Published in final edited form as:

Exp Eye Res. 2011 February ; 92(2): 104–111. doi:10.1016/j.exer.2010.12.010.

Imaging the Human Aqueous Humor Outflow Pathway in Human Eyes by Three Dimensional Micro-Computed Tomography (3D micro-CT)

Cheryl R. Hann^a, Michael D. Bentley^b, Andrew Vercnocke^c, Erik L. Ritman^c, and Michael P. Fautsch^a

Cheryl R. Hann: hann.cheryl@mayo.edu; Michael D. Bentley: michael.bentley@mnsu.edu; Andrew Vercnocke: vercnocke.andrew@mayo.edu; Erik L. Ritman: elran@mayo.edu; Michael P. Fautsch: fautsch.michael@mayo.edu

^aDepartment of Ophthalmology, Mayo Clinic, Rochester, Minnesota

^bDepartment of Biological Sciences, Minnesota State University, Mankato, Minnesota

^cDepartment of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, Minnesota

Abstract

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 μ m or 5 μ m voxel resolution. In the immersion fixed eye, 24 collector channels were identified with an average orifice size of 27.5 ± 5 μ m. In comparison, the perfusion fixed eye had 29 collector channels with a mean orifice size of 40.5 ± 13 μ 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 noninvasive study of the role of the trabecular outflow pathway as a whole unit.

© 2010 Elsevier Ltd. All rights reserved.

Address for corresponding author: Michael P. Fautsch, Department of Ophthalmology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905 Telephone 507-284-2244, Fax 507-284-8566.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

3D micro-CT; three dimensional micro-computed tomography; collector channels; outflow pathway; Schlemm's canal; intrascleral vasculature; anterior segment

INTRODUCTION

Increased outflow resistance is the primary cause of elevated intraocular pressure in primary open-angle glaucoma (POAG). Failure to lower intraocular pressure leads to retinal ganglion cell death and blindness. Studies supporting the trabecular outflow pathway as the main site of outflow in the human eye have focused on Schlemm's canal inner wall endothelium, its basement membrane and the adjacent juxtacanalicular tissue as the site of increased outflow resistance in POAG (Grant, 1958). However, numerous light microscopic, electron microscopic, and molecular studies have failed to identify the key mechanism and/or molecules involved in outflow resistance in this region (Acott, Kelley, 2008; Alvarado, 1984; Alvarado et al., 1986; Grierson et al., 1982; Rohen et al., 1981). Early light microscopic work identified numerous changes in POAG eyes in the distal outflow pathway. These changes included narrowing and collapse of collector channels and intrascleral veins, proliferation of Schlemm's canal outer wall endothelium with adhesion to the collector channel wall, and blockage of collector channel ostia by herniations of the juxtacanalicular tissue (Chi et al., 1957; Dvorak-Theobald, 1955; Teng et al., 1955). More recently, in normal eyes preferential flow and "foamy" regions have been identified adjacent to collector channels (Battista et al., 2008; Hann, Fautsch, 2009; Johnson, 2007; Lee, Grierson, 1974; Melamed, Epstein, 1987; Svedbergh, 1976). In POAG, blockage of collector channels with expanded regions of the JCT (Gong, IOVS, 2007, 48: E-abstract 2079), reduction in Schlemm's canal cross-sectional area and length (Allingham et al., 1996) and a reduction in Schlemm's canal volume have been found (Hann, IOVS, 2010 51; E-Abstract 2735). These findings suggest changes in the distal region of the outflow pathway (collector channel, Schlemm's canal, and intrascleral vasculature) may also contribute to the increase in overall resistance seen in glaucoma.

The study of the trabecular meshwork outflow pathway as an organ system has been difficult to accomplish due to limitations in imaging and microscopy techniques. Much of the information about Schlemm's canal, collector channels and intrascleral vessels originate from serial sections, or injection of dyes, neoprene, latex, or silicone rubber (Ashton, 1951; Ashton, 1952; Ashton, Smith, 1953; Dvorak-Theobald, 1955; Jocson, Grant, 1965; Thomassen, 1950). Other studies of the conventional outflow pathway have been limited to sampling of selected limbal regions around the circumference of the eye using light microscopy with dyes (Rohen, Rentsch, 1968), transmission electron microscopy (TEM) (Hann et al., 2005; Lutjen-Drecoll et al., 1981; Parc et al., 2000), confocal (Battista et al., 2008; Hann, Fautsch, 2009; Zhu et al.), and scanning electron microscopy (SEM) (Hoffman, Dumitrescu, 1971; Johnstone, 2004; Morrison et al., 1996). With the exception of SEM, these techniques rely on regional sampling, sectioning, and examination of numerous small tissue areas. While it is possible to sample and section an entire organ system, registration of completed sections and time needed to complete the study may limit accuracy and usefulness. Imaging of the conventional outflow pathway of the eye *in vivo* has been done with optical coherence tomography (OCT) in humans (Kagemann et al., 2010) and with ultrasound biomicroscopy in cats (Irshad et al., 2010). While this is encouraging, the image resolution of these modalities limits their usefulness for the visualization of the complex relationships existing between the trabecular meshwork, Schlemm's canal, collector channels and intrascleral vasculature.

