

Clinical Evidence of Valvelike Structures in Schlemm Canal and Their Anatomic Links Detected by Optical Coherence Tomography



RONALD L. FELLMAN, STEVEN PADILLA, AVERY LIU, MATTHEW HOLLIMAN, CHEN XIN, ELIZABETH MARTIN, DAVINDER GROVER, AND RUIKANG K. WANG

- **PURPOSE:** To determine if the valvelike structures in Schlemm canal detected by OCT in the clinic setting are comparable to established intracanalicular ex vivo laboratory images.
- **DESIGN:** Retrospective observational case series
- **PARTICIPANTS:** Pre- and postoperative angle images of 10 human subjects from Glaucoma Associates of Texas who underwent interventional valve-enhancing trabeculotomy (iVENt) were compared to 24 human and non-human primate (NHP) ex vivo eyes from the Johnstone Laboratory.
- **METHODS:** To determine the origin of the newly observed in vivo OCT intracanalicular structures, the OCT images were compared to 6 known laboratory outflow images from the Johnstone Laboratory: (1) hinged collagen flaps (HCFs), (2) Schlemm canal (SC) inlet valve (SIV), (3) circumferential vascular channels (CVCs), (4) collector channel ostium (CCO), (5) septum of the outer wall of SC (OWSC), and (6) SC.
- **MAIN OUTCOME MEASURES:** A comparison of the shape, location, and dimensions of the 6 ex vivo structures to the undefined in vivo clinic OCT angle structures.
- **RESULTS:** Two hundred seventy outflow laboratory images from 15 human and 9 nonhuman primates (NHPs) were sourced from the Johnstone Laboratory to define the measurements of the 6 key outflow structures that served as the comparators for the undefined in vivo OCT structures of 277 clinic angle images. The shape and location of the clinic in vivo valvelike structures in SC and their anatomic links correlated with the shape and location of all 6 known ex vivo laboratory structures. A limited

number of preoperative eyes also demonstrated valvelike intracanalicular structures. The in vivo OCT dimensions on average were greater than ex vivo measurements, likely because of tissue shrinkage from scanning electron microscopy (SEM) processing.

- **CONCLUSION:** This is the first clinical OCT evidence of valvelike structures in Schlemm canal, which were visualized in all 10 postoperative eyes. Based on structural and dimensional similarities, the authors hypothesize that the 3 in vivo clinical OCT structures closely resemble the SIV, HCF, and CVC. Our preliminary results bridge the gap from the laboratory to the clinic and add support to the addition of these 3 structures to the anatomy of the living human aqueous outflow system. (Am J Ophthalmol 2026;285: 324–334. © 2026 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>))

LABORATORY EVIDENCE OF BOTH HUMAN AND nonhuman primate (NHP) intracanalicular structures has existed for more than a half century.¹ Initially thought to prevent collapse of the canal, these structures were named the endothelial tubule of Johnstone,² transcanalicular septa, tissue bridges,³ and septa.⁴ Continuing ex vivo studies revealed an association of the spanning structures with collector channel openings,⁵ and eventually a valvelike role in motion-dependent aqueous outflow.⁶ However, in vivo confirmation in the living human eye of these laboratory structures has been missing.

On reviewing a high-definition optical coherence tomography (HD-OCT) angle image following canal-based minimally invasive glaucoma surgery (MIGS), the authors unexpectedly visualized a spanning canal structure. Schlemm canal (SC) is typically slitlike as seen on routine OCT; however, dilation of the canal after interventional valve-enhancing trabeculotomy or iVENt, allowed visualization of structures in the lumen (Figure 1). The authors were cognizant of previous images of ex vivo intracanalicular structures and realized a similarity. This study compares in vivo OCT images acquired pre- and post-canal-based iVENt to established ex vivo laboratory structures to determine the nature of the valvelike intracanalicular structures.



Supplemental Material available at [AJO.com](http://ajoc.com).
Accepted for publication January 27, 2026.

From the Glaucoma Associates of Texas (R.L.F., D.G.), Dallas, Texas, USA; Department of Bioengineering, University of Washington (S.P., R.K.W.), Seattle, Washington, USA; Department of Global Health, School of Public Health, University of Washington (A.L.), Seattle, Washington, USA; Biological Sciences Collegiate Division, University of Chicago (M.H.), Chicago, Illinois, USA; Department of Ophthalmology, Beijing Anzhen Hospital, Capital Medical University (C.X.), Beijing, China; Achieve Eye and Laser (E.M.), Silverdale, Washington, USA.

Inquiries to Ronald L. Fellman, Glaucoma Associates of Texas, Dallas, Texas, USA; e-mail: rfellman1982@gmail.com

