Three-Dimensional Reconstruction of Normal and Early Glaucoma Monkey Optic Nerve Head Connective Tissues

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PURPOSE. To introduce high-resolution, digital three-dimensional (3-D) reconstruction of the connective tissues of the optic nerve head (ONH).

METHODS. Trephinated ONH and peripapillary sclera from both eyes of three monkeys with early glaucoma (EG; one eye normal, one eye given laser-induced EG) were embedded in paraffin and serial sectioned at 3-μm thickness from the vitreous surface through the orbital optic nerve, with the embedded tissue block face stained and imaged after each cut. Each image was aligned, and then the scleral canal wall, sclera, border tissue of Elschnig, Bruch’s membrane, lamina cribrosa, optic nerve septa, pial sheath, and vasculature were delineated as unique objects. Delineated images were stacked, color mapped, and volume rendered and then serial sagittal and transverse digital sections of the resultant voxel geometries were viewed and measured.

RESULTS. Substantial differences in the 3-D architecture of the peripapillary sclera, scleral canal wall, and lamina cribrosa were present among the three normal eyes. All three EG eyes displayed permanent posterior deformation of the central lamina cribrosa, as well as expansion of the anterior and posterior neural canal openings in comparison with their respective contralateral normal control eyes. Peripherally, whereas laminar deformation was greatest inferiorly or superiorly in all three EG eyes, statistically significant deformation was present in all four quadrants of all three eyes.

CONCLUSIONS. High-resolution, digital 3-D reconstructions of the load-bearing connective tissues of the monkey ONH confirm that the ONH connective tissues are profoundly altered at the onset of detectable ONH surface change in experimental glaucoma. (Invest Ophthalmol Vis Sci. 2004;45:4388–4399) DOI:10.1167/iovs.04-0022

The connective tissues of the peripapillary sclera, scleral canal wall, and lamina cribrosa have long been thought to play a central role in the pathophysiology of glaucomatous damage to the retinal ganglion cell axons within the optic nerve head (ONH). To date, these tissues have been extensively studied using light and electron microscopy1–4 and initial attempts have been made at three-dimensional (3-D) reconstruction.45,46 However, until now, the high-resolution, digital, 3-D reconstructions necessary to model accurately the levels of intraocular pressure (IOP)-related stress (force/cross-sectional area) and strain (local deformation) to which these structures are subjected have not been attempted.

Modeling IOP-related stress and strain within the ONH connective tissues is important for several reasons. First, regardless of the mechanism of insult or the level of IOP at which it occurs, we propose that IOP-related connective tissue stress and strain underlies and predicts the onset and progression of glaucomatous axonal insult. From a biomechanical standpoint, the pathophysiology of the axons in the glaucomatous optic neuropathies is likely to be multifactorial, with the principal mechanisms of axonal insult (astrocyte and glial cell activation, ischemia, and physical compression) acting alone or together according to the region of the ONH and the stage of underlying connective tissue and cellular involvement.45,46 However, we propose that it is a defining feature of the glaucomatous optic neuropathies that, regardless of mechanism, the distribution of IOP-related stress and strain within the ONH connective tissues underlies and predicts the location of axonal insult within the ONH, whether the insult occurs at normal or elevated levels of IOP.45,46

Second, we propose that the distribution of IOP-related stress and strain also underlies and predicts the pattern of connective tissue damage (referred to as mechanical failure) and that this pattern of connective tissue damage both explains the clinical phenomenon known as glaucomatous cupping and is a second defining feature of the neuropathy. By this, we mean that for an individual ONH to demonstrate classic glaucomatous cupping, its connective tissues must become damaged and undergo mechanical failure in a process that is again governed by the distribution of IOP-related stress and strain, regardless of the level of IOP at which it occurs.45,46

IOP-related stress and strain within the connective tissues of the ONH can be studied using finite element modeling, which is an engineering technique for modeling the distribution of stress and strain within complicated structures. Constructing a finite element model requires a digital geometry of the structure being modeled and estimates of the structure’s material properties (stiffness) and boundary and loading conditions.47–50 For the past 8 years, we have worked on all three components of this process to build finite element models of the normal and EG monkey ONH.51–54

We have previously demonstrated permanent deformation of the ONH connective tissues (the onset of mechanical failure) within serial histologic sections of monkey eyes with early experimental glaucoma.41 In the present report, we introduce our method for high-resolution, digital, 3-D reconstruction of the ONH connective tissues and describe its use to compare normal and EG ONH connective tissue architecture within three new monkeys with early experimental glaucoma in one eye.
Materials and Methods

Animals
All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Three male cynomolgus monkeys, approximately 8 years of age, were used (Table 1).