Three dimensional micro-computed tomography (3D micro-CT) (Jorgensen et al., 1998; Ritman et al., 1997) provides the opportunity to examine the whole anterior segment of the eye. In the past, 3D micro-CT has been used successfully to study a variety of normal and pathologic tissues (Bentley et al., 2007; Borah et al., 2006; Gossel et al., 2003; Ritman, 2005; Zhu et al., 2007). Increases in the sensitivity of 3D micro-CT with resolutions down to 1 μm make it feasible to examine the small structures of the eye (Ritman, 2004). In fact, tomographic images at 60 nm resolution can be obtained with the use of X-ray microscopic tomography equipped with Fresnel zone plates (Larabell, Le Gros, 2004).

Most studies analyzing aspects of the aqueous outflow pathway rely on imaging of the trabecular meshwork and Schlemm's canal. Unfortunately, the trabecular meshwork and Schlemm's canal only represent part of the structures involved in the aqueous outflow pathway. The purpose of the present study was to look at the feasibility of using 3D micro-CT to visualize the distal outflow pathway composed of Schlemm's canal, collector channels and the intrascleral vasculature around the circumference of the human anterior chamber.

MATERIAL AND METHODS

Preparation of Tissue for 3D micro-CT

Immersion fixation—One human eye (88 year old male) was immersion fixed in 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.2. The anterior segment was removed and divided into quadrants. Each quadrant was rinsed twice in 0.1M phosphate buffer for ten minutes followed by immersion in 1% osmium tetroxide for 90 minutes to increase the X-ray opacity (Bentley et al., 2007). The tissue was again rinsed twice in 0.1M phosphate buffer, pH 7.2. Each quadrant was oriented cornea tip down in a conical microfuge tube. Formol-saline moistened cotton was packed around the tissue to hold it in position. All quadrants were scanned at 5 μm voxel resolution using the 3D micro-CT scanner at the Mayo Clinic (Jorgensen et al., 1998). For resolution comparison, one quadrant was embedded in Epon-Araldite and was scanned at 2 μm voxel resolution at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratories (BNL) in New York.

Perfusion fixation—The anterior chamber of one human eye (74 year old male) was perfused at 10 mmHg with 5.5 mM glucose in phosphate buffered saline for two hours. The anterior chamber perfusion was performed as previously described (Parc et al., 2000). Briefly, a needle was inserted just anterior to the limbal region and passed to the opposite side of the anterior chamber and inserted underneath the iris into the posterior chamber. An anterior chamber exchange was performed with 4% paraformaldehyde/2% glutaraldehyde in 0.1M phosphate buffer, pH 7.2 followed by continuous perfusion of the fixative for eight hours at 10 mmHg. The anterior segment was divided into quadrants. The tissue was rinsed twice for 10 minutes each in 0.1M phosphate buffer followed by immersion in 1% osmium for 90 minutes. Tissue was rinsed in 0.1M phosphate buffer, dehydrated in an ascending series of ethanols and incubated in two changes of propylene oxide for 10 minutes each. The tissue quadrants were embedded, cornea tip down, in the bulb of a disposable 1 ml plastic transfer pipette. The resin used was Epon-Araldite. All quadrants were scanned at 5 μm voxel resolution using the 3D micro-CT scanner at Mayo Clinic in the Department of Physiology and Biomedical Engineering.

3D micro-CT scans

Wedges of tissue from either the immersed or perfused eye were mounted on a 360° rotating stage and scanned at 5 μm voxel resolution using a molybdenum X-ray source as previously

described (Jorgensen et al., 1998) (Fig. 1). The X-rays are converted to visible light by projection onto a crystal scintillator plate. The multi view projection X-ray images were used to reconstruct the 3D image using a modified Feldkamp cone beam algorithm (Jorgensen 1998). The 3D images were examined using ANALYZE, a 3D image software analysis and display program (Robb 2001). One quadrant of the immersed eye was selected for scanning at Brookhaven National Light Source at 2 μm voxel resolution using a synchrotron X-ray source. The advantages of using the synchrotron source include higher radiation flux for shortened scan times and parallel, tunable, monochromatic illumination to maximize resolution and tissue contrast (Peyrin et al., 1998).