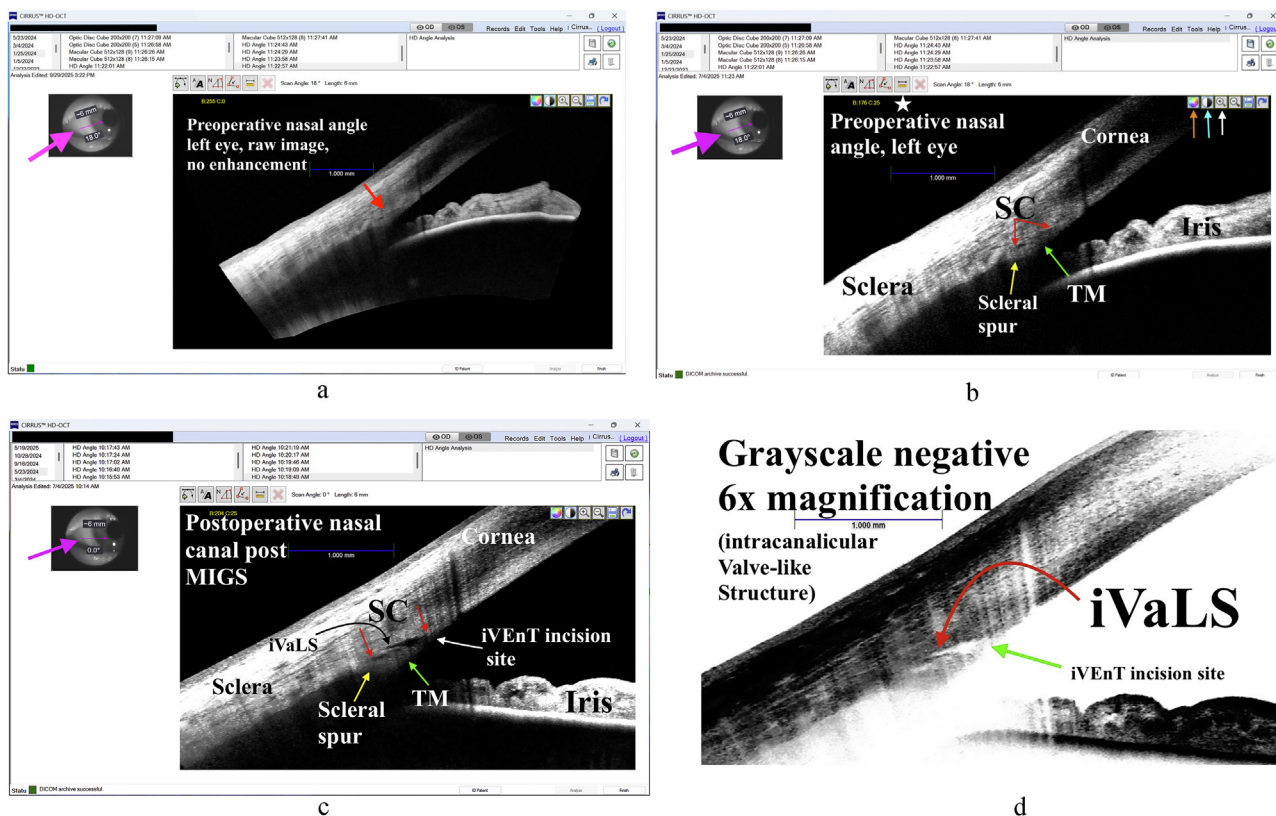


FIGURE 1. High-definition ocular coherence tomography (HD-OCT) imaging of Schlemm canal in a left eye before and after iVENT surgery. **A.** Preoperative raw image. Unprocessed nasal-angle scan showing Schlemm canal (red arrow) and scan site (~8:45 clock hour, purple arrow). At native magnification, angle details are indistinct, and no display adjustments are applied. **B.** Preoperative enhanced image. Four-click relative magnification and display optimization improve visualization of intracanalicular contents. The scleral spur (yellow arrow) and trabecular meshwork (green arrow) are identified. Brightness and contrast were adjusted to 176 and 25 (default = 225 and 0). Image quality depends on coordinated use of magnification, ruler placement, brightness, contrast, and grayscale adjustments. **C.** Postoperative image (4 months after iVENT). Canal dilation reveals an intracanalicular valvelike structure (iVaLS; curved black arrow). The iVENT incision below Schwalbe's line (white arrow) minimizes canal trauma. Dilated Schlemm canal (between red arrows) shows enhanced visibility of intraluminal structures. **D.** Postoperative enhanced image. Same eye under 6-click magnification with reversed grayscale, highlighting the valvelike spanning structure (curved red arrow). The appearance closely resembles previously described ex vivo histologic images. iVENT = interventional valve-enhancing trabeculotomy.

METHODS

In vivo OCT angle images from Glaucoma Associates of Texas were compared to the known aqueous outflow system (AOS) structures from ex vivo human and NHP eyes sourced from the Johnstone Laboratory, University of Washington, Department of Ophthalmology, Seattle, Washington. Historically, the laboratory employed 3 primary methods for investigations: scanning electron microscopy (SEM), OCT with innovative imaging platforms, and confocal microscopy. Complementary techniques included lumen expansion with viscodilation, variable luminal SC pressures, and immunofluorescence. The fundamental preparatory procedures applied to both eye bank and NHP eyes are outlined below, with detailed descriptions of

each specific method available in previously published literature.

Laboratory images were derived from a combination of 5 human eyes from the eye bank of Lions World Vision Institute (Seattle, WA) and 9 NHP eyes—7 from *Macaca nemestrina* and 2 from *Macaca mulatta*, sourced from the Washington State National Primate Center Tissue Program. Basic preparation included excising their posterior halves. The vitreous was carefully removed using eye spear-cellulose sponges, followed by trimming of the sclera and ciliary muscle. The suspensory ligaments of the lens were severed with Vannas scissors to facilitate lens extraction. Subsequently, the anterior halves were sectioned into 4 quadrants using scissors: superonasal (SN), inferonasal (IN), superotemporal (ST), and inferotemporal (IT). For detailed descriptions of all the methodologies employed, readers are referred to the following publications: OCT

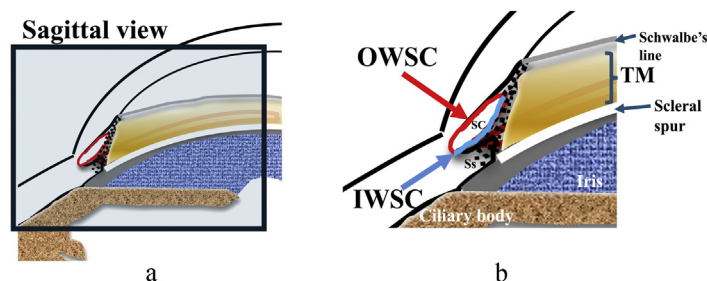


FIGURE 2. Orientation and anatomical reference for Schlemm canal (SC) imaging. **A.** The sagittal plane (black rectangle with light-blue tint) defines the standard reference view used for all in vivo OCT images. **B.** Schematic showing key structures: outer wall (OWSC, red arrow), inner wall (IWSC, blue arrow), scleral spur (Ss), and trabecular meshwork (TM). The aqueous outflow system extends from the scleral spur to the Schwalbe line.

platform,⁷⁻⁹ SEM,¹⁰ confocal microscopy,¹¹ and Supplemental Tables S1 and S2. Ten additional human eyes from a previous joint laboratory study were available for review of ex vivo OCT dimensions.