ONH Surface Compliance Testing and EG
We have described our confocal scanning laser tomography (CSLT)-based (TopSS, Laser Diagnostics Technology, San Diego, CA) ONH surface compliance testing strategy. Briefly, compliance testing involved measurement of the parameter mean position of the disc (MPD), which describes the position of the surface of the ONH relative to the peripapillary retinal nerve fiber layer. Both eyes of each monkey were compliance tested on three separate occasions to provide the prelaser MPD baseline ± 95% confidence interval (CI) for normal eyes. Then one eye of each monkey was given laser-induced experimental glaucoma (EG eye), and compliance testing of both eyes was repeated at 2-week intervals until the onset of permanent posterior deformation of the ONH surface (defined as MPD baseline ± 95% CI below prelaser MPD baseline ± 95% CI) in the EG eye on two successive postlaser imaging sessions, at which point the monkey was killed (Fig. 1).

Monkey Euthanasia and Fixation
For each monkey, deep pentobarbital anesthesia was induced, both eyes were cannulated with a 27-gauge needle, and the IOP was set to 10 mm Hg by using an adjustable saline reservoir. After a minimum of 30 minutes, the monkey was perfusion fixed via the descending aorta with 1 L of 4% buffered hypertonic paraformaldehyde solution followed by 6 L of 5% buffered hypertonic glutaraldehyde solution. Both the paraformaldehyde and glutaraldehyde were buffered in 1 M Sorenson’s solution (0.15 M K2HPO4 and 0.85 M Na2HPO4). Osmolarity of each solution was approximately 0.4 osmol/L. After perfusion fixation, IOP was maintained for 1 hour, after which each eye was enucleated, all extraorbital tissues were removed, and the anterior chamber was removed 2 to 3 mm posterior to the limbus. By gross inspection, perfusion was excellent in all six eyes. The posterior scleral shells with intact ONH, choroid, and retina were placed in 5% glutaraldehyde solution for long-term storage.

Generation of the Aligned Serial Section Images for Each ONH
For each eye, the ONH and surrounding peripapillary sclera were trephined (6-mm diameter), pierced with four to seven 10-0 polypropylene alignment sutures (Prolene; Ethicon, Somerville, NJ), and photographed to document the position of the alignment sutures in the clinical ONH surface (Fig. 2). The tissue was dehydrated through a graded series of ethanols, cleared in chloroform, and infiltrated with paraffin (TissuePrep 2; Fisher Scientific, Pittsburgh, PA) using an automatic tissue processor (Tissue-Tek II; Ames Division, Miles Laboratories, Inc., Elkhart, IN). The specimen was oriented in the desired position, embedded in paraffin, and mounted on a microtome (RM2165; Leica, Wetzlar, Germany), so that the vitreous side of the ONH surface was visible in the block in a clinically appropriate orientation (Fig. 2). A 1080 × 1520-pixel chip camera (Spot RT; Diagnostic Instruments, Sterling Heights, MI) was positioned so that an image of the centered, embedded tissue block surface could be acquired at a resolution of 2.5 × 2.5 µm per pixel (Fig. 3). The block was then sectioned without imaging until the cutting plane was just superficial to the internal limiting membrane of the retina. At that point, a 1:1 (vol/vol) mixture of Ponceau S and acid fuchsin stains, which stained only the exposed connective tissues, was manually applied to the surface of the tissue block, the excess stain was blotted away, and the stained surface of the embedded tissue block was imaged. Then another 3.0-µm section was cut away, and the staining and imaging process was repeated.

