in both depth and visibility  
311 of the LC were substantial. Median depth changed between -53% and 24% and visibility changed  
312 between -33% and 20% (**Supplemental Figure 1**).  
313

### 314 **Heterogeneity of Responses to IOP/ICP**

315 The variable responses in structural parameters are best visualized in **Figures 5-9** and  
316 **Tables 2 and 3**. To help readers interpret the figures and understand their implications, we will  
317 consider some examples. For instance, consider the ALC Median depth of M1 in **Figure 8**. As  
318 ICP was increased at baseline IOP (blue line) we measured an increase in the depth of the ALC.  
319 All blue points representing the data subsets created for bootstrapping were neatly clustered  
320 around each median depth, indicating a relative homogeneity of depth measurements and thus  
321 good confidence in the values. A regression line was fit between the clusters at baseline and  
322 elevated ICP settings for visualization. The positive, non-zero slope represented a significant  
323 increase in ALC depth as a function of ICP. When we repeated the experiment at elevated IOP,  
324 we observed a similar relationship, but the ALC depths were shifted shallower at both ICP  
325 settings. The parallel lines indicate that ICP and IOP affected the deformations independently  
326 and that there was no interaction between variables. This first row of **Figure 8** summarizes a  
327 case in which ICP elevation had a significant effect at each IOP, but there was no interaction  
328 between ICP and IOP. The following subject, M2, showed a similar relationship for elevations of  
329 ICP at baseline IOP (blue line), but at high IOP the relationship was not present (red line). This  
330 indicates that the variables interacted strongly. For M3L, ALC depth was reduced with increasing  
331 ICP. No significant interaction was observed between the effects of ICP and IOP for ALC depth  
332 in M3L. These variable structural response are summarized in **Table 2**, where it can be seen that  
333 each column has at least one negative and one positive value. **Figure 9** summarizes the  
334 statistical results and the significance of IOP and ICP effects independently and in interaction.  
335 These examples are representative of the heterogeneity in the ONH response between eyes that  
336 was present for all parameters.

337 Interestingly, in most cases, setting the translaminar pressure difference (TLPD) to the  
338 baseline levels did not deform the canal and lamina back to baseline configurations. For example,  
339 cases in which IOP was 15 and ICP was 10 mmHg (baseline for all eyes) can be compared with  
340 cases in which IOP was 30 and ICP was 25 mmHg (such as in M1, M2, and M3R). Each of these  
341 cases had a TLPD of 5 mmHg. However, we observed changes in scleral canal displacement  
342 (**Figure 6**), ALC visibility (**Figure 7**), and ALC depth (**Figure 8**) upon pressures increases despite  
343 conserved TLPD. In M1 and M2, scleral canal area, ALC visibility, ALC depth were all increased

344 as IOP and ICP were increased from baseline to elevated pressures. In M3R, these factors were  
345 respectively decreased, decreased, and similar to their respective baselines. Based on this data,  
346 we did not observe a strong relationship between TLPD and ONH morphology.

347 Figure 10 shows the location of where forces from IOP and CSFP are applied in effort to  
348 illustrate why IOP and ICP do not balance each other out. Provided this context, it is clear why  
349 TLPD is not a strong predictor of ONH morphology. IOP and ICP can undergo equal changes in  
350 magnitude, maintaining a consistent TLPD, but they cannot be expected to have opposite  
351 effects due to the locations at which they are applied.

352

## 353 4. DISCUSSION

354 In this study, we set out to quantify *in vivo* deformations of the ALC and scleral canal under  
355 acute, controlled variations of IOP and/or ICP in a monkey model. Four main findings arise from  
356 this work: (1) changes in either IOP or ICP caused significant, detectable ONH deformations, (2)  
357 there were strong interactions between the effects of IOP and ICP, (3) elevating both pressures  
358 by the same amount, to maintain TLPD, did not cancel out the effects, and (4) a high degree of  
359 heterogeneity in response to pressure manipulation was observed among eyes. Our findings are  
360 important because they demonstrate the crucial need to consider both IOP and ICP to fully  
361 understand pressure effects and likely susceptibility to glaucoma. Our results highlight the  
362 complexity of LC biomechanics, and the challenge to understand the multiple interacting factors.  
363 We expand upon these points below.

364 **Changes in either IOP or ICP caused significant, detectable ONH deformations.** IOP  
365 and ICP were both manipulated, allowing us to detect effects of IOP and ICP individually. Whereas  
366 many studies have described effects on the ONH of variations in IOP<sup>10,35–40</sup>, far less understood  
367 are the effects of modulating ICP and its interactions with IOP. Our results demonstrate that the  
368 effects of ICP on the ONH can be detected *in vivo*, and that they can have a magnitude  
369 comparable to the effects of IOP.

370 ONH structures were manually delineated at relatively high density compared with  
371 previous studies<sup>41–43</sup>. For instance, we marked the ALC every 31  $\mu\text{m}$ , about six times higher than  
372 previously<sup>43</sup>. The dense markings allowed us to reconstruct detailed BMO planes and ALC  
373 surfaces, which were then used to quantify their changes and deformations in 3D. A major  
374 concern of ONH studies based on OCT data is the variable visibility, particularly that of the deep  
375 structures like the LC<sup>26,44–46</sup>. Inconsistent visibility means that a simple comparison of parameters  
376 across conditions has a substantial risk of being biased by region visibility. To avert this bias, all  
377 the LC analyses in this work were based on uniform sampling of regions that were clearly visible

378 in all pressure conditions of an eye. This is similar to the shared sectors used by Strouthidis et al  
379 <sup>31</sup> and the overlap restriction in Wang et. al. <sup>21</sup>. This approach reduced the size of the lamina  
380 regions analyzed from an average of 57% to 34%, which still compares well with those of other  
381 studies <sup>21,31</sup>. In contrast with previous work which considered deformations of ONH microstructure  
382 <sup>21</sup> this study places focus on large-scale parameters. This allowed us to observe changes in the  
383 overall size and shape of the scleral canal.

384 Acute modulation of ICP and IOP allowed us to measure the deformations without  
385 potential confounding from remodeling or inflammation associated with chronic pressure changes  
386 <sup>47,48</sup>. Another strength of this work is that we studied monkeys, the animal model that most  
387 resembles humans <sup>49</sup>. The procedures we performed were highly invasive and pose multiple risks  
388 in human subjects. Recently, Fazio et al have overcome many of these by studying IOP and  
389 CSFP in brain-dead human organ donors.<sup>27</sup> Fazio et al <sup>27</sup> reported on ONH deformations in  
390 response to changes in IOP and CSFP in brain-dead human organ donors. In this study, ICP  
391 was estimated based on donor body position and not probed or controlled directly as done here.  
392 Our in vivo measurements also avoid postmortem effects, such as tissue processing, histology  
393 and the absence of blood pressure <sup>26</sup>.