Six previously published key laboratory outflow anatomic structures served as the basis for in vivo comparisons: (1) hinged collagen flaps (HCFs), (2) SC inlet valve (SIV), (3) circumferential vascular channels (CVCs), (4) collector channel ostia (CCO), (5) septum of the outer wall of SC (OWSC), and (6) SC. The data were analyzed comparing the shape, location, and dimensions of the in vivo OCT clinic structures to the 6 key ex vivo structures.

For clarification concerning both ex vivo and in vivo images, the septum was defined as a partitioning wall that separates 2 distinct compartments, specifically SC from the CVC. Intraluminal structures refer to images seen within SC. An HCF was defined as a flaplike structure that constitutes part of the outer wall of the Schlemm canal (ie, OWSC). The methodology used to measure the flap is outlined in Figure 3, A. In vivo OCT measurements were conducted using the built-in measurement tools of the Zeiss Cirrus system, whereas laboratory measurements were performed using ImageJ software. Whenever feasible, measurements were reported as the mean $\pm$ SD, followed by the range in parentheses.

At Glaucoma Associates of Texas, Dallas, Texas, HD-OCT angle scans were acquired using the Zeiss Cirrus 6000 OCT system (Zeiss). The HD-OCT angle analysis software package was used for all measurements because of its superior resolution compared with other anterior segment imaging modalities. The HD-OCT angle analysis tools were used to measure, visualize, and enhance the clarity of the OCT images, with particular emphasis on the contrast and brightness adjustment features, as explained and illustrated in Figure 1, A and B. Patients who underwent phaco-iVEnT between June 2023 and September 2024 and had both preoperative and postoperative HD-angle OCT images were selected for study inclusion. This study adhered to the principles of the Declaration of Helsinki and received approval from the Institutional Review Board (Sterling IRB) of the

affiliated clinical practice. Figure 2, A, defines the sagittal horizontal cross-sectional plane used for the OCT images, whereas Figure 2, B, illustrates the location and orientation of key anatomical structures.

To acquire enough high-quality images, 2 to 5 HD-OCT angle scans were performed at the 3- and 9-o'clock meridians. Scans exhibiting segmentation errors, limbal shadows, or motion artifacts were excluded from analysis. OCT images from every session were classified as readable or unreadable, along with the reason for exclusion, SC morphology was graded (Supplemental Table S3) and all visible outflow structures measured (Supplemental Tables S4-S7).

HD-OCT angle scans were captured as a “raw” image with an A-scan density of 1024, yielding a pixel resolution of 5.8 μ m. On board internal fixation and signal strength for posterior pole imaging is not available for anterior segment imaging. For this reason, additional postprocessing controls (eg, magnification, contrast, and brightness) were adjusted as necessary and described in the accompanying figure text.

All ex vivo images were reviewed and measured by the SEM laboratory group from the Johnstone Laboratory and final adjudication of the measurements reviewed by the senior laboratory technician. OCT image grading and structural identification were performed using a predefined ordinal visibility scale for Schlemm canal and luminal features, as detailed in Supplemental Table S3. All in vivo OCT images were reviewed and measured by the senior glaucoma clinician (R.L.F.) and independently reviewed by an experienced laboratory investigator with extensive expertise in Schlemm canal anatomy, providing independent confirmation of the anatomical correspondence between in vivo OCT findings and established ex vivo structures. Structural labels (eg, HCF, SIV, CVC) were assigned based on predefined operational criteria including anatomical location, morphology, and relationship to adjacent outflow structures, rather than image contrast alone.

Guided by predefined structural criteria and expert consensus review, the subsequent analyses focused on comparing the morphology, location, and dimensions of in