Analysis of 3D micro-CT volumes

The entire periphery of two anterior segments comprising 68.3 mm of Schlemm's canal and the adjacent inflow and outflow tissues were examined. 3D micro-CT volumes of the anterior segments from both the immersion and perfusion fixed eyes were evaluated using ANALYZE®, a software package developed for the analysis and 3D reconstruction of medical imaging modalities (Robb, 2001). In this multifunctional software, the modules of segmentation, volume rendering and computation were used for analysis of 3D image volumes. 3D volumes were viewed in the Display module at the indicated voxel size of the scan. Reconstructed image volumes of 9 mm tissue wedges were viewed in the sagittal or coronal orientation. Collector channels were located within the volume and the collector channel orifice was measured at its widest point using the Measure module. The length of Schlemm's canal was measured by auto tracing 25 μm sub-volumes of Schlemm's canal at selected intervals within the wedge, reconstructing the 3D images from the segmented images and measuring length using the line profile tool in the Measure module. Collector channels and Schlemm's canal were auto-traced in the Segment module and the total volume was rendered to view the final 3D image of the circumference of the eye. The number of collector channels was computed from each quadrant and the orifice size of each collector channel was measured. For both the immersed and the perfused eye, the mean and standard deviation was calculated for total collector channel number and collector channel orifice size.

Correlative Light Microscopy

Validation of structures identified by reconstruction of the 3D micro-CT images was performed by light microscopy. A representative tissue wedge from the immersed eye was serially sectioned at 1 μm and stained with toluidine blue. The total number of sections examined was 356. Distal outflow structures examined included a complete collector channel, Schlemm's canal, adjacent trabecular meshwork, and intrascleral vessels. Morphologic details and measurements of the collector channel orifices in the 3D images were compared with the light microscopic sections. The collector channel orifice present in the 3D volumes of both the 2 μm and 5 μm voxel scans were measured and compared to measurements of the same collector channel in the light microscopic sections. Intrascleral vessels and ciliary body projections in the 3D micro-CT multiplanar sections were used as landmarks to identify the same collector channel region in the light microscopic sections.

RESULTS

Schlemm's canal, collector channels and large intrascleral vessels were detected at both 5 μm and 2 μm resolution by 3D micro-CT (Fig. 2A and 2B). The collector channel orifice identified in the 5 μm scan measured $26.0 \pm 5.47 \mu\text{m}$ (n=5) compared to $18.6 \pm 3.51 \mu\text{m}$ (n=15) in the 2 μm scan. Correlative serial sectioning of tissue followed by toluidine blue staining confirmed the identification of Schlemm's canal, collector channels and intrascleral vessels and the orifice size (Fig. 2C).

In the 5 μm voxel scans, the trabecular meshwork beams, intertrabecular spaces and septae within Schlemm's canal were not resolved. In the 2 μm voxel scans, smaller caliber intrascleral vessels were visible in one multiplanar section but could not be followed over several microns. In contrast, images scanned at 2 μm voxels gave morphologic detail similar to correlative plastic sections (compare Fig. 2B and 2C). Trabecular meshwork spaces and regional variations in Schlemm's canal such as septae and undulation of the orifice walls could be observed. The 2 μm scans provided more detailed images of the interconnections between Schlemm's canal, collector channel and intrascleral vessel regions (compare Fig. 2A and 2B). Vessel walls were apparent and vessels ranging from 10–40 μm were detected. At 2 μm resolution, smaller vessels in the range of 10–20 μm could be followed within the volume. However, use of BNL scanner for large numbers of specimens is limited by beam time scheduling and cost.

Schlemm's canal, collector channels and larger intrascleral vessels identified in volumes could be followed through sequential sections (Fig. 3A–F). Intrascleral vessels ranging in size from 10–40 μm in diameter were visible at 5 μm voxel resolution in both the immersed and perfused eye. In some wedges, a vessel could be found paralleling Schlemm's canal for some distance (Fig. 4A and 4B). Connections between this vessel and collector channels were sometimes observed. Collector channel orifices were distinguished by parallel areas on either side at or immediately adjacent to the widest portion of the orifice. Some collector channels, extending from the posterior or middle region of the canal were found to proceed without interruption to an aqueous vein. More often collector channels joined other vessels in the intrascleral vasculature. Several times, two collector channels were observed in close proximity to one another, separated by only 20 μm (Fig. 4C and 4D). These collector channels would often join together to form a horseshoe and extend into the same intrascleral vessel (Fig. 4D).

Comparison of the immersion and perfusion fixed eyes showed more collector channels in the perfused eye than the immersed eye (29 versus 24). Orifice size of the collector channels was slightly increased in the perfused eye when compared to the immersed eye (40.5 ± 13.0 μm versus 27.5 ± 5 μm). Schlemm's canal length was 33.2 mm in the immersed eye versus 35.1 mm in the perfused eye. Schlemm's canal was attenuated in regions and was usually more dilated at collector channel orifices in both immersed and perfused eyes.