394 **There were strong interactions between the effects of IOP and ICP on the ONH.**  
395 Here, we presented robust evidence that the effects of ICP on the ONH can be influenced by the  
396 level of IOP, and conversely that the effects of IOP can be influenced by the level of ICP.  
397 Interaction of ICP and IOP-mediated effects were observed in all eyes and in the majority of  
398 parameters. This demonstrates the importance of considering the effects of both ICP and IOP  
399 together rather than only independently. When possible, incorporation of ICP as an experimental  
400 variable in studies of IOP-induced deformation is warranted.

401 An important consequence of the complex interactions between IOP and ICP was that  
402 **elevating both pressures by the same amount, and thus maintaining TLPD constant, did**  
403 **not cancel out the pressure effects on ONH visibility and morphology.** We found that none  
404 of the tested eyes had the same structural measurements when subject to the same TLPD but  
405 different IOP/ICP conditions. A constant translaminar pressure did not ensure constant ONH  
406 morphology. A better understanding of how these interactions are influenced by both micro- and  
407 macro-scale ONH structure can allow us to determine the factors necessary to better predict these  
408 complex structural responses and how they might impact the health of the resident neural tissue.  
409 This indicates that TLPD is unlikely to be a strong parameter to predict morphologic changes in  
410 the LC, as has been proposed <sup>50</sup>.

411        **A high degree of heterogeneity in responses to pressure manipulation was**  
412        **observed among eyes.** With variations in either IOP or ICP, no parameters changed consistently  
413        in one direction for all eyes. This heterogeneity cannot be explained by variability in marking or  
414        measuring of ONH structures, as demonstrated by the high repeatability of measurements, the  
415        narrow confidence intervals for many parameters, and consistent findings in the bootstrap  
416        analysis. The bootstrap analysis increased confidence of the observed gross structural changes.  
417        Out of all experimental configurations considered in this study, ~70% (44/65) of structural changes  
418        in response to elevations in either ICP or IOP were significant. This indicates that we observed  
419        robust changes following pressure manipulation. Furthermore, the absolute observed percentage  
420        changes were as large as 123%, 15%, 53%, and 33% for canal planarity, canal area, LC depth,  
421        and LC visibility, respectively. Conversely, changes in canal aspect ratio were relatively small:  
422        within 4%. It is worth reminding the reader that the experiment and analysis were not designed to  
423        determine whether the responses to IOP and ICP variations measured in these monkey eyes will  
424        extend to other eyes or monkeys. This cannot be determined from the small set of eyes studied.  
425        The bootstrap analysis shows that the deformations measured are likely to be 'true' changes in  
426        the structures as visible in the OCT scans, and not due to statistical noise or variability in the  
427        markings. Additional studies with more eyes and animals are necessary to characterize the  
428        population and the variable directionality of the observed tissue responses.

429        Our previous study on the response of microstructure to IOP and ICP modulation showed  
430        that the best fit statistical models included an interaction between ICP and TLPD<sup>21</sup>. In that study,  
431        there was similarly a marked variability in responses between eyes. Both the prior and current  
432        studies emphasize the importance of considering both IOP and ICP in evaluation of the ONH.  
433        The current study, focused on macro-scale ONH features, suggests that the variable micro-scale  
434        responses previously observed extend to global measures of deformation. This may help to  
435        explain the variable responses of patients' eyes to elevated pressure in certain disease states  
436        (i.e. glaucoma, intracranial hypertension). These findings also suggest that a more personalized  
437        medicine approach to optic neuropathy may be optimal for determining the risk and best course  
438        of treatment for individual patients. Further work is necessary to understand how ONH structural  
439        factors are associated with increased glaucoma risk.

440        A few studies have characterized the effects of acute manipulations of IOP and ICP on  
441        prelaminar neural tissue displacement. Zhao et al. showed posterior movement of the ONH  
442        surface and surrounding peripapillary retina with IOP elevation, and greater displacement at lower  
443        ICP using a rat model<sup>18</sup>. These results are concordant with the study conducted by Morgan et  
444        al. with dogs showing posterior displacement of the disc surface with IOP elevation, whereas

445 CSFP elevation prompted anterior displacement. This study also reported non-linear surface  
446 deformations as a function of TLPD where most displacement occurred in the low range  
447 translaminar pressure gradients <sup>15</sup>. In our 2017 study of 5 monkeys <sup>21</sup>, we similarly modulated  
448 IOP and ICP, stepwise, at a range of pressure settings. We observed a significant interaction  
449 between the effects of IOP and ICP on changes in LC microstructure. It is possible that the model  
450 used for the study described here also exhibits similar non-linear behavior. A comprehensive  
451 characterization of the deformations in the monkey ONH at more pressure combinations, as done  
452 previously in dogs<sup>15,51</sup>, is necessary to determine this.

453 Feola et al. <sup>14</sup> utilized phase-contrast micro-CT to capture CSFP-induced deformations of  
454 the LC and retrolaminar neural tissue in an ex vivo porcine eye model. They found that variation  
455 of cerebrospinal fluid pressure greatly impacted the distribution of strain within the RLNT and to  
456 a lesser degree, the LC as well. In line with the heterogeneity of the observations reported here,  
457 the spatial distribution of strains within the LC differed greatly among individual eyes.  
458 Understanding the factors that contribute to this heterogeneity of responses would be of great  
459 value in prediction of medical risk. Numerical models are shedding valuable light in the  
460 mechanistic interactions between the forces acting on the ONH, including IOP, ICP, blood  
461 pressure and tension from the optic nerve <sup>20,52,53</sup>.

462 Epidemiologic work has reported a correspondence between TLPD and both structural  
463 and functional glaucomatous changes <sup>13,54-56</sup>. In human subjects, the Valsalva maneuver was  
464 similarly shown to cause a greater acute increase in cerebrospinal fluid pressure than IOP,  
465 resulting in changes of ONH morphology <sup>50</sup>. Transiently altered TLPD was associated with  
466 decreased cup/disc ratio as well as maximum optic cup depth. Many of these studies involve  
467 subjects with chronic elevation or suppression in either ICP or IOP. As different IOP/ICP  
468 combinations with the same TLPD did not result in consistent deformation of the ONH, our results  
469 suggest that with acute manipulation, the interaction may be more involved. This is consistent  
470 with the findings of the numerical studies mentioned above. For instance, Hua et al found that the  
471 overall influence of TLPD was 28 times smaller than that of IOP, and weaker even than CSFP  
472 whose effect were 16 times smaller than those of IOP. In contrast, it is also important to note that  
473 there are a number of excellent papers which do discuss IOP/ICP in more detail. They do not  
474 trivialize TLPD and instead argue for the importance of dealing with IOP and ICP in a more  
475 nuanced way <sup>20,52,57,58</sup>. Our results support this conclusion as well.