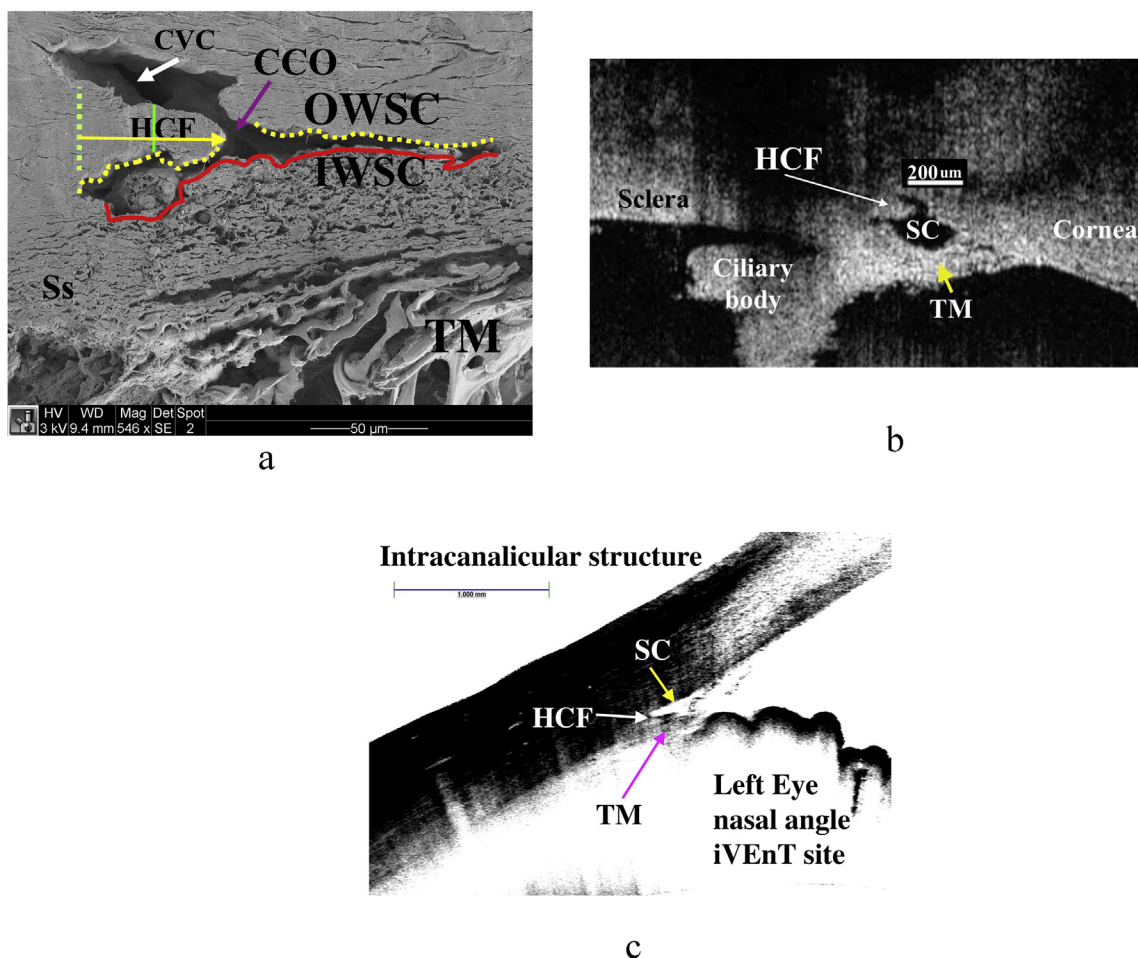


FIGURE 3. Hinged collagen flap (HCF), a component of the outer wall of Schlemm canal (OWSC). **A.** Scanning electron micrograph of a nonhuman primate eye showing the HCF. The vertical green dotted line is the base or hinge of the HCF, facilitating motion of the flap into SC or toward the CVC. Note the length of the HCF (solid horizontal yellow arrow) composes part of the OWSC (yellow dotted line). The measurement of the length of the HCF was made from the base of the flap (green dotted line) to its tip, at the yellow arrow. The vertical solid green line denotes where the width of an HCF was measured. The yellow dotted line is the OWSC. The inner wall (IWSC, solid red line) and canal lumen are delineated. The collector channel ostium (CCO, purple arrow) and circumferential vascular channel (CVC, white arrow) are also visible. The motion of the HCF and Schlemm canal inlet valve (SIV) act together as a valvelike unit regulating aqueous flow. **B.** Ex vivo OCT image of canal and HCF. Appearance of HCF from an OCT perspective. All 3 images, A through C, are oriented so the reader can correlate the morphology of the SEM along with its orientation, with both ex vivo and in vivo OCT. The morphology of the ex vivo OCT best fits the dimensions of the in vivo OCT, for there is minimal shrinkage of the HCF with ex vivo OCT preparation. The mean length (Table 1) of the OCT ex vivo HCF = 147.5 μm compared with the mean in vivo preoperative OCT HCF measurement of 163 μm . The SEM of HCF was 85 μm , significantly shorter, likely because of SEM processing. **C.** Postoperative HD-OCT image 9 months after iVenT showing an HCF (white arrow) prolapsing into SC (yellow arrow) near the trabecular meshwork (purple arrow). Acquired with grayscale negative mode and ~ 6 to $8\times$ relative magnification, this HCF measured 225 μm in length and 26 μm in height. There may be a component of HCF stretch induced by iVenT surgery. iVenT = interventional valve-enhancing trabeculotomy, SC = Schlemm canal, Ss = scleral spur, TM = Trabecular meshwork.

vivo OCT-visible structures with well-characterized ex vivo aqueous outflow anatomy.

The interventional valve enhancing trabeculotomy or iVenT is a novel surgery developed by 2 of the authors (R.L.F. and D.S.G.) that selectively creates a minimally invasive incision in the anteriormost aspect of the nonfiltering trabecular meshwork. This procedure mini-

mizes disruption of intracanalicular structures, which are typically located in the posterior portion of the canal. As such, the authors feel this specific surgical technique has allowed us to visualize the intracanalicular structures on OCT because the Schlemm canal is minimally disturbed, in contrast to more traditional methods of trabeculotomy.

TABLE 1. Aqueous Outflow Structure Measurements

Aqueous Outflow Structure	Ex Vivo						In Vivo			
	SEM				OCT (Variable IOP)		OCT			
	Human		NHP		Human		Preoperative		Postoperative	
	E/I	Mean ± SD	E/I	Mean ± SD	E/I	Mean ± SD	E/I	Mean ± SD	E/I	Mean ± SD
HCF: length	4/6	85.4 ± 41.2 (39.9-138.9)	7/33	64.4 ± 31.5 (22.9-132.9)	10/29	147.5 ± 49.4 (41-268)	6/6	163 ± 18 (141-185)	9/29	209 ± 50 (97-298)
HCF: height	4/6	20.2 ± 9.7 (8.0-31.5)	7/33	21.2 ± 9.2 (5.2-43.6)	10/31	70.6 ± 25.5 (35-138)	6/6	29 ± 10 (23-49)	8/27	35 ± 9 (24-48)
Septum: width	2/2	99.4 ± 24.5 (82.0-116.7)	3/7	88.6 ± 9.3 (71.7-108.7)			1/1	185	2/2	167 ± 90 (103-230)
Septum: height	2/2	13.9 (11.1-16.7)	3/7	25 ± 9.4 (14.2-40.2)			1/1	42	1/2	36.0 ± 8 (31-45)
CCO: height	2/16	14.4 ± 12 (9.4-22.2)							4/4	26.0 ± 9 (18-50)
Schlemm inlet valve SIV: length	2/12	76.3 ± 3.0 (69.6-83.0)	1/7	101.2 ± 11.5 (72.9-129.4)			4/5	193 ± 84 (97-290)	4/7	185 ± 37 (130-219)
Schlemm inlet valve SIV: diameter	2/12	19.1 ± 0.5 (18.1-20.2)	1/7	19.2 ± 1.7 (15.1-23.3)			4/5	14 ± 5 (8-18)	4/7	20 ± 2 (19-22)
CVC: width	1/40	150.6 ± 113.8 (50-400)					3/4	235 ± 91 (137-318)	7/16	254 ± 79 (142-346)
CVC: height	1/40	21.9 ± 11.5 (10-65)					3/4	25 ± 10 (14-34)	6/13	37.0 ± 13 (33-61)
Schlemm canal SC: length	5/113	268.2 ± 62.3 (129.7-443.1)			10/30	360.3 ± 83.6 (130-528)	8/14	461 ± 73 (310-532)	10/100	452 ± 80 (344-592)
Schlemm canal SC: height	5/112	56.4 ± 27.5 (11.2-132.9)			10/30	101.4 ± 41.5 (40-198)	8/14	43 ± 9 (28-55)	10/87	69 ± 10 (50-91)