Table 1. Animal and Eye Data for Three Cynomolgus Monkeys

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<th>Monkey ID</th>
<th>Age (y)</th>
<th>Eye</th>
<th>Weight (kg)</th>
<th>Axial Length (mm)</th>
<th>Duration of Test Elevation (day)*</th>
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* Estimated time from detection of elevated IOP to death.
† Axial length at an IOP of 10 mm Hg during the final compliance testing session.
‡ Connective tissues of the pial sheath not included in the calculation.
§ Ratio of neural area to total area (excluding the pial sheath).
Thus, for each ONH, imaging began at the vitreous–retinal interface and continued through the lamina cribrosa, approximately 200 \( \mu m \) into the orbital optic nerve. Depending on the degree of tilt of the embedded tissue, the number of sections obtained per eye ranged from 222 to 411. Each section image was aligned in the \( z \)-direction (anterior to posterior) by using custom software to register the cut ends of the embedded polypropylene sutures that were visible within each section (Fig. 4).

**Voxel Geometry Construction and Visualization**

The principal steps of voxel geometry construction were performed for both eyes of each monkey by a single operator. A separate operator processed the voxel geometry for visualization. The entire process is depicted in Figures 5 and 6 and outlined in the following sections.

**Object Segmentation within Each Section Image.** Manual and semiautomated selection tools developed within custom and
commercial software (Photoshop, ver 7.0; Adobe Systems, Inc., San Jose, CA) were used to isolate the connective tissues in the individual section images. First, the neural boundary, defined as the outermost extent of the neural tissues in the scleral canal, was segmented in each section image (Fig. 5). This line marked the boundary between the neural and connective tissues starting from the anteriormost sclera as it forms the anterior scleral canal wall, and continuing through the posterior pial sheath. The neural boundary thus demarcates the area in which the lamina cribrosa and retrolaminar optic nerve septa are located. Second, a mask for the nonconnective tissue areas was generated for each section image, covering the choroid, retina, nerve fiber layer, embedding medium, and subarachnoid space. The inverse of this mask covered all connective tissue objects present in each section image, including the sclera, Bruch’s membrane, the border tissue of Elschning, and the pial and dural sheaths of the optic nerve. Third, the central retinal vessels in each section image were manually segmented about the vertical axis of the intact geometry (Fig. 6C), to obtain images in 2° to 5° increments (not shown). Second, standard views of serial transverse, sagittal (vertical), and coronal (horizontal) sections through each geometry were generated (Fig. 5). Custom software constructed in Visual Basic C++ (Microsoft, Redmond, WA) was used to visualize each standard view for both eyes of a given monkey (Fig. 7).

**Voxel Geometry Visualization.** Voxel geometries were rendered with the open-source OpenDX data visualization software (http://www.opendx.org; provided in the public domain by IBM, Armonk, NY) or custom visualization software built using the Visualization Toolkit (VTK) library (http://www.vtk.org; provided in the public domain by Kitware, Clifton Park, NY). Because of the size of the geometries, the ability to interact dynamically with the entire geometry was not initially possible. Therefore, four sets of standard views were generated for each ONH. First, a software-viewing camera was rotated about the vertical axis of the intact geometry (Fig. 6C), to obtain images in 2° to 5° increments (not shown). Second, standard views of serial transverse, sagittal (vertical), and coronal (horizontal) sections through each geometry were generated (Fig. 5). Custom software constructed in Visual Basic C++ (Microsoft, Redmond, WA) was used to visualize each standard view for both eyes of a given monkey (Fig. 7).