476 In addition to the strengths, the limitations of this study must be recognized to best inform  
477 future directions. To evaluate the ONH response to acute, controlled changes in IOP and ICP,  
478 we focused on two structures with well-established relevance in analyzing effects of IOP, the ALC

479 and the scleral canal <sup>9,11,59</sup>. Future studies will need to incorporate analysis of additional factors  
480 known to influence mechanical insult in the ONH, such as cerebrospinal fluid pressure <sup>14</sup> and  
481 additional ONH structures, such as blood vessels <sup>60</sup> or even cellular components <sup>61-63</sup>. This can  
482 allow us to better understand the full effects of the pressure-induced changes. Besides  
483 macroscopic deformations of the scleral canal and ALC that were investigated in our study,  
484 microarchitecture, such collagen crimp <sup>64</sup>, could be an important measurement as well. Analysis  
485 of these factors will help to better explain the reason behind and the implications of macroscopic  
486 deformations reported here. Additionally, this will aid in assessment of whether particular regions  
487 of ONH could be loaded with more or less force in response to changes in IOP and/or ICP, and  
488 how this may eventually contribute to neural tissue insult <sup>65</sup>. We accounted for transverse scaling  
489 by relating OCT scans to histology in order to ensure an accurate baseline scale. Changes in  
490 axial length due to IOP changes could potentially result in scaling differences that were not  
491 accounted for. Lastly, the experimental protocol included setting several more IOP and ICP  
492 conditions than what we analyzed in this work. We decided to select a subset of pressures as a  
493 first analysis to evaluate the effects and potential interactions between IOP and ICP. Future  
494 studies should use a more comprehensive set of the data acquired. It is important to acknowledge  
495 the additional IOP and ICP conditions between the ones studied. These steps are important  
496 because they allow the eyes to stabilize after pressure changes, ensuring that our measurements  
497 are free from viscoelastic effects.<sup>21,24,26,27</sup>

498 We focused on the effects of acute variations in ICP and IOP. It is likely that the effects  
499 of chronic exposure to variable levels of ICP and/or IOP will have effects that are different from  
500 the acute ones, such as remodeling and inflammation. Understanding the role of ICP and IOP in  
501 glaucoma will require a careful characterization of the chronic effects of these pressures. We  
502 posit that understanding the acute effects of the pressures, as advanced in this work, is an  
503 essential and necessary step. In other words, to understand the long term process of glaucoma,  
504 we must also understand the short term biomechanics of the ONH. An improved understanding  
505 of acute and short term interactions between ICP and IOP may also be relevant in the  
506 development and screening of techniques to measure ICP non-invasively. Some techniques,  
507 for instance, are based on the concept of TLPD, which our study suggests may be  
508 problematic.<sup>14,66</sup>

509 A technical challenge for our analysis was the lack of an absolute frame of reference. This  
510 is a limitation that our study shares with other work on ONH morphometrics <sup>67</sup>. Although the BMO  
511 plane has been commonly used as a reference for measurements within the ONH <sup>38,68,69</sup>, pressure  
512 changes and pathology can cause BMO surface deformations. In this work, the effects of these

513 deformations were minimized by using BMO best-fit plane. For this reason, analysis between  
514 pressure settings and structural registration was based on the centroid and principal axes of the  
515 BMO best-fit plane. We chose this method because it is objective and repeatable, facilitating inter  
516 and intra-study comparisons. However, as we and others <sup>70</sup> have shown, the BMO itself is affected  
517 by IOP and ICP. Hence, it is possible that changing the registration would produce slightly different  
518 results. Future work should consider other potential methods of registration and measures of the  
519 ONH that are independent of the BMO <sup>27,71,72</sup>.

520 Because the subarachnoid space of both eyes and the brain are directly connected, it is  
521 not possible to manipulate ICP independently between two eyes within the same animal. The  
522 effects of ICP could be more profound in an eye that had been exposed to elevated ICP for a  
523 longer period of time. This would be the case in monkeys where both eyes were imaged, such  
524 as M3. One eye of M3 was exposed to ICP elevation prior to imaging due to earlier imaging of  
525 the contralateral eye. Interestingly, deformations of the scleral canal and ALC were larger in M3L  
526 when compared those in M3R. This was particularly profound in the cases of canal planarity and  
527 ALC depth.

528 It is important to articulate not only which changes were statistically significant, but which  
529 may be physiologically impactful. With limited information available about the risks associated  
530 with these particular degrees of ONH deformation on tissue health and vulnerability, it is not yet  
531 straightforward to determine which of these factors are associated with medically relevant risk.  
532 From a biomechanical perspective, studies suggest that it is often not the magnitude of the  
533 displacements, but their gradient (deformations) that are best predictors of damage.<sup>73,74</sup>  
534 Answering these questions will require separate investigations in future work.

535 We manipulated and measured ICP at the brain, whereas it is the cerebrospinal fluid  
536 pressure immediately behind the globe that directly impacts the ONH. <sup>12,13,15,51,54</sup> Although these  
537 two pressures are closely related, they are not necessarily identical, with likely differences in their  
538 magnitude and potentially even a time lag between changes in ICP translating to pressures within  
539 the orbit. It is still unknown if the 5 minutes we waited before imaging after a change in pressure  
540 are sufficient to allow the changes in ICP to fully translate to the orbit.

541 In summary, our study provided evidence of substantial changes in gross ONH  
542 morphology caused by acute changes in IOP and ICP that were unique to each individual eye.  
543 Additionally, we describe a significant interaction between the effects of ICP and IOP on the ONH  
544 scleral canal and LC. Altogether, our results show that ICP affects sensitivity to IOP, and thus that  
545 it can potentially also affect susceptibility to glaucoma.