CCO = collector channel ostium, CVC = circumferential vascular channels, D = diameter, E = number of eyes, HCF = hinged collagen flap, H = height, I = number of images, L = length, NHP = nonhuman primate, OCT = optical coherence tomography, SC = Schlemm canal, SEM = scanning electron microscopy.

All measurements are in micrometers. The morphology of structures is described in the text, while their measurements are noted in the table. The table is divided into ex vivo and in vivo measurements, both SEM and OCT for ex vivo (nasal vs temporal was not always specified), and the measurements of in vivo are from the nasal angle, for the data for temporal angle were very limited. In vivo is divided into preoperative and postoperative measurements. A recurrent theme throughout the measurements is that the in vivo measurements are larger, especially the diameter, compared to ex vivo. This is likely due to the 10% to 30% tissue shrinkage associated with tissue processing for SEM. Note the ex vivo OCT measurements, which do not undergo the tissue shrinkage associated with SEM, have longer dimensions, closer to the in-clinic in vivo measurements. One area to note is the increased height of SC seen with ex vivo OCT, which is due to dilation of the canal for experiments, this will artificially increase the height of SC. The E and I before each structural category reflects the number of eyes (E) evaluated with their number of respective images (I). All OCT scans were evaluated in a consecutive fashion, only scans that were unreadable were omitted.

RESULTS

Two hundred seventy ex vivo tissue specimens from 15 human (220 images) and 9 NHP eyes (50 images) were sourced from the Johnstone Laboratory. The measurements and morphology of these specimens is summarized in

Table 1 under ex vivo. Ten eyes from 10 patients from Glaucoma Associates of Texas revealed 277 scans, of which 187 (67.5%) were readable and of sufficient quality (Table 1, under "In Vivo"). Scans were acquired over a mean follow-up of 15 months (range 1-24 months). The mean age of patients was 76 ± 6 years (range 68-81). The cohort comprised 6 right eyes and 4 left eyes, with an equal gender distribution of 5 male and 5 female. Nine patients were diag-

nosed with primary open angle glaucoma and 1 with pigmentary glaucoma.

All patients underwent combined phaco-nasal iVenT, and HD-OCT imaging of both the nasal and temporal angles was obtained. The authors systematically reviewed all OCT images for aqueous outflow system (AOS) structures in the nasal and temporal angles, and an in-depth analysis of each image classification and measurement is available in Supplemental Tables S4 to S7. The raw OCT image for each enhanced OCT figure listed below, is contained in Supplemental Figures S1-S7.

HCFs were the most consistently observed intracanalicular structure in the postoperative OCT images and were detected in 8 of 10 clinic eyes. The morphology of the ex vivo and in vivo OCT images (Figure 3, B and C) closely matched previous ex vivo SEM descriptions (Figure 3, A), and OCT images often revealed connections between HCFs and smaller spanning elements, likely Schlemm canal inlet valves (SIV) (Figure 4, B). In vivo OCT image Figure 3, C, shows the hinged collagen flap protruding into the canal lumen, consistent with its hypothesized valvelike function.

Postoperative HCFs measured $209 \pm 50 \mu\text{m}$ in length (range 97-298 μm) and $35 \pm 9 \mu\text{m}$ in thickness (range 24-48 μm). Six preoperative eyes also demonstrated HCFs, although visualization required increased magnification, brightness/contrast adjustment, and repeated inspection. The preoperative structures were easier to identify knowing their clock hour location from the postoperative images. Preoperative mean HCF dimensions were slightly smaller (length: $163 \pm 18 \mu\text{m}$, thickness $29 \pm 10 \mu\text{m}$).

High-resolution OCT laboratory ex vivo platform images (Figure 3, B) revealed an HCF length of $147.5 \pm 49.4 \mu\text{m}$ (range 41-268 μm) with a height of $70.6 \pm 25.5 \mu\text{m}$ (range 35-138 μm) (Table 1), nearly twice the length of the human and NHP SEM measurements, but almost exactly the same length as the preoperative in vivo OCT HCF of $163 \pm 18 \mu\text{m}$ (Figure 3, C). These findings exemplify tissue shrinkage with SEM and demonstrate that HCFs can be visualized both in vivo and ex vivo and support their potential role as Schlemm canal outlet valves, regulating flow between Schlemm canal and distal collector channels.

