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3D morphometry of the human optic nerve head☆

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ABSTRACT

Human optic nerve head (ONH) anatomy is of interest in glaucoma. Our goal was to carry out a morphometric study of the human ONH based on 3D reconstructions from histologic sections. A set of 10 human ONHs (from four pairs of eyes plus two singles) were reconstructed in an iterative procedure that required the resulting geometries to satisfy a set of quality control criteria. Five models corresponded to eyes fixed at 5 mmHg and the other five models to eyes fixed at 50 mmHg. Several aspects of ONH morphology were measured based on surface and point landmarks: the thicknesses of the lamina cribrosa (LC), the peripapillary sclera and the pre-laminar neural tissue (peripapillary and within the cup); the minimum distance between the anterior surface of the LC and the subarachnoid space; the surface area of the anterior and posterior surfaces of the LC; and the diameter of the scleral canal opening. Our results showed that about one third of the anterior LC surface was obscured from view from the front by the sclera. In all eyes the LC inserted into the pia mater, and not only into the sclera. The variations in ONH morphology between eyes of a pair exceeded, or were of the same order as, changes in morphology due to acute changes in IOP. The reconstruction and morphometry techniques introduced are suitable for application to the ONH. Comparison of measurements in eyes fixed at different pressures suggested small effects on geometry of the increase in IOP. A large variability in ONH morphology, even between contralateral eyes of different IOP, was observed. We conclude that reconstruction of human ONH anatomy from 3D histology is possible, but that large inter-individual anatomic variations make morphometric analysis of the ONH very difficult in the absence of large sample numbers. The insertion of the pia mater into the LC may have biomechanical implications and should be further investigated. Emerging clinical imaging techniques such as deep-scanning OCT will be limited to investigation of the central and mid-peripheral regions of the LC due to optical "occluding" by the peripapillary sclera.

1. Introduction

The causes of retinal ganglion cell dysfunction - and eventual retinal ganglion cell loss – in glaucomatous optic neuropathy are poorly understood, but likely involve some combination of biomechanical and ischemic factors acting on optic nerve head (ONH) tissues (Burgoyne et al., 2005). Previous work has sought to identify links between anatomic features of the ONH and an individual's susceptibility to the development and progression of glaucomatous optic neuropathy (Burgoyne et al., 2005; Downs et al., 2007; Jonas et al., 2003, 2004; Quigley et al., 1981).

Characterization of ONH geometry is complicated because of the difficulty of accessing ONH tissues directly. Some researchers have therefore measured ONH surface morphology, searching for correlations between surface features, such as cup depth or volume, and the risk of glaucomatous neuropathy (Jonas and Budde, 2000; Wells et al., 2008). Although these studies are valuable, they have limitations. Specifically, recent work suggests that the ONH surface is probably not a good surrogate for IOP-induced deformations of the interior (Agoumi et al., 2009; Sigal et al., 2004, 2009a; Williams et al., 2009), and that IOP-related ONH biomechanics depend more strongly on the morphology of the ONH interior than on that of the ONH surface (Sigal, 2009; Sigal et al., 2005a, 2004). These limitations can be overcome by using histological samples, as has been

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done from monkeys (Burgoyne and Morrison, 2001; Burgoyne et al., 1994; Downs et al., 2007; Yang et al., in press, 2007b), dogs (Morgan, 1999), tree shrews (Albon et al., 2007) and human donors (Jonas et al., 2003, 2004; Oyama et al., 2006; Quigley and Addicks, 1981; Yan et al., 1998). Histological tissue samples can be stained to show tissue composition and imaged with high spatial resolution. However, many of these studies have also been limited since measurements taken from 2D histologic sections are susceptible to artifacts, e.g. related to the angle and location of the section. Recent histomorphometric analysis of the monkey ONH has shown that more robust measurements can be obtained through 3D analysis (Downs et al., 2007; Yang et al., 2007a).

The goal of this manuscript was to demonstrate a method to study human ONH morphology in 3D. In addition to traditional measures of ONH morphology, such as lamina cribrosa (LC) and sclera thickness, this approach enables us to obtain novel measures, such as the area of the LC insertion into the surrounding load-bearing tissues (which is important in the transfer of IOP-induced loads to the LC (Sigal, 2009; Sigal et al., 2005a, 2009a)), and the area of the LC visible through the scleral canal opening (which is important in the interpretation of images of the ONH obtained with deep-scanning OCT).