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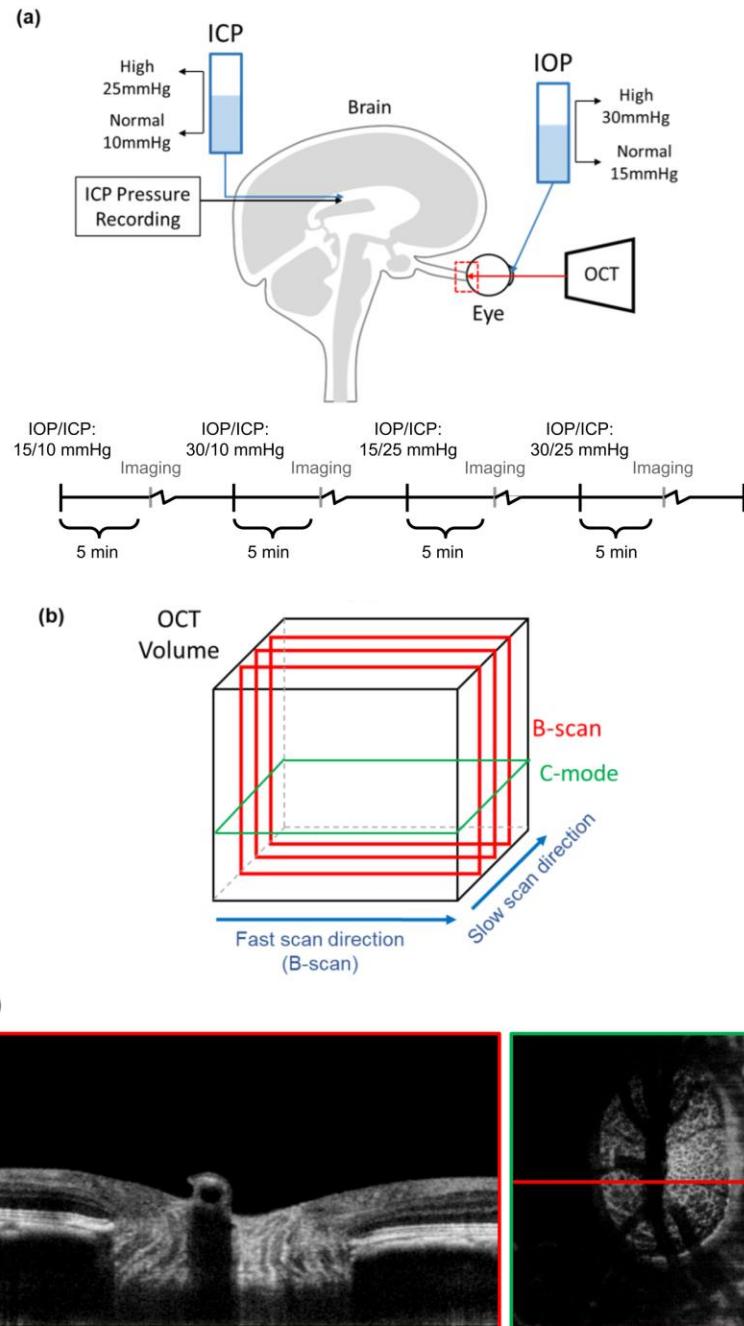
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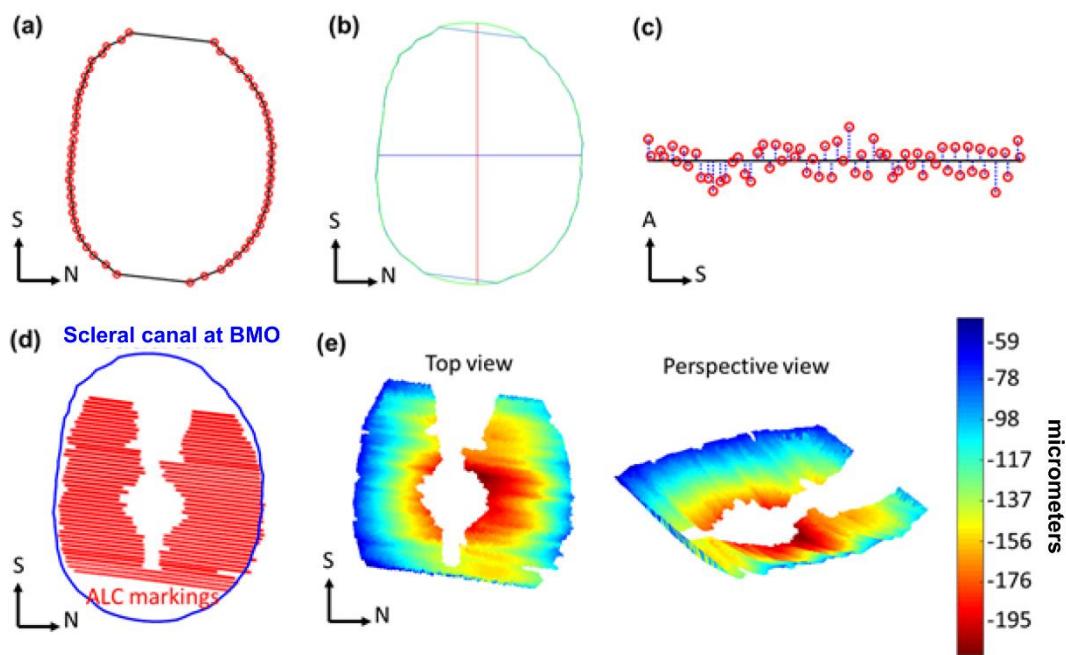
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## 746 Tables and Figures



748 **Figure 1:** (a) Diagram of in vivo experimental set up and timeline, in which both intraocular and  
749 intracranial pressures were controlled via gravity perfusion while the optic nerve head region (red) was  
750 imaged with optical coherence tomography. This study focuses on analysis of the four IOP/ICP  
751 conditions highlighted. The experimental protocol included other IOP/ICP conditions between the ones  
752 highlighted. See the main text for details. Motion artifacts in the slow scan direction were removed (b).  
753 Example B-scan and C-mode views at the lamina cribrosa level acquired with an IOP of 15 mmHg and  
754 ICP of 8 mmHg (c).

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757 **Figure 2:** Example markings of Bruch's membrane opening, (BMO, red) (a). Example scleral canal area  
758 (within green perimeter), interpolated from BMO markings, and its corresponding principal axes (red, blue)  
759 (b). Scleral canal planarity was calculated as the average of distances (blue) from BMO markings (red) to  
760 BMO best-fit plane (black) (c). Example scleral canal (blue) and anterior lamina cribrosa (ALC) markings  
761 (red) used to reconstruct ALC surface and compute ALC depth (d). Heat maps of ALC depth (shallow to  
762 deep: blue to red) (e). S: Superior, N: Nasal, A: Anterior.