Schlemm canal inlet valves (SIVs) were noted in 4 of 10 postoperative eyes, appearing as spanning tubular structures, connecting the IWSC to the OWSC (Figure 4, A and B). Postoperative SIVs on average measured $185 \pm 37 \mu\text{m}$ in length (range 130-219 μm) and $20 \pm 2 \mu\text{m}$ in diameter (range 19-22 μm). Four preoperative eyes demonstrated a mean SIV measurement of $193 \pm 84 \mu\text{m}$ in length (range 97-290) and $14.5 \pm 5 \mu\text{m}$ in diameter (range 8-18). These in vivo dimensions of length were longer than ex vivo SEM images from human and NHP eyes (Table 1). This may partially reflect tissue shrinkage associated with SEM, which may be 15% to 30%. Even though the in vivo lengths were greater, the diameters were similar.¹² Figure 4, B, demon-

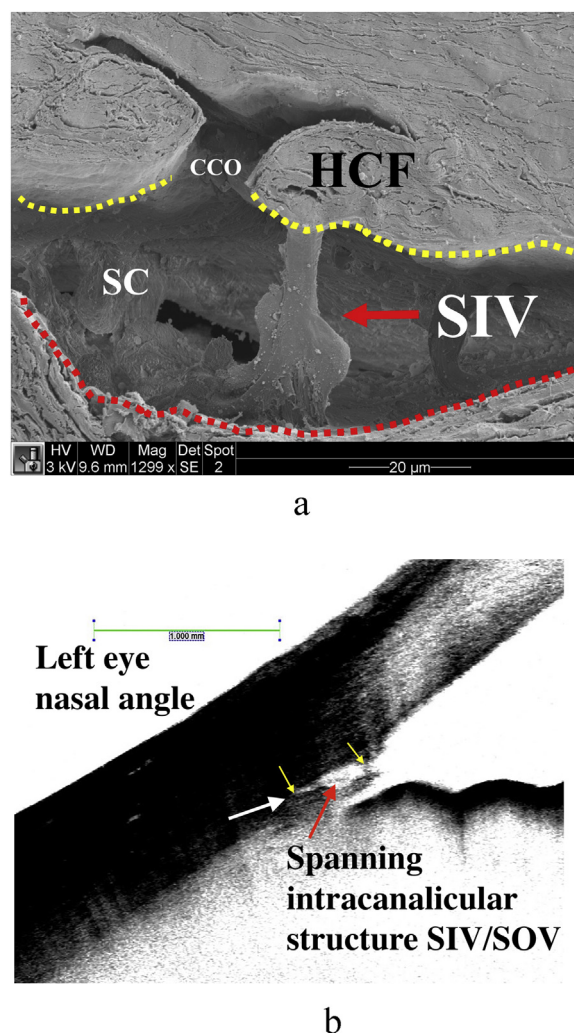


FIGURE 4. Spanning intracanalicular valvelike structures within Schlemm canal (SC). **A.** Scanning electron micrograph showing a canal-spanning structure extending from the inner wall (IWSC, red dotted line) to the outer wall (OWSC, yellow dotted line), often attached to a hinged collagen flap (HCF). These thinner elements bridge the canal lumen and are now recognized as elastic, valvelike structures, or Schlemm canal inlet valves (SIVs) formerly termed “septa.” **B.** In vivo HD-OCT image 1 year after iVenT demonstrating a tubular Schlemm canal inlet valve (SIV, red arrow) attached to an HCF (white arrow). The SIV measured 265 μm in length and 25 μm in diameter; the canal segment between yellow arrows measured 389 μm . The attachment of the SIV to the HCF supports prior concepts of a synchronous Schlemm canal outlet valve (SIV/SOV) complex. CCO = collector channel ostium, iVenT = interventional valve-enhancing trabeculotomy.

strates the likely attachment of an SIV to an HCF, spanning the canal.

The CVC, an outer parallel collector to SC, was detected in 7 of the 10 postoperative eyes (Figure 5, A and B). Their shape and location closely matched previous ex vivo SEM

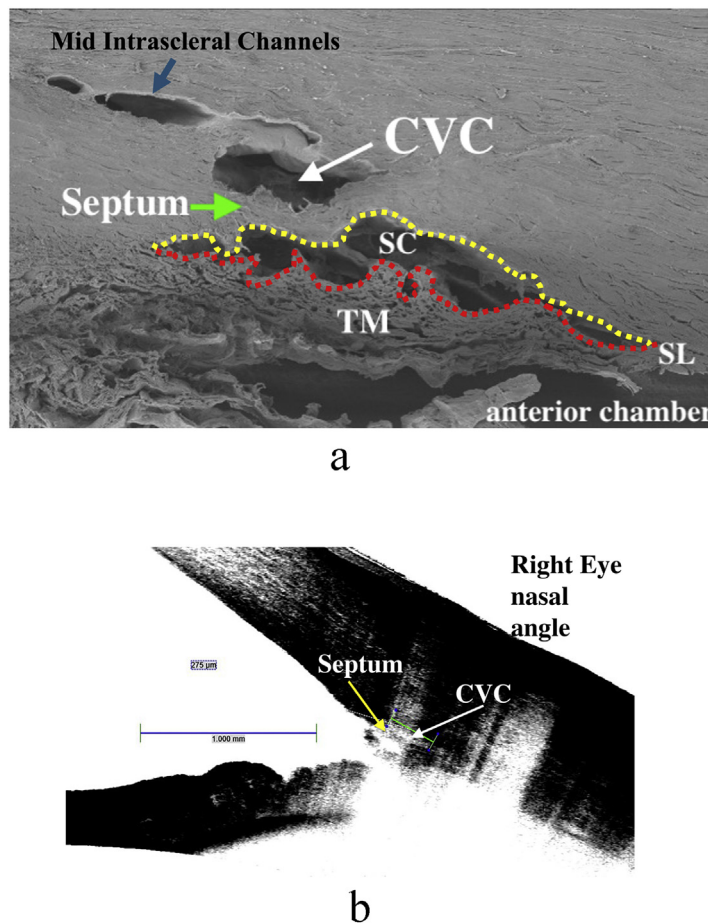


FIGURE 5. Circumferential vascular channels (CVCs) adjacent to Schlemm canal (SC). **A.** Scanning electron micrograph showing a CVC (white arrow) running parallel and external to SC. The green arrow points to the septum, a component of the outer wall of Schlemm canal (OWSC), yellow dotted line. The septum separates the CVC from SC. The inner wall (IWSC) is marked by the red dotted line. Hinged collagen flaps (HCFs) may also separate the CVC from SC and are thus continuous on the back wall of SC with the septum, an important 3-dimensional conceptualization (see [Figure 3, A](#)). Posterior hinging of the HCF could transiently restrict CVC flow, supporting motion-dependent regulation. **B.** In vivo grayscale-negative HD-OCT image 1 year after iVenT at 6-fold relative magnification. The CVC (white arrow) and septum (yellow arrow) are clearly delineated. The CVC measured 275 μm in width and 30 μm in height, with hyporefectivity consistent with aqueous transport. iVenT = interventional valve-enhancing trabeculotomy.