Using 3D micro-CT, collector channels could be isolated from within the quadrant volume (Fig. 5A), traced and viewed by volume reconstruction (Fig. 5B). Within these sub-volumes, single collector channel reconstructions could be isolated and viewed from different angles (Fig. 5B and D). Variations in orifice shape, volume and connections with intrascleral vasculature could be examined in this manner (Fig. 5C). Reconstructions were made of the entire anterior segment of the perfused eye showing Schlemm's canal and the location of collector channels (Fig. 6). Collector channel distribution followed no set pattern in any quadrant. Attenuation of Schlemm's canal was apparent in the superior quadrant.

DISCUSSION

Most studies analyzing the aqueous outflow pathway have focused on imaging of the trabecular meshwork and Schlemm's canal. However, the trabecular meshwork and Schlemm's canal only represent part of the structures involved in the aqueous outflow pathway. As shown in this study, 3D micro-CT enabled the visualization of the trabecular meshwork, Schlemm's canal, collector channels and intrascleral and episcleral vasculature around the circumference of the eye. Imaging with 3D micro-CT provides a mechanism to study the anatomical and pathological state of the whole conventional outflow pathway in human eyes.

Total collector channel numbers detected using 3D micro-CT (24 in the immersed eye; 29 in the perfused eye) corresponded to previous studies that used serial sectioning of tissue followed by light microscopy. Dvorak-Theobald reported 29, 24, and 31 collector channels respectively in three eyes immersion fixed with formalin (Dvorak-Theobald, 1955). A corresponding study by Rohen confirmed the presence of 25–30 collector channels per eye (Rohen, Rentsch, 1968). Similar to these studies, our study found collector channels randomly distributed around the eye. Imaging with 3D micro-CT also confirmed variability in collector channel orifice size in immersion fixed eyes ($27 \pm 5.0 \mu\text{m}$ compared to $5\text{--}50 \mu\text{m}$ (Dvorak-Theobald, 1955) and $50\text{--}70 \mu\text{m}$ (Rohen, Rentsch, 1968). Schlemm's canal length of 33 mm in the immersed eye and 35 mm in the perfused eye was consistent with previous measurements of 36 mm in normal eyes (Moses, 1979).

Differences between perfusion and immersion fixed eyes were noted. In perfusion fixed eyes, collector channel orifices were larger ($40.5 \pm 13 \mu\text{m}$) and Schlemm's canal length was longer (35 mm) compared to immersion fixed collector channel orifices ($27.5 \pm 5 \mu\text{m}$) and Schlemm's canal length (33 mm). Similar to previous studies, perfusion fixation appears to open flow pathways that may not be evident in immersion fixed tissue (Ten Hulzen, Johnson, 1996; Ainsworth, Lee, 1990; Grierson, Lee, 1974).

3D micro-CT provides the ability to reconstruct 3D images and study the volumetric data of collector channels and Schlemm's canal. In this way, structures connecting to and surrounding the collector channel orifice and channel may be analyzed. Collector channel outflow structures can be compared regionally in the entire anterior segment. If outflow facility is decreased by segmental change in preferential flow pathways underneath or within collector channels in aging or POAG, collector channels in one quadrant may contribute to an increase in outflow resistance more than in other regions. Understanding where collector channels are or which region of the eye has more collector channels may be beneficial to ophthalmic surgeons in placing devices aiding filtration, or optimally identifying preferred trabeculotomy or trabeculectomy sites.

The intrascleral vessels identified in our study ranged in size from $10\text{--}40 \mu\text{m}$. However, in 3D images obtained using both the 2 and $5 \mu\text{m}$ voxel scans, we did not observe the complex network of the deep scleral plexus reported following Neoprene corrosion casting (Ashton, Smith, 1953). This may be due to two factors. The first is the use of immersed tissue in our initial scans in which these small vessels may have collapsed. The second is the use of osmium as the contrast agent. Use of osmium for 3D micro-CT is useful for correlative studies where TEM is going to be pursued. Section contrast variability in the smaller vessels made analysis of these structures difficult to detect and trace. Use of Microfil, a radio-opaque silicone polymer has been successful in other tissues allowing accurate reconstruction of vascular trees (Bentley et al., 2002; Herrmann et al., 2001; Zhu et al., 2004). In preliminary perfusion studies by our laboratory, Microfil failed to penetrate past the uveal trabecular meshwork beams. Perfusion of other contrast agents into the anterior chamber such as gadolinium chelate (Alric et al., 2008), iron oxide preparations (Ruehm et al., 2001) or magnetic nanoparticles (Tai et al., 2006) may be possible. Additional studies are required with alternative contrast agents so that analysis of the intrascleral vasculature is less cumbersome.