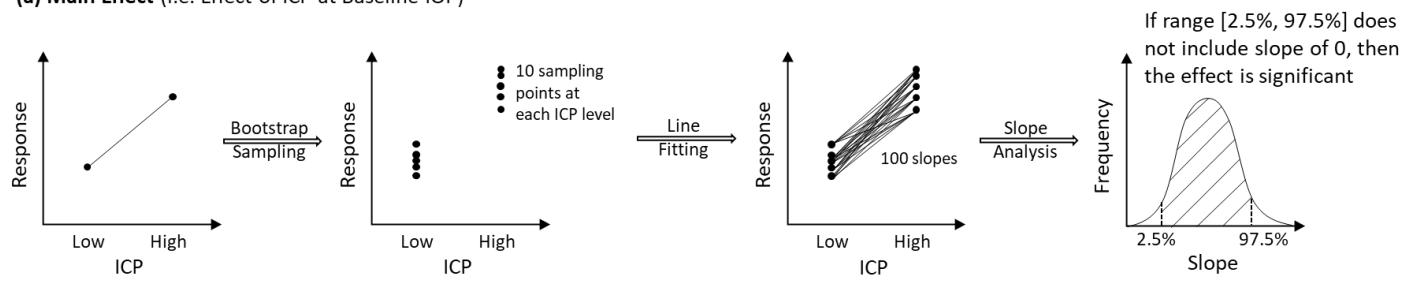
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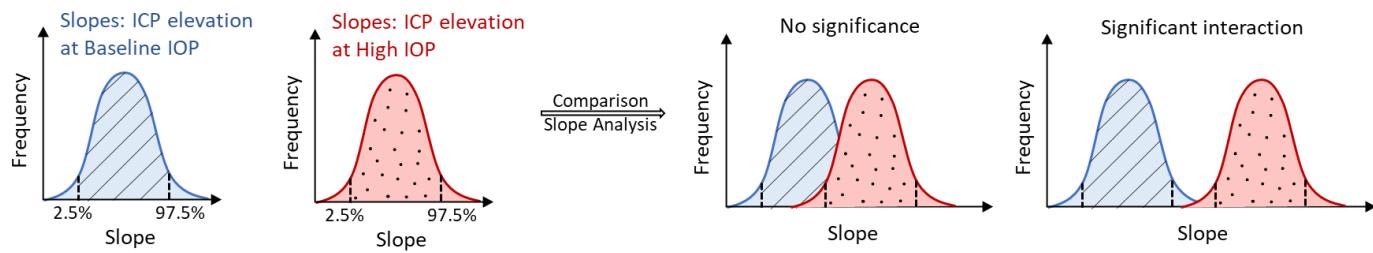
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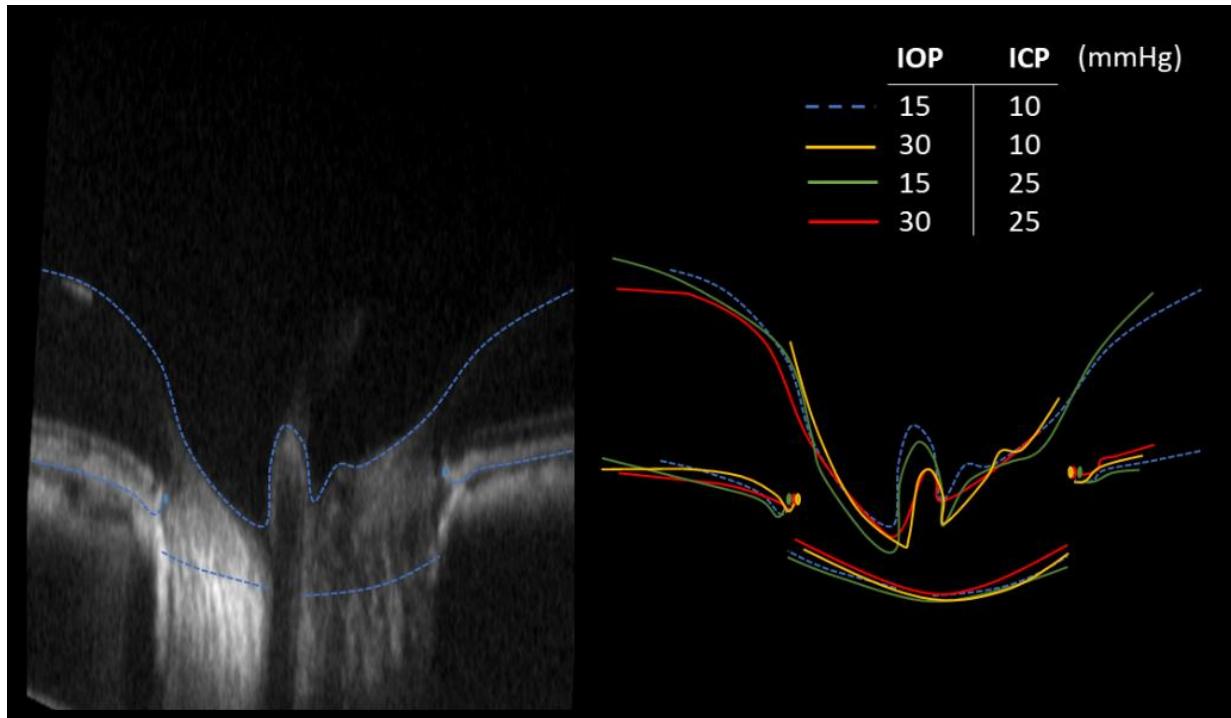
**(a) Main Effect (i.e. Effect of ICP at Baseline IOP)**



**(b) Interaction Effect**

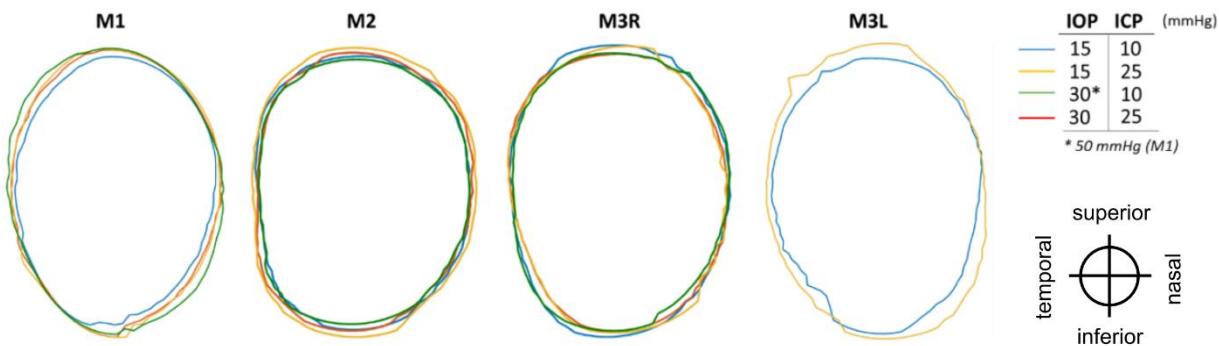


767 **Figure 3:** Diagram of statistical tests for the main effects of IOP, ICP (a) and the interaction of their effects  
768 (b). (a) Main effect: Bootstrap sampling was used to generate 20 sampling points, 10 sampling points at  
769 each of 2 ICP levels. Fitting lines through these points, 100 slopes and their 95% range (between 2.5%  
770 and 97.5%), were computed. A significant main effect was detected if the range did not include a slope of  
771 0. (b) Interaction effect: From left to right: Similar procedure in (a) is performed to generate slopes and  
772 their two corresponding ranges due to ICP elevation at baseline and at high IOP. A significant interaction  
773 between ICP effects and IOP effects was detected if these two ranges did overlap.