2. Methods

2.1. Building 3D reconstructions of the ONH

For the present study, ten eyes from six human donors were used (Table 1). Eyes were obtained and managed in accordance with the provisions of the Declaration of Helsinki for research involving human tissue. When a pair of eyes was available, one eye was randomly chosen to be fixed and reconstructed at 5 mmHg, whereas the contralateral eye was processed at 50 mmHg. A full description of the histologic techniques has been presented elsewhere (Sigal et al., 2005b). Briefly, after imaging (which typically took 2.5 h per eye, 3 h per pair), the eyes were fixed using 2.5% paraformaldehyde/2.5% glutaraldehyde in Sorensen's phosphate buffer and the ONH and peripapillary sclera were dissected free from the eye, dehydrated and embedded in JB-4 plastic using a special mould that allowed the placement of tensioned collagen sutures for use as fiducial markers. Serial sagittal sections (2 μm thick at 100 μm spacings across the ONH) were cut at right angles to the axes of the fiducial markers and digitally photographed under dark-field illumination (2048 × 1536 pixels, 8 RGB bits per channel per pixel) ensuring uniform sample illumination and non-saturation of the brightest image regions (typically the sclera). Sections were then stained with picrisirius red to identify collagen and solochrome cyanin to identify myelin, nuclei and blood cells. The dual-stained sections were digitally photographed under bright field illumination using the same procedure.

Images were aligned and unwarped to correct the deformations that occurred during the sectioning process (Fig. 1, top row). This was carried out using a custom modified version of TPSSuper (F. James Rohlf, SUNY, Stony Brook, NY), and was based on the known fiducial marker positions as cast into the histologic block during the embedding procedure. This method avoided the use of anatomical features for alignment, which can lead to artefacts (Hecksher-Sorensen and Sharpe, 2001). The final magnification of images was determined by using measurements of inter-fiducial distances taken prior to sectioning.

Table 1
Summary of donor information and fixation pressure.

Donor	1	1	2	3	4	4	5	5	6	6
Eye	OD	OS	OD	OD	OD	OS	OD	OS	OD	OS
Age [Years]	83		79	91	76		84		70	
Gender	M		M	M	M		M		M	
Time to enucleation [Hrs]	2		2	10	3		7		7	
Time to imaging [Hrs]	17		23.5	29	19		24		16	
Axial length [mm]	24.5	24.5	23.2	24.4	23.7	23.8	24.7	25.0	24.0	24.0
Fixation pressure [mmHg]	50	5	5	50	5	50	5	50	5	50

The digital sections were manually segmented to define five tissue regions: sclera, LC, pre-laminar neural tissue (including retina), post-laminar neural tissue (including the optic nerve) and pia mater (Fig. 1, middle row). We used both the dark-field and dual-stained images to different extents depending on the tissue being identified. The concurrent use of both dark and bright field images for the same section improved the decisions taken during reconstruction, since not all features of the geometry were equally visible in all imaging modalities. The sclera was dense and fibrous in the dark-field images, and in the stained images it had the most intense picrisirius staining. The pia mater also stained well for collagen and was bright and amorphous in the dark-field images. The anterior boundary of the LC was defined by the termination of the laminar beams and the insertion points at the sclera, whereas the posterior boundary was defined by two features: the termination of solochrome cyanin staining, indicating a lack of myelination inside the LC (Hernandez, 2000), and the "stacked plate" morphology of the connective tissues typical of the LC (Jonas and Budde, 2000; Quigley and Addicks, 1981). The vitreo-retinal interface was clearly distinguishable in the double-stained images, as were the boundaries where the ONH had been cut from the rest of the eye. To ensure consistency across all eyes, all the segmentations were checked and adjusted by a single observer (JGF).

To generate smooth and anatomically accurate 3D reconstructions it was useful to enrich the image stack by adding virtual sections between the ones obtained from the histology. The new sections were cubic interpolations inserted evenly (~33 μm apart, i.e. two interpolated sections between a pair of original sections). The interpolated sections were then cleaned of artefacts of interpolation and smoothed in all three projections. Although interpolation did not add any new information, it simplified the production of 3D geometries while preserving a physiologically reasonable morphology.

2.2. Segmentation quality control

Segmentation and 3D reconstruction produced good draft models, but a crucial element of our reconstruction process was its iterative nature. Small iterative refinements were made to the image alignment (translation and rotation), and the segmentation to reduce irregularities. At each step of the iterative process the segmentation could be seen in the same planes as the original and interpolated sections, or in any other plane. To be included in this work, the reconstructed models had to satisfy the following six quality criteria:

1. All tissue interfaces had to be anatomically plausible, e.g. the pre and post-laminar neural tissue regions could not touch.
2. Each region (tissues and exterior) had to be topologically connected in a single volume, i.e. no "islands" or "bubbles".
3. Tissue surfaces had to be smooth, with no bumps, ripples or waves, due for example to the discrete nature of the serial sections.
4. Tissue labelling in the model had to match tissue labelling in the histologic sections. If small adjustments were required during the iterative process to satisfy quality requirements, changes in the segmentation were only accepted if they matched the histologic images. This meant that changes were made mostly to the interpolated sections, leaving the original sections unchanged.