

# Imaging the aqueous humor outflow pathway in human eyes by three-dimensional micro-computed tomography (3D micro-CT)

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The site of outflow resistance leading to elevated intraocular pressure in primary open-angle glaucoma is believed to be located in the region of Schlemm's canal inner wall endothelium, its basement membrane and the adjacent juxtacanalicular tissue.

Evidence also suggests collector channels and intrascleral vessels may have a role in intraocular pressure in both normal and glaucoma eyes. Traditional imaging modalities limit the ability to view both proximal and distal portions of the trabecular outflow pathway as a single unit. In this study, we examined the effectiveness of three-dimensional micro-computed tomography (3D micro-CT) as a potential method to view the trabecular outflow pathway.

Two normal human eyes were used: one immersion fixed in 4% paraformaldehyde and one with anterior chamber perfusion at 10 mmHg followed by perfusion fixation in 4% paraformaldehyde/2% glutaraldehyde. Both eyes were postfixed in 1% osmium tetroxide and scanned with 3D micro-CT at 2  $\mu\text{m}$  or 5  $\mu\text{m}$  voxel resolution.

In the immersion fixed eye, 24 collector channels were identified with an average orifice size of  $27.5 \pm 5 \mu\text{m}$ . In comparison, the perfusion fixed eye had 29 collector channels with a mean orifice size of  $40.5 \pm 13 \mu\text{m}$ . Collector channels were not evenly dispersed around the circumference of the eye.

There was no significant difference in the length of Schlemm's canal in the immersed versus the perfused eye (33.2 versus 35.1 mm). Structures, locations and size measurements identified by 3D micro-CT were confirmed by correlative light microscopy.

These findings confirm 3D micro-CT can be used effectively for the non-invasive examination of the trabecular meshwork, Schlemm's canal, collector channels and intrascleral vasculature that comprise the distal outflow pathway.

This imaging modality will be useful for non-invasive study of the role of the trabecular outflow pathway as a whole unit.

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