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776 **Figure 4:** Example qualitative comparison of effects of IOP and ICP. (Left) Baseline B-scan and markings.  
777 (Right) Overlay markings from all pressure conditions on baseline B-scan to demonstrate deformations of  
778 ONH structures. For this study we analyzed the scleral canal at BMO and the anterior boundary of the LC.  
779 In this image we also show delineations of the BM and the inner limiting membrane (including over the  
780 central retinal vessels). The dashed lines are delineations at the baseline IOP and ICP levels. Note that to  
781 simplify discerning the differences, the B-scan and outlines are shown exaggerated 3 times in axial  
782 direction, as is the common for presenting OCT.  
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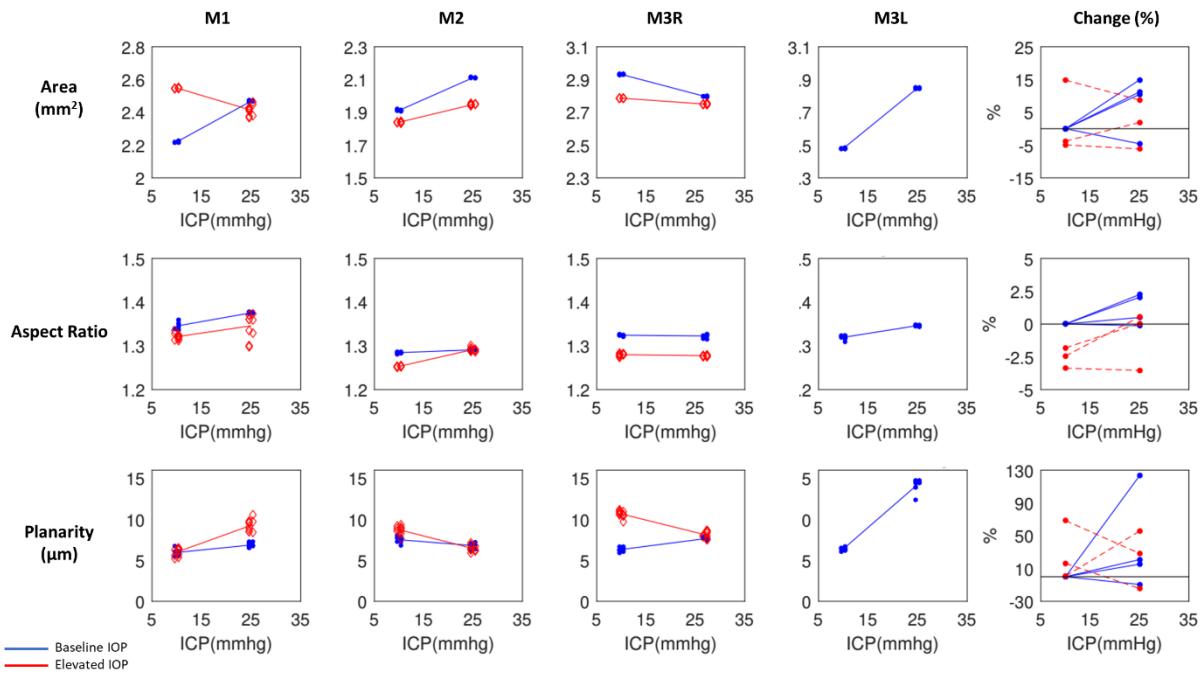


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**Figure 5:** Outlines of the scleral canal at the Bruch's membrane openings, for each eye at 4 pressure conditions: baseline (blue), base IOP/high ICP (yellow), high IOP/base ICP (green), and high IOP/high ICP (red). Outlines were registered rigidly by the centroid and principal axes. Images of M3L at elevated IOP had poor LC visibility and were therefore excluded from analysis. Orientation of eyes as displayed is indicated at the lower right-hand side.