Since visualization of the distal outflow pathway using 3D micro-CT is possible, it is feasible to study and consider the conventional outflow pathway as being composed of individual functional regions. Each region would be composed of a collector channel, the adjacent Schlemm's canal, and the trabecular meshwork tissue adjacent to the orifices. This would be similar in concept to areas drained by renal papillae in kidney, respiratory bronchi branching into lobes and terminating in alveoli, and the vasculature trees of individual organ

systems. While study of the individual functional regions is important, questions arise concerning regional function in relation to one another. The identification of preferential (Hann, Fautsch, 2009) and segmental flow regions (Ethier, Chan, 2001; Gottanka et al., 2001; Hann et al., 2005; Overby et al., 2009) within the conventional outflow pathway make it a necessity to study the complete outflow pathway as an anatomical unit composed of individual sub regions. The use of 3D micro-CT as an imaging modality of the outflow pathway makes identification of individual and regional changes in aging and the pathogenesis of glaucoma feasible. Examination of the relationships between Schlemm's canal, collector channels and the intrascleral vasculature in normal and POAG eyes will help to delineate the anatomical changes that are present in the distal portion of the outflow system.

RESEARCH HIGHLIGHTS

- 3D micro-CT is an effective, non-invasive imaging method to study the trabecular outflow pathway
- 3D micro-CT enables outflow pathways to be studied in the context of the whole eye
- 3D micro-CT enables identification of collector channel number, size, and location around the eye
- 3D micro-CT will enable study of regional differences in normal and diseased eyes

Acknowledgments

Use of the National Synchrotron Light Source, Brookhaven National Laboratory was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-AC02-98CH10886.

Supported in part by National Institutes of Health research grants EY 15736 (MPF), EY 15736-S1 (MPF); EY 07065 (MPF); EB000305 (ELR) Mayo Foundation, Rochester, MN; and Research to Prevent Blindness, New York, NY (MPF is a recipient of a Lew R. Wasserman Merit Award and the Department of Ophthalmology, Mayo Clinic is the recipient of an unrestricted grant).

REFERENCES

- Acott TS, Kelley MJ. Extracellular matrix in the trabecular meshwork. *Exp. Eye Res* 2008;86:543–561. [PubMed: 18313051]
- Ainsworth JR, Lee WR. Effects of age and rapid high-pressure fixation on the morphology of Schlemm's canal. *Invest. Ophthalmol. Vis. Sci* 1990;31:745–750. [PubMed: 2186010]
- Allingham RR, de Kater AW, Ethier CR. Schlemm's canal and primary open angle glaucoma: correlation between Schlemm's canal dimensions and outflow facility. *Exp. Eye Res* 1996;62:101–109. [PubMed: 8674505]
- Alric C, Taleb J, Le Duc G, Mandon C, Billotey C, Le Meur-Herland A, Brochard T, Vocanson F, Janier M, Perriat P, Roux S, Tillement O. Gadolinium chelate coated gold nanoparticles as contrast agents for both X-ray computed tomography and magnetic resonance imaging. *J. Am. Chem. Soc* 2008;130:5908–5915. [PubMed: 18407638]
- Alvarado J, Murphy C, Juster R. Trabecular meshwork cellularity in POAG and nonglaucomatous normals. *Ophthalmology* 1984;91:564–579. [PubMed: 6462622]
- Alvarado JA, Yun AJ, Murphy CG. Juxtacanalicular tissue in primary open angle glaucoma and in nonglaucomatous normals. *Arch. Ophthalmol* 1986;104:1517–1528. [PubMed: 3767685]
- Ashton N. Anatomical study of Schlemm's canal and aqueous veins by means of neoprene casts. Part I. Aqueous veins. *Br. J. Ophthalmol* 1951;35:291–303. [PubMed: 14830741]