**Qualitative and Quantitative Assessment of the Digital Geometries**

All qualitative comparisons were performed between the two eyes of each monkey using the simultaneous bilateral viewing software, as just described (Fig. 7). Measurements of (1) anterior laminar position, (2) laminar thickness, (3) horizontal and vertical neural canal diameter, and (4) peripapillary scleral thickness were performed within the five central horizontal and vertical digital section images (each section, 1 voxel wide) for each eye by a single observer, using a modified version of software that has been described previously (Fig. 8). Measurements of (1) anterior laminar position and thickness data for each eye were assessed overall and by central, superior, and inferior (vertical sections) or central, nasal, and temporal (horizontal sections) regions. Neural canal diameter was measured at its internal (Bruch’s membrane) and external (posterior scleral canal) openings within each section and regionalized into horizontal and vertical data for each eye. Peripapillary scleral thickness was measured at the posterior scleral canal opening within each section and pooled into superior and inferior (vertical sections) or nasal and temporal (horizontal sections) regions for each eye.
A nested ANOVA was used to assess the effects of region (central, superior, inferior, nasal, and temporal) and treatment group (normal and EG) on the parameters anterior laminar position and laminar thickness, both overall and between the eyes of each monkey. A second nested ANOVA evaluated the effects of quadrant (superior, inferior, nasal, and temporal) and treatment group on posterior scleral thickness, both overall and between the eyes of each monkey. A third nested ANOVA assessed the overall effects of treatment group on the parameters vertical and horizontal anterior neural canal diameter at Bruch’s membrane and the posterior scleral canal opening, both overall and between the eyes of each monkey.

Optic Nerve Neural Area Measurements
For each eye, the cross-sectional areas of the neural and connective tissues of the optic nerve were measured in a single section image located 20 sections (approximately 60 μm) beyond the last section image having recognizable lamina cribrosa (i.e., the last section image containing transversely oriented connective tissue fibers). The selected section image was binarized and the area within the neural boundary (excluding the pial sheath connective tissues immediately surrounding the nerve) was thresholded using image-processing software (Photoshop ver. 5.5; Adobe Systems, Inc.) so as to isolate the connective tissue septa. The areas of the neural and connective tissues within the neural boundary were then calculated with custom software. To increase our ability to compare these areas among eyes that had been cut at different angles, the ratio of neural area to total area (percent neural area relative to total ONH area) was then calculated for each eye.

RESULTS
Descriptive data for the three monkeys and six eyes are reported in Table 1.

Experimental Glaucoma
Monkeys 2 and 3 were killed three weeks and monkey 1 six weeks after the onset of CSLT detection of a change (permanent posterior deformation) in the ONH surface. The delay in killing monkey 1 was the result of an unexpected decrease in the MPDbaseline in the normal eye, which eventually returned to normal. In monkeys 1 and 2, IOP elevations were moderate, with only one measurement higher than 30 mm Hg. In monkey 3, elevated IOP was not detected (Table 1, Fig. 1). Postlaser IOP and MPDbaseline data for the study eye and the contralateral normal eye are shown for each monkey in Figure 1.

In monkeys 1 and 2, the percentage of neural area of the EG eye was less than that of the normal eye (4.7% and 4.5% less, respectively), whereas in monkey 3, the percentage of neural area of the EG eye was 0.6% larger than that of the normal eye (Table 1).
Peripheral deformation of the lamina at its insertion into the scleral canal wall was visible in the superior, inferior, nasal, and temporal peripheral canal sections of monkey 1 (Fig. 11). Although the greatest peripheral laminar deformation was seen in the inferior (monkeys 1 and 3) and superior (monkey 2) laminar insertion zones (Table 2), statistically significant deformation of the peripheral lamina occurred in all four quadrants of all three EG eyes, relative to their respective contralateral normal control eyes (Table 2).

**Mild to moderate thickening of both the lamina and sclera was present at the posterior scleral canal opening in most regions of the EG eyes (Table 2). Expansion of the neural canal at both its internal (Bruch’s membrane) and external (posterior scleral canal) openings was present in both the horizontal and vertical axes of two of the three EG eyes (Table 2).**

**DISCUSSION**

Accurately characterizing the 3-D geometry of the lamina cribrosa within the scleral canal has been the goal of a large and important body of research. In the present report, we introduce a new method for high-resolution, digital 3-D reconstruction of the ONH tissues and use it to visualize and measure differences within the connective tissue voxel geometries of both eyes of three monkeys with induced EG in one eye. Several aspects of both the method and findings are important to discuss.