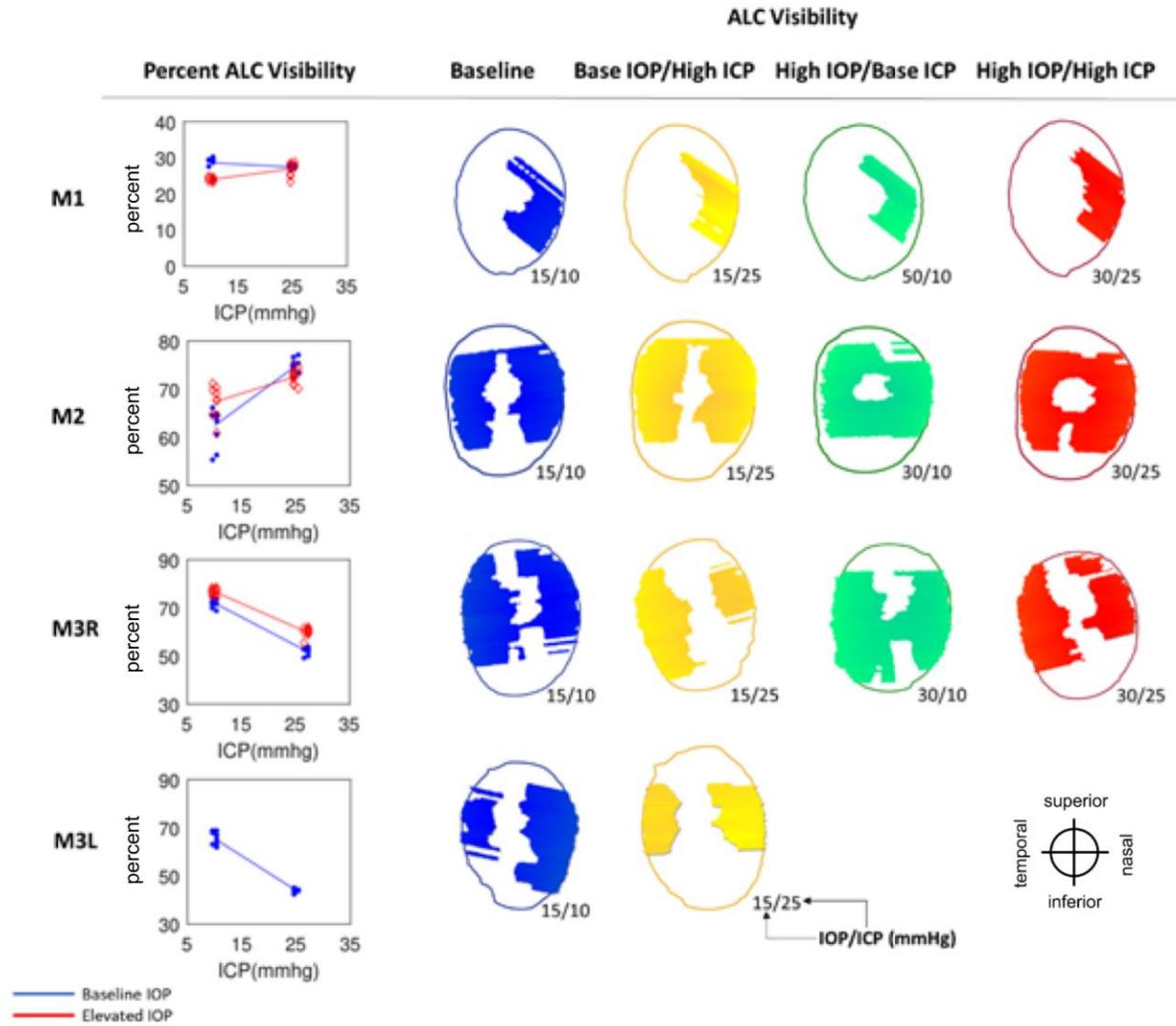
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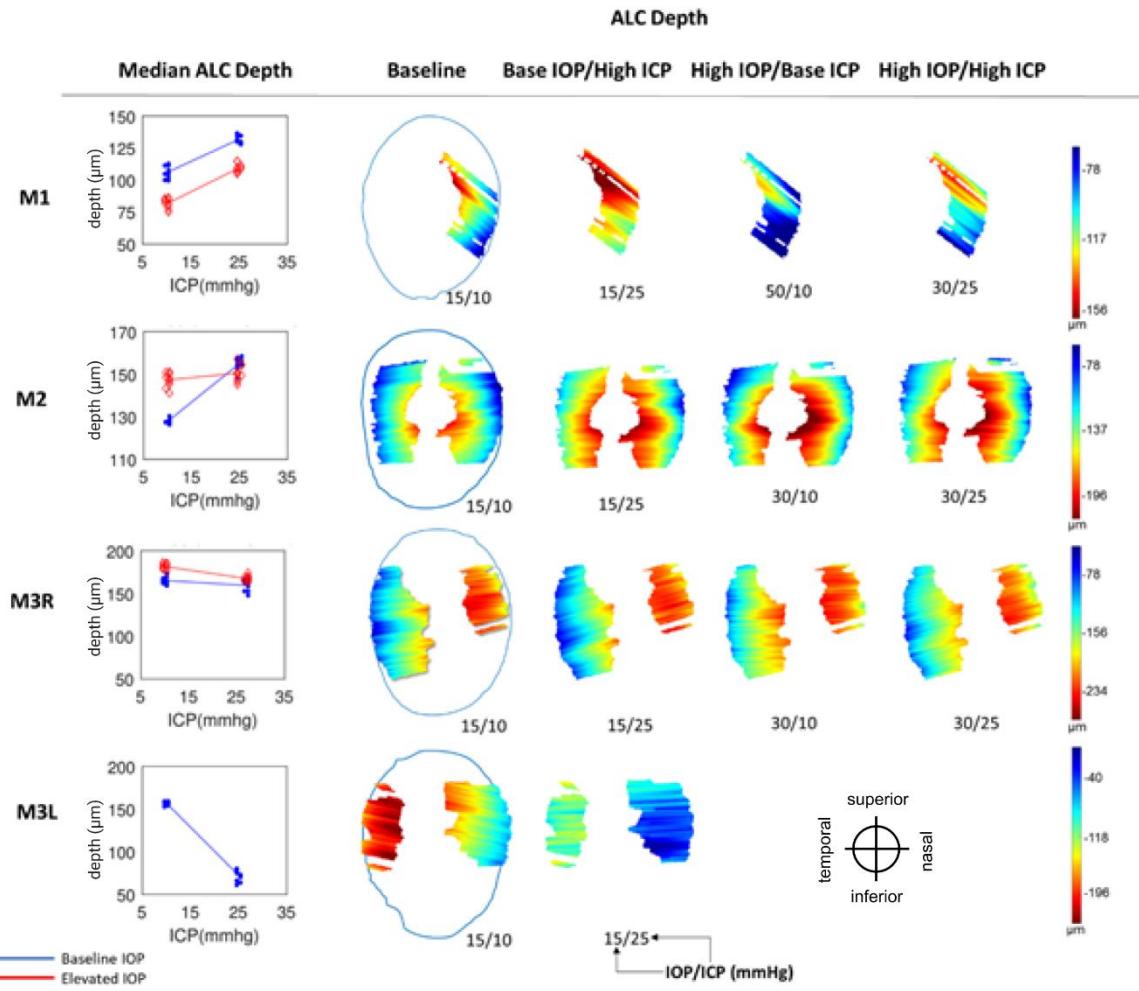
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797 **Figure 6:** Scleral canal displacements due to variations in intraocular (IOP) and intracranial (ICP)  
798 pressures. Percentage changes of BMO area, aspect ratio, and planarity with respect to baseline values  
799 due to ICP elevation at baseline IOP (blue) and elevated IOP (red). Each line represents the regression of  
800 the estimates, or average of 10 bootstrap sampling points, at each ICP. To reduce overlap, the symbols  
801 were scattered laterally slightly.



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803 **Figure 7:** Anterior lamina cribrosa (ALC) visibility. (Left) Percentage of ALC visibility at baseline IOP  
 804 (blue) and elevated IOP (red). Each line represents the regression of the estimates, or average of 10  
 805 bootstrap sampling points, at each ICP. (Right) Maps of ALC visibility. Shown are canal outline (thin line)  
 806 and ALC for 4 pressure conditions: baseline (blue), baseline IOP/high ICP (yellow),  
 807 high IOP/base ICP (green), and high IOP/high ICP (red). Pressures for each condition are indicated as IOP/ICP.