- Ashton N. Anatomical study of Schlemm's canal and aqueous veins by means of neoprene casts. II. Aqueous veins. *Br. J. Ophthalmol* 1952;36:265–267. contd. [PubMed: 14925298]
- Ashton N, Smith R. Anatomical study of Schlemm's canal and aqueous veins by means of neoprene casts. III. Arterial relations of Schlemm's canal. *Br. J. Ophthalmol* 1953;37:577–586. [PubMed: 13093933]
- Battista SA, Lu Z, Hofmann S, Freddo T, Overby DR, Gong H. Reduction of the available area for aqueous humor outflow and increase in meshwork herniations into collector channels following acute IOP elevation in bovine eyes. *Invest. Ophthalmol. Vis. Sci* 2008;49:5346–5352. [PubMed: 18515571]
- Bentley MD, Jorgensen SM, Lerman LO, Ritman EL, Romero JC. Visualization of three-dimensional nephron structure with micro-computed tomography. *The Anatomical Record (Hoboken)* 2007;290:277–283.
- Bentley MD, Rodriguez-Porcel M, Lerman A, Sarafov MH, Romero JC, Pelaez LI, Grande JP, Ritman EL, Lerman LO. Enhanced renal cortical vascularization in experimental hypercholesterolemia. *Kidney Int* 2002;61:1056–1063. [PubMed: 11849461]
- Borah B, Dufresne TE, Ritman EL, Jorgensen SM, Liu S, Chmielewski PA, Phipps RJ, Zhou X, Sibonga JD, Turner RT. Long-term risedronate treatment normalizes mineralization and continues to preserve trabecular architecture: sequential triple biopsy studies with micro-computed tomography. *Bone* 2006;39:345–352. [PubMed: 16571382]
- Chi HH, Katzin HM, Teng CC. Primary degeneration in the vicinity of the chamber angle; as an etiologic factor in wide-angle glaucoma. II. *Am. J. Ophthalmol* 1957;43:193–203. [PubMed: 13394666]
- Dvorak-Theobald G. Further studies on the canal of Schlemm. Its anastomoses and anatomic relations. *Am. J. Ophthalmol* 1955;39:65–89. [PubMed: 14361607]
- Ethier RC, Chan DWH. Cationic ferritin changes outflow facility in human eyes whereas anionic ferritin does not. *Invest. Ophthalmol. Vis. Sci* 2001;42:1795–1802. [PubMed: 11431444]
- Gossel M, Malyar NM, Rosol M, Beighley PE, Ritman EL. Impact of coronary vasa vasorum functional structure on coronary vessel wall perfusion distribution. *Am. J. Physiol. Heart Circ. Physiol* 2003;285:H2019–H2026. [PubMed: 12855425]
- Gottanka J, Johnson DH, Martus P, Lutjen-Drecoll E. Beta-adrenergic blocker therapy and the trabecular meshwork. *Graefes Arch. Clin. Exp. Ophthalmol* 2001;239:138–144. [PubMed: 11372544]
- Grant WM. Further studies on facility of flow through the trabecular meshwork. *Arch. Ophthalmol* 1958;60:523–533.
- Grierson I, Lee WR. Changes in the monkey outflow apparatus at graded levels of intraocular pressure: a qualitative analysis by light microscopy and scanning electron microscopy. *Exp. Eye Res* 1974;19:21–33. [PubMed: 4412389]
- Grierson I, Wang Q, McMenamin PG, Lee WR. The effects of age and antiglaucoma drugs on the meshwork cell population. *Res. Clin. Forums* 1982;4:69–92.
- Hann CR, Bahler CK, Johnson DH. Cationic ferritin and segmental flow through the trabecular meshwork. *Invest. Ophthalmol. Vis. Sci* 2005;46:1–7. [PubMed: 15623746]
- Hann CR, Fautsch MP. Preferential fluid flow in the human trabecular meshwork near collector channels. *Invest. Ophthalmol. Vis. Sci* 2009;50:1692–1697. [PubMed: 19060275]
- Herrmann J, Lerman LO, Rodriguez-Porcel M, Holmes DR Jr, Richardson DM, Ritman EL, Lerman A. Coronary vasa vasorum neovascularization precedes epicardial endothelial dysfunction in experimental hypercholesterolemia. *Cardiovasc. Res* 2001;51:762–766. [PubMed: 11530109]
- Hoffman F, Dumitrescu L. Schlemm's canal under the Scanning Electron Microscope. *Ophthalmic Res* 1971;2:37–45.
- Irshad FA, Mayfield MS, Zurakowski D, Ayyala RS. Variation in Schlemm's canal diameter and location by ultrasound biomicroscopy. *Ophthalmology* 2010;117:916–920. [PubMed: 20079926]
- Jocson VL, Grant WM. Interconnections of Blood Vessels and Aqueous Vessels in Human Eyes. *Arch. Ophthalmol* 1965;73:707–720. [PubMed: 14281992]
- Johnson DH. Histologic findings after argon laser trabeculoplasty in glaucomatous eyes. *Exp. Eye Res* 2007;85:557–562. [PubMed: 17822700]