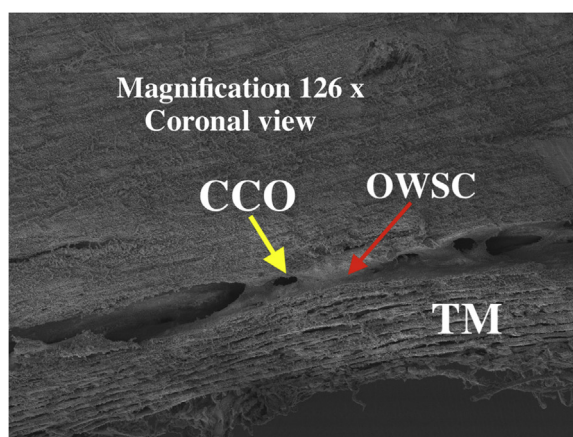
descriptions. As anticipated, the SEM dimensions of CVC were smaller than the average in vivo images. Postoperative CVCs measured $254 \pm 79 \mu\text{m}$ in width (range 142-346 μm) and $37 \pm 13 \mu\text{m}$ in height (range 33-61 μm). Three preoperative eyes also demonstrated CVCs, with similar dimensions, averaging 235 μm in width and 25 μm in height. [Figure 5, A and B](#), defines the location of the CVC, outer and parallel to SC. Note that the CVC is separated from the canal by either the septum ([Figure 5, A](#)) or an HCF ([Figure 3, A](#)). This is the first in vivo documentation of the location and dimensions of the CVC, demonstrating their height and width, which appear to convey aqueous from the canal ([Figure 6, B](#)) to distal collector channels.

CCOs are present on the OWSC, but none were seen in preoperative eyes, although CCOs were detected in 5 of the 10 postoperative eyes ([Figure 6, A and B](#)). Their shape

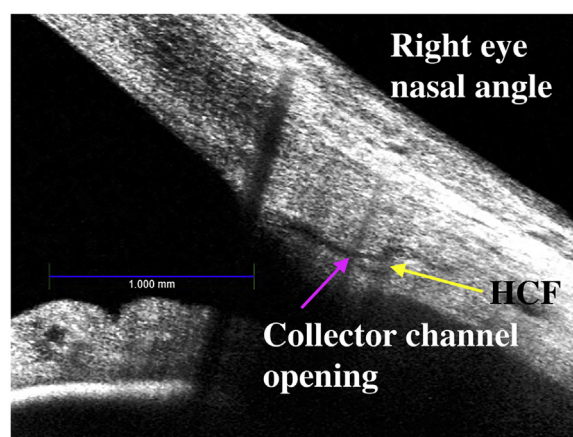
and location closely matched previous ex vivo SEM descriptions, considering some shrinkage with SEM ([Table 1](#)). Postoperative CCO measured $26 \pm 9 \mu\text{m}$ in height (range 18-50 μm). The horizontal plane is not measurable with in vivo OCT.

Two human ex vivo eyes exhibited visible septa, yielding average dimensions of $99.4 \pm 24.5 \mu\text{m}$ in width (range 82.0-116.7 μm) and $13.9 \pm 4.0 \mu\text{m}$ (range 11.1-16.7 μm) in thickness. Seven images from 3 NHP eyes revealed a width of $88.6 \pm 14.2 \mu\text{m}$ (range 71.7-108.7 μm) and thickness of $25 \pm 9.4 \mu\text{m}$ (range 14.2-40.2 μm). Septa were identified in 2 of 10 postoperative in vivo OCT images, and only 1 preoperative eye ([Figure 7, A and B](#)).

Schlemm canal was seen in all 10 postoperative eyes and measured $452 \pm 80 \mu\text{m}$ in diameter (range 344-592 μm), and $69 \pm 10 \mu\text{m}$ in height (range 50-91 μm) ([Figure 8](#)).