Our method achieves high resolution for three reasons. First, we acquired images at relatively high magnification compared with the structures being modeled (2.5 × 2.5 μm pixel). Second, we acquired new z-axis data every 3 μm (achieving a

**Physiologic Variation within the Three Normal Monkey Eyes**

**Surface Views of the Intact Geometry.** The clinical (CSLT) and histologic (voxel geometry) anterior scleral canal openings of each ONH are visible in Figure 9. Because the scleral voxels are partially translucent, the peripapillary sclera immediately adjacent to the canal wall is most transparent due to its relative thinness as the neural tissues become myelinated and expand to form the orbital optic nerve.

**Standard Serial Digital Sections.** Representative horizontal and vertical sections through the midcanal are shown in Figure 10. Quantitative measurements of the central five horizontal digital sections from each eye are reported in Table 2. Note that the peripapillary sclera and lamina of the normal eye of monkey 2 are substantially thicker than they are in monkeys 1 and 3. In addition, the scleral canal is less oblique relative to the plane of the sclera in monkey 2, compared with monkeys 1 and 3. In monkey 2, the canal passes almost perpendicular to the sclera, whereas in monkeys 1 and 3 the canal shows the more common nasal-to-temporal oblique orientation.

**Comparison of the Normal and EG ONH of Each Monkey**

**Standard Serial Digital Sections.** The horizontal and vertical sections in Figure 10 and the quantitative measurements in Table 2 demonstrate marked posterior deformation of the central lamina in the EG eyes of all three monkeys. Central deformation was greater than peripheral deformation in all three EG eyes (greater in monkeys 1 and 3 than in monkey 2).

Thus, the section plane in (A) proceeds through the dura (d), expanded arachnoid space (a), pia (p), retrolaminar optic nerve (on), lamina (l), central vascular tree (cv), prelaminar neural bundles (pla), Bruch’s membrane flange, the junction with the border tissues of Elschnig (bt), and the choroid (c). The oblique embedding angle can be further appreciated within the central vertical (E) and horizontal (F) digital sagittal sections, which demonstrate the position of the connective tissues within the stacked sections (top, vitreous; bottom, orbit) relative to the orientation of the cutting plane, which is horizontal (proceeding from top to bottom) in these views.

**Three EG eyes (greater in monkeys 1 and 3 than in monkey 2).***

**Peripheral deformation was greater than peripheral deformation in all three EG eyes.***

**The peripapillary sclera and lamina of the normal eye of monkey 2 are substantially thicker than they are in monkeys 1 and 3.***

**In monkey 2, the canal passes almost perpendicular to the sclera, whereas in monkeys 1 and 3 the canal shows the more common nasal-to-temporal oblique orientation.***

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by cutting a 3-μm section and obtaining a fresh, unstained block face after each cut. Third, we acquired each of the 200 to 600 section images for a given ONH with perfect 3-D registration, because we imaged the cut surface of the embedded tissue block rather than free histologic sections that shrink and become distorted relative to one another once cut from the block.

The voxel geometries reported herein reconstruct the entire passageway for the ganglion cell axons through the wall of the eye. We propose that this passageway should be referred to, both clinically and histologically, as the neural canal, which extends from its internal opening at Bruch’s membrane to its external opening at the posterior scleral canal and consists of prescleral and scleral components. The prescleral portion of the neural canal extends from its internal opening at the optic disc (the clinically visible opening in Bruch’s membrane) along the inner surface of the border tissues of Elschnig to their insertion into the sclera. The scleral canal portion of the neural canal extends from the anterior to the posterior surface of the sclera.

Our concept of the neural canal has several important implications regarding the relationship between the clinical disc (Bruch’s membrane opening or the internal entrance to the neural canal) and the actual anterior and posterior openings in the sclera. First, characterizing the architecture of the entire neural canal may be more important than estimating the size and shape of the optic disc (its clinically visible internal opening). We have previously reported that differences in the architecture of the scleral portion of the neural canal (large size, elliptical shape, and thin peripapillary sclera) should markedly increase the level of IOP-related connective tissue stress for a given level of IOP.51 The 3-D reconstructions presented herein suggest that the size and shape of the clinical disc may not adequately convey the architectural complexity of the underlying scleral canal.