- Johnstone MA. The aqueous outflow system as a mechanical pump: Evidence from examination of tissue and aqueous movement in human and non-human primates. *J Glaucoma* 2004;13:421–438. [PubMed: 15354083]
- Jorgensen SM, Demirkaya O, Ritman EL. Three-dimensional imaging of vasculature and parenchyma in intact rodent organs with X-ray micro-CT. *Am. J. Physiol* 1998;275:H1103–H1114. [PubMed: 9724319]
- Kagemann L, Wollstein G, Ishikawa H, Bilonick RA, Brennen PM, Folio LS, Gabriele ML, Schuman JS. Identification and assessment of Schlemm's canal by spectral domain optical coherence tomography. *Invest. Ophthalmol. Vis. Sci.* 2010 In press.
- Larabell CA, Le Gros MA. X-ray tomography generates 3-D reconstructions of yeast, *saccharomyces cerevisiae*, at 60-nm resolution. *Mol. Biol. Cell* 2004;15:957–962. [PubMed: 14699066]
- Lee WR, Grierson I. Relationships between intraocular pressure and the morphology of the outflow apparatus. *Trans. Ophthalmol. Soc. U. K* 1974;94:430–449. [PubMed: 4219862]
- Lutjen-Drecoll E, Futa R, Rohen JW. Ultrahistochemical studies on tangential sections of the trabecular meshwork in normal and glaucomatous eyes. *Invest. Ophthalmol. Vis. Sci* 1981;21:563–573. [PubMed: 7287346]
- Melamed S, Epstein DL. Alterations of aqueous humour outflow following argon laser trabeculoplasty in monkeys. *Br. J. Ophthalmol* 1987;71:776–781. [PubMed: 3676149]
- Morrison JC, Fahrenbach WH, Bacon DR, Wilson DJ, Van Buskirk EM. Microvasculature of the ocular anterior segment. *Microsc. Res. Tech* 1996;33:480–489. [PubMed: 8800753]
- Moses RA. Circumferential flow in Schlemm's canal. *Am. J. Ophthalmol* 1979;88:585–591. [PubMed: 484689]
- Overby DR, Stamer WD, Johnson M. The changing paradigm of outflow resistance generation: towards synergistic models of the JCT and inner wall endothelium. *Exp. Eye Res* 2009;88:656–670. [PubMed: 19103197]
- Parc CE, Johnson DJ, Brilakis HS. Giant vacuoles are found preferentially near collector channels. *Invest. Ophthalmol. Vis. Sci* 2000;41:2984–2990. [PubMed: 10967055]
- Peyrin F, Salome M, Cloetens P, Laval-Jeantet AM, Ritman E, Rueggsegger P. Micro-CT examinations of trabecular bone samples at different resolutions: 14, 7 and 2 micron level. *Technol. Health Care* 1998;6:391–401. [PubMed: 10100941]
- Ritman EL. Micro-computed tomography-current status and developments. *Ann. Rev. Biomed. Eng* 2004;6:185–208. [PubMed: 15255767]
- Ritman EL. Micro-computed tomography of the lungs and pulmonary-vascular system. *Proc. Am. Thoracic Soc* 2005;2:477–480.
- Ritman EL, Jorgensen SM, Lund PE, Thomas PJ, Dunsmuir JH, Jomero JC, Turner RT, Bolander ME. Synchrotron-based micro-CT of in situ biological basic functional units and their integration. *SPIE* 1997;3149:13–24.
- Robb RA. The biomedical imaging resource at Mayo Clinic. *IEEE Trans. Med. Imaging* 2001;20:854–867. [PubMed: 11585203]
- Rohen JW, Futa R, Lutjen-Drecoll E. The fine structure of the cribriform meshwork in normal and glaucomatous eyes as seen in tangential sections. *Invest. Ophthalmol. Vis. Sci* 1981;21:574–585. [PubMed: 7287347]
- Rohen JW, Rentsch FJ. [Morphology of Schlemm's canal and related vessels in the human eye]. *Albrecht Von Graefes Arch. Klin. Exp. Ophthalmol* 1968;176:309–329. [PubMed: 5305008]
- Ruehm SG, Corot C, Vogt P, Kolb S, Debatin JF. Magnetic resonance imaging of atherosclerotic plaque with ultrasmall superparamagnetic particles of iron oxide in hyperlipidemic rabbits. *Circulation* 2001;103:415–422. [PubMed: 11157694]
- Svedbergh B. Protrusions of the inner wall of Schlemm's canal. *Am. J. Ophthalmol* 1976;82:875–882. [PubMed: 826163]
- Tai JH, Foster P, Rosales A, Feng B, Hasilo C, Martinez V, Ramadan S, Snir J, Melling CW, Dhanvantari S, Rutt B, White DJ. Imaging islets labeled with magnetic nanoparticles at 1.5 Tesla. *Diabetes* 2006;55:2931–2938. [PubMed: 17065328]
- Ten Hulzen RD, Johnson DH. Effect of fixation pressure on juxtacanalicular tissue and Schlemm's canal. *Invest. Ophthalmol. Vis. Sci* 1996;37:114–124. [PubMed: 8550315]