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**Figure 8:** Anterior lamina cribrosa (ALC) depth. (Left) Median ALC depth at baseline IOP (blue) and elevated IOP (red). Each line represents the regression of the estimates, or average of 10 bootstrap sampling points, at each ICP. (Right) Heat maps of ALC depth (blue to red: shallower to deeper) with respect to scleral canal (blue outline), shown only on regions visible across all 4 pressure conditions within an eye. Pressures for each condition are indicated as IOP/ICP.

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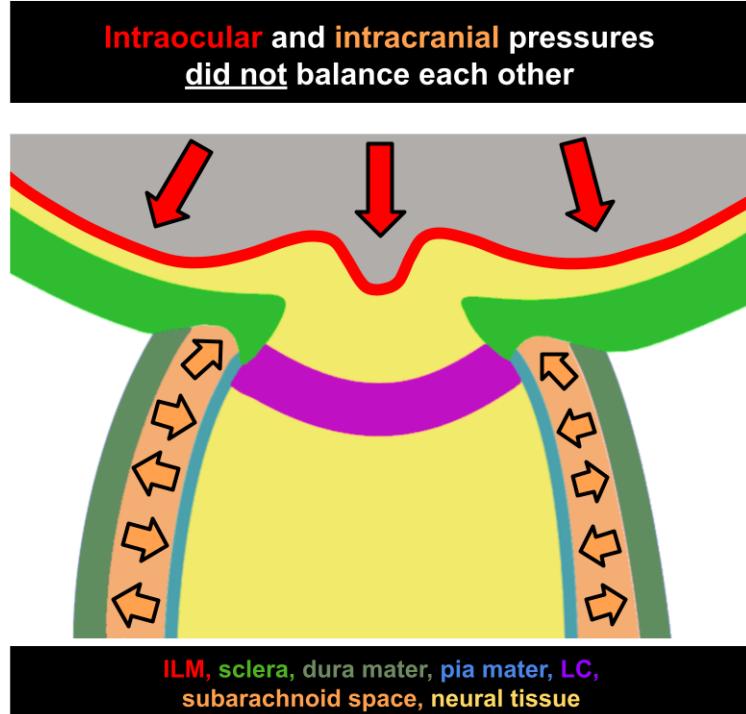
Effect of	BMO Area		BMO Aspect Ratio		BMO Planarity		ALC Median Depth		ALC Visibility		<i>Under Condition</i>
	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH	
ICP	M1	○	○	○		○	○	○			IOP
	M2	○	○		○		○	○		○	
	M3R	○	○			○	○		○	○	
	M3L	○	-	○	-	○	-	○	-	○	
IOP	M1	○	○	○	○	○	○	○	○	○	ICP
	M2	○	○	○			○				
	M3R	○	○	○	○	○	○		○	○	
	M3L	-	-	-	-	-	-	-	-	-	

"○"=Significant, "-"=N/A

 =Significant interaction

819 **Figure 9:** Summary of statistical results showing the significance of IOP and ICP independent effects  
820 ("○") as well as the significance of their interaction effects (red box) on ONH structures. Scleral canal  
821 measurements taken at BMO.

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**Figure 10:** Intraocular and intracranial pressures do not balance each other. Diagram of the ONH with representations of the inner limiting membrane (ILM, red), sclera (green), dura mater (gray-green), pia mater (blue), lamina cribrosa (LC, purple), subarachnoid space (orange), and neural tissue (yellow). Direction of force placed by intraocular pressure (red arrows) and intracranial pressure (orange arrows.)

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**Table 1:** Baseline parameters of the scleral canal, measured at the Bruch's membrane opening, and of the anterior lamina cribrosa across 4 eyes. Overlap visibility (\*) is the common visible region of the lamina across all analyzed conditions within each eye.

Parameters		M1	M2	M3R	M3L	Average
Scleral Canal at BMO	Area (mm <sup>2</sup> )	2.2	1.9	2.9	2.5	2.4
	Aspect ratio	1.3	1.3	1.3	1.3	1.3
	Planarity (μm)	6.0	7.5	6.3	6.4	6.5
Anterior Lamina Cribrosa	Visibility (%)	29	62	72	65	57
	Median depth (μm)	107	128	166	157	139
	Overlap visibility (%)*	15	45	38	37	34

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**Table 2:** Summary of changes of ONH parameters as a response to pressure variations. LOW: baseline pressure; HIGH: elevated pressure. These ranges of the parameters were used to test the significance of parameter effects and interactions. Dashes indicate relationships that could not be computed due to images of insufficient quality.

Effect of	BMO Area (mm <sup>2</sup> )		BMO Aspect Ratio		BMO Planarity (μm)		ALC Median Depth (μm)		ALC Visibility (%)		
	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH	
ICP	M1	0.25	-0.13	0.030	0.025	0.9	3.3	25	28	-1	3
	M2	0.20	0.11	0.006	0.039	-0.7	-2.3	28	3	12	5
	M3R	-0.14	-0.04	-0.002	-0.002	1.3	-2.5	-8	-14	-20	-17
	M3L	0.37	-	0.027	-	7.9	-	-83	-	-22	-
IOP	M1	0.33	-0.05	-0.024	-0.030	0.0	2.4	-25	-22	-5	0
	M2	-0.07	-0.16	-0.031	0.001	1.2	-0.4	20	-5	5	-2
	M3R	-0.14	-0.05	-0.045	-0.045	4.3	0.5	15	9	5	8
	M3L	-	-	-	-	-	-	-	-	-	-

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**Table 3:** Summary of mean regression slopes corresponding with cases which demonstrated significant effects of IOP, ICP, and IOP-ICP interaction. LOW: baseline pressure; HIGH: elevated pressure. Dashes indicate relationships that could not be computed due to images of insufficient quality. Blank cells indicate results that were not significant.

Effect of	BMO Area (mm <sup>2</sup> )		BMO Aspect Ratio		BMO Planarity (μm)		ALC Median Depth (μm)		ALC Visibility (%)	
	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH
ICP	M1	0.017	-0.009	0.002			0.217	1.736	1.857	
	M2	0.013	0.007		0.003		-0.155	1.821		0.818
	M3R	-0.008	-0.002			0.078	-0.170		-0.927	-1.173 -1.114
	M3L	0.025	-	0.002	-	0.527	-	-5.650	-	-1.435 -
IOP	M1	0.009	-0.004	-0.001	-0.002		0.159	-0.693	-1.497	
	M2	-0.005	-0.011	-0.002				1.304		
	M3R	-0.010	-0.002	-0.003	-0.003	0.290		1.087		0.314 0.529
	M3L	-	-	-	-	-	-	-	-	-

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