Second, two features of the neural canal determine the thickness of the peripapillary sclera (Fig. 5C): (1) the degree to which the nerve expands within the neural canal, which can be defined by the difference in size of the posterior relative to the anterior neural canal opening; and (2) the oblique orienta-
the anterior and posterior surfaces of the lamina cribrosa (red), the posterior scleral canal opening, defined as the posteriormost aspect of the insertion of the load-bearing sclera into the neural canal (orange), and the anterior and posterior surfaces of the peripapillary sclera (dark green). Once the section was marked, custom software divided the distance between the two Bruch’s membrane termination points into nine equidistant measurement locations, dropped perpendicular lines to the anterior lamina cribrosa, and extended those lines normal to the anterior lamina surface down to the posterior laminar surface. Automatic measures of the following parameters were thus generated: nine equally spaced measurements of anterior laminar position relative to Bruch’s membrane opening and laminar thickness (vertical yellow lines); one measurement each of the diameter of the neural canal opening at Bruch’s membrane (horizontal yellow line) and the posterior scleral canal opening (horizontal orange line); and measurements of peripapillary scleral thickness beginning at the posterior scleral canal opening and repeated every 100 μm (vertical orange lines) to the peripheral edge of the section. For this report, only scleral thickness at the posterior scleral canal opening is reported because more peripheral measurements were not available for all four quadrants of all six eyes.

The principal findings within the normal and EG connective tissue voxel geometries are as follows. First, substantial differences in the 3-D architecture of the peripapillary sclera, scleral canal wall, and lamina cribrosa architecture are apparent within the normal eyes of the three monkeys. Second, posterior deformation of the central lamina cribrosa is evident within each of the EG eyes, compared with the respective contralateral normal eye. Third, in the periphery, laminar deformation beneath Bruch’s membrane is greatest inferiorly or superiorly in all three EG eyes; however, mild to moderate excavation is also present in the other three quadrants in each of these eyes. Fourth, mild thickening of the lamina and peri-

**FIGURE 9.** Voxel geometry surface image and CSLT reflectance image for the right and left optic nerve head of each monkey. The surface of the voxel geometry and the CSLT reflectance image are shown. All structures within the voxel geometries have been made partially transparent to improve visibility of the deeper structures. The orientation of the voxel geometry relative to the CSLT image has not been aligned and is shifted somewhat clockwise in the left eye of monkey 1 and counterclockwise in the right eye of monkey 3. In the voxel geometries for monkeys 1 and 3 (right eye, normal; left eye, EG), the border and flange tissues of the anterior scleral canal opening are orange. The innermost aspect of this circle corresponds to the clinically apparent anterior scleral canal opening within the CSLT reflectance image of each eye. In monkey 2 (right eye, EG; left eye, normal), a slightly altered protocol was used, resulting in the border and flange tissues being assigned the same yellow color as the sclera. N, nasal; T, temporal.
papillary sclera (at the posterior scleral canal opening) is present in most quadrants of all three EG eyes.

In a previous study, we cut 16 immersion-fixed (IOP, 0 mm Hg) and 18 perfusion-fixed (IOP, 10, 30, or 45 mm Hg) monkey ONHs from four separate groups of monkeys into serial, 4-μm sagittal histologic sections and measured anterior laminar position and thickness and scleral canal diameter in digitized images of every sixth section to study the lamina cribrosa and anterior scleral canal wall in the normal and EG monkey eye at various levels of IOP.\(^4\) That study was the first to report that the lamina cribrosa demonstrates both central and peripheral fixed posterior deformation at this early stage of the neuropathy. The implications of this early connective tissue deformation were extensively discussed in that report. The present study confirms these findings in a new group of three EG eyes.

The laminar position data in the current report are similar to the values we have previously reported for histologic specimens.\(^4\) However, laminar thickness, peripapillary thickness, and neural canal diameter at Bruch’s membrane are substantially smaller in the 3-D reconstructed cynomolgus eyes of this study, compared with histologic data reported previously from nine rhesus monkey eyes perfusion fixed at an IOP of 10 mm Hg.\(^5\) Overall laminar thickness in the three normal eyes in this report ranged from 87 to 115 μm, compared with 182 to 215 μm for two groups of eyes perfusion-fixed at an IOP of 10 mm Hg in our previous report.\(^4\) Overall peripapillary scleral thickness at the posterior scleral canal opening ranged from 120 to 151 μm in this study, compared with 200 to 250 μm in another of our reports.\(^5\) Finally, the horizontal and vertical diameters of the neural canal at Bruch’s membrane in the normal cynomolgus monkey eyes of the current report (horizontal, 1023–1069 μm; vertical, 1298–1430 μm) are smaller than previously reported within histologic sections of rhesus monkey eyes (horizontal, 1262 ± 22 μm; vertical, 1821 ± 31 μm).