- Teng CC, Paton RT, Katzin HM. Primary degeneration in the vicinity of the chamber angle; as an etiologic factor in wide-angle glaucoma. *Am. J. Ophthalmol* 1955;40:619–631. [PubMed: 13268529]
- Thomassen TL. On the exit of aqueous humour in normal eyes. *Acta Ophthalmol. (Copenh.)* 1950;28:479–487. [PubMed: 14846560]
- Zhu JY, Ye W, Gong HY. Development of a novel two color tracer perfusion technique for the hydrodynamic study of aqueous outflow in bovine eyes. *Chinese Medical Journal (Engl)* 2010;123:599–605.
- Zhu XY, Bentley MD, Chade AR, Ritman EL, Lerman A, Lerman LO. Early changes in coronary artery wall structure detected by micro-computed tomography in experimental hypercholesterolemia. *American Journal of Physiology, Heart and Circulatory Physiology* 2007;293:H1997–H2003. [PubMed: 17573460]
- Zhu XY, Chade AR, Rodriguez-Porcel M, Bentley MD, Ritman EL, Lerman A, Lerman LO. Cortical microvascular remodeling in the stenotic kidney: role of increased oxidative stress. *Arterioscler. Thromb. Vasc. Biol* 2004;24:1854–1859. [PubMed: 15308558]

Figure 1. Schematic diagram of 3D micro-CT imaging

Anterior segment tissue mounted on a mechanical stage was scanned using an X-ray tube containing a molybdenum anode (left side of figure). The X-rays were converted to visible light by projection onto a crystal scintillator plate. Visible light was focused by a lens onto a CCD-camera (right side of the figure) and the images were captured digitally. The ANALYZE© software program was used to construct, analyze, and display the 3D images.

Figure 2. Comparison of 3D micro-CT volume slices at 2 μ m and 5 μ m with correlative plastic section

(A) 3D micro-CT slice from volume scanned at 5 μ m. Schlemm's canal (SC) visible as dumbbell shape with narrow region centrally. Collector channel (CC) opens from the right hand side of the dumbbell. Scale Bar = 200 μ m. (B) 3D micro-CT slice from volume scanned at 2 μ m. Schlemm's canal (SC) visible as two regions (arrows) with the anterior region adjacent to the collector channel orifice (CCO). Episcleral vessel = (EPV). Scale Bar = 100 μ m. (C) Correlative 1 μ m plastic section stained with Toluidine blue. Schlemm's canal (SC) opens in two regions anteriorly and posteriorly (arrows) with collector channel orifice (CCO) visible centrally. Episcleral vessel (EPV) which connects to the CC outside of the section is visible above the CCO. Scale bar = 200 μ m.

Figure 3. Multiplanar sections of the conventional outflow pathway in the perfused eye scanned at 5 μm

Visibility of trabecular meshwork (TM), Schlemm's canal (SC), a collector channel (CC) and episcleral vessels (EPV). Distance between sections: (A) original image, (B) 45 μm distal to original image, (C) 95 μm distal to original image, (D) 120 μm distal to original image, (E) 135 μm distal to original image, (F) 150 μm distal to original image. In Figure 3F, a large vessel, possibly of a ciliary artery is visible. Scale bar = 300 μm .

Figure 4. Unique images of the distal aqueous outflow pathway at 5 μm

(A) Vessel observed paralleling Schlemm's canal (SC). (B) A collector channel (CC) is shown extending from Schlemm's canal (SC) and joining intrascleral vessel (INV) traveling posteriorly to either join a ciliary vessel or proceeding to merge with an episcleral vessel. Image is 165 μm proximal to (A). (C) One collector channel (CC1) leaves the anterior portion of Schlemm's canal (SC) and extends towards an intrascleral vessel. (D) Two CCs (CC1 and CC2) are visible with a small island of tissue extending between them. Both join the same intrascleral (INV) vessel leading away from Schlemm's canal (SC). Image is 20 μm distal to (C). Scale bar = 300 μm .

Figure 5. 3D micro-CT sub-volume of representative collector channel, Schlemm's canal and episcleral vessel at 2 μ m

(A) Region of representative collector channel (CC) shows two part Schlemm's canal (SC) and episcleral vessel (arrows). Scale bar = 100 μ m. (B) 3D micro-CT sub-volume showing the entire episcleral vessel (blue) separated from Schlemm's canal (pink) by a collector channel (yellow arrow). (C) Wedge from the center of the 3D micro-CT sub-volume showing the narrow collector channel (CC) joining the episcleral vessel and the anterior portion of Schlemm's canal (pink arrow). (D) 3D micro-CT sub-volume viewed from the base of Schlemm's canal (pink) showing collector channel orifice (CCO) opening off of the canal. Scale bar = 100 μ m.

Figure 6. 3D micro-CT reconstruction of anterior segment of the perfused eye
 Reconstructed 3D wedges from 5 μm voxel scans (gray) with the pars plicata of ciliary body processes visible adjacent to Schlemm's canal and collector channels (magenta). Collector channels are indicated with black arrows. Asterisk denotes two closely adjacent collector channels in the inferior quadrant. Schlemm's canal is attenuated in the superior quadrant. Scale bar = 2 mm.