Although this difference may be the result of tissue preparation alone (Historesin embedding for histologic measurements, paraffin embedding for 3-D reconstruction), or species differences (cynomolgus versus rhesus), we believe that these three monkeys just happen to be at the small end of the dimensions of normal cynomolgus and rhesus monkeys. To date, we have completed the initial phases of 3-D reconstruction of both eyes of 15 monkeys (six cynomolgus, nine rhesus). Of these, initial estimates of the long and short axes of an ellipse fitted to Bruch’s membrane opening have been completed for five cynomolgus and five rhesus monkeys (Pottle J.
et al. *IOVS* 2004;45:ARVO E-Abstract 1118). Within these data (not shown), although the cytomologus data may cluster at the smaller end of the spectrum, both cytomologus and rhesus data demonstrate a wide range of sizes of the opening in Bruch’s membrane. For the five cynomolgus monkeys, the range for Bruch’s membrane opening is 1031 to 1171 μm. In the five rhesus monkeys, the range of the long axis of Bruch’s membrane opening was 1233 to 1745 μm and the range of the short axis was 844 to 1345 μm.

Although it is possible that our paraffin-embedding protocol induced greater shrinkage of the tissues, the fact that the laminar position data are not different from our previous reports argues against this. Experiments in which both eyes of rabbits are perfusion fixed, then individually embedded (one in the 3-D architecture of the neural canal.

Our method of 3-D reconstruction has the following limitations. First, anterior-to-posterior resolution was limited to 3 μm by the fact that the current stain penetrates approximately 2.5 μm into the block. Thus, cutting a 3-μm section was necessary to obtain an unstained block surface after each cut. Second, because the stain is applied by hand to the block with a cotton-tipped swab and then the excess is manually removed with lens paper, staining variation between section images can be substantial for some eyes. This problem, combined with variations in illumination, has made automation of object delineation difficult. Third, the current method is laborious, requiring on average 1 month to complete the construction and visualization of the connective tissue voxel geometry for a single ONH.

Two subsequent analyses are planned for each geometry. First, additional methods for quantitative connective tissue architecture characterization are under development, to enable a 3-D characterization of the location and pattern of connective tissue damage in early, and eventually in moderate and severe, experimental glaucoma.

Second, continuum and micro-finite element models will be built of each eye to characterize the distribution of IOP-related stress and strain within the connective tissues of each ONH. This characterization, once completed, will allow us to establish the relationships between IOP-related stress and strain and EG connective tissue damage. These relationships, once elucidated, will direct a new generation of experiments designed to colocalize IOP-related stress and strain, early connective tissue damage, astrocyte and glial cell activation, and axonal distress within the ONHs of individual monkey eyes.

The geometries presented herein reconstruct the lamina cribrosa, central retinal vessels, peripapillary sclera, and optic nerve sheath insertions. However, the internal limiting membrane of the retina, peripapillary Bruch’s membrane, subarachnoid space, and principal branches of the posterior ciliary arteries within the peripapillary sclera are also present and will be included in future reconstructions. The addition of these structures will allow future models to include not only the underlying connective tissues but also the prelaminar and retrolaminar neural tissues, posterior ciliary arteries, and cerebro-
spinal fluid pressure. Because the method ensures continuous anterior-to-posterior registration from the clinically recognizable ONH surface through the cut surface of the optic nerve, these enhanced reconstructions will offer an unprecedented opportunity to correlate the location of astrocyte activation, axonal insult, capillary collapse, and tissue oxygenation relative to the stresses and strains within the adjacent connective tissues. The reproducibility and accuracy of ONH voxel geometry and finite element model construction for individual monkey eyes will be presented in a series of future reports.

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