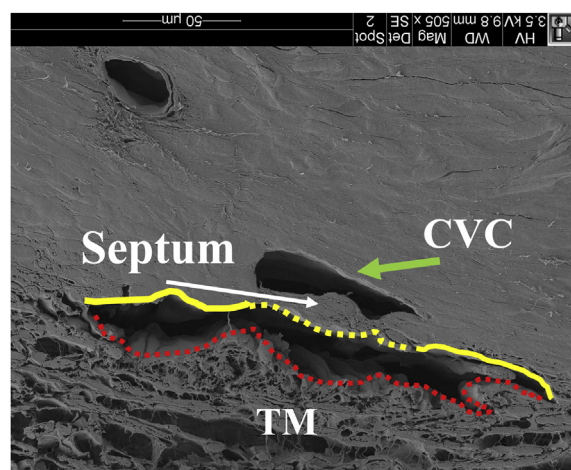
a



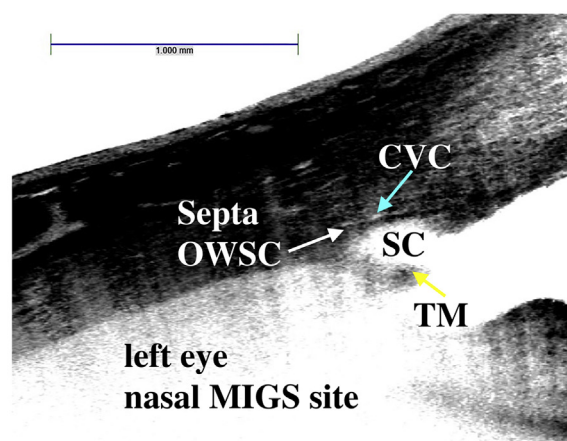
b

FIGURE 6. Collector channel ostium (CCO) of the outer wall of Schlemm canal (OWSC). **A.** Scanning electron micrograph (coronal view) showing an oval CCO (yellow arrow) within the OWSC (red arrow), its long axis oriented circumferentially. **B.** In vivo HD-OCT image 3 months after iVenT, meridional view at $\sim 8\times$ relative magnification. The CCO (purple arrow) lies along the posterior OWSC, typically near a hinged collagen flap (HCF, yellow arrow) that acts as a valvelike structure controlling ostial opening and closure. The CCO height here corresponds to the vertical dimension of the oval lumen. iVenT = interventional valve-enhancing trabeculotomy.

Eight preoperative eyes demonstrated similar SC dimensions. The ex vivo SEM average of $268\text{ }\mu\text{m}$, is smaller than the vivo images, potentially related to tissue shrinkage and the ex vivo OCT images, less prone to shrinkage, demonstrated a mean length of $360\text{ }\mu\text{m}$. The shape and location of in vivo SC images closely matched their ex vivo locations. When SC was only a slit, as seen by OCT image, it was recorded, but not measured; these examples are noted in the supplemental data, both preoperative and postoperative (Supplemental Tables S4-S7).



a



b

FIGURE 7. Septum, a structural component of the outer wall of Schlemm canal (OWSC). **A.** Scanning electron micrograph showing the septum (white arrow), a collagenous partition separating SC from the circumferential vascular channel (CVC). One face borders SC (yellow dotted line) within the OWSC (solid yellow line); the opposite face borders the CVC. The inner wall (IWSC) is indicated by the red dotted line. **B.** In vivo HD-OCT image 6 months after iVenT, grayscale negative, 6-fold magnification. The septum (white arrow) separates SC from CVC and measured $122\text{ }\mu\text{m}$ in width and $26\text{ }\mu\text{m}$ in height. Although the septum extends circumferentially for several millimeters, its full length is beyond current OCT imaging range. iVenT = interventional valve-enhancing trabeculotomy.

DISCUSSION

In 1974, Johnstone proposed that SC spanning “septa” were endothelial tubules that exhibited pressure-dependent changes, indicating a valvelike function.¹³ Laboratory studies over the past 2 decades suggest a valvelike mechanism interconnected to synchronous components, although

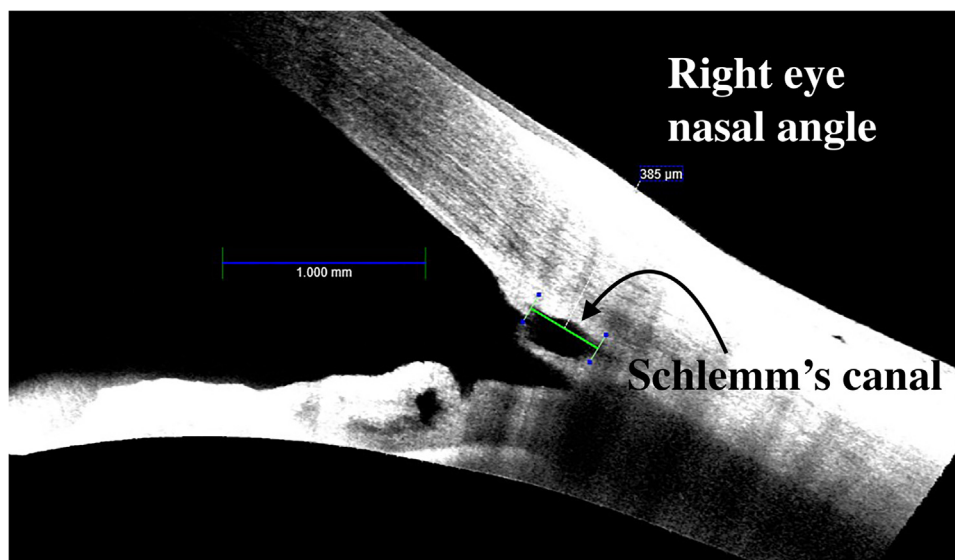


FIGURE 8. Marked dilation of Schlemm canal (SC) 1.5 years after iVENT. Postoperative HD-OCT image showing pronounced canal dilation with a diameter of 385 μm and height of 190 μm . The expanded lumen allows clear visualization of intracanalicular details compared with the slitlike SC morphology typical of glaucomatous eyes. At this location, no intracanalicular valvelike structures (iVaLS) were observed. Across all analyzable images, iVaLS were detected in 42 of 187 readable scans (22.5%). iVENT = interventional valve-enhancing trabeculotomy.

without in vivo support. This article supports the OCT presence of in vivo HCF and SIV in SC along with existence of an outer parallel collector channel to SC, the CVC. These structures anatomically substantiate the concept of a valvelike mechanism of aqueous outflow in the living human eye.

Innovations such as canal viscodilation, confocal microscopy, immunofluorescence (IF), OCT imaging platforms, and phase-sensitive (PhS) OCT¹⁴ have collectively advanced the understanding of the roles of each of the 3 anatomical components. The variable prolapse of the HCF into the lumen of the canal has never before been documented and points to the importance of a valvelike function with integrated movement of aqueous through the human AOS. The identification of these in vivo structures adds support to their role in the synchronous motion of aqueous outflow.

HD-OCT angle imaging is commonly used in the clinic to measure narrow angle parameters, such as angle opening distance. However, the capability of this imaging modality to visualize SC and its potential clinical applications remains underrecognized. Continued improvements in anterior segment imaging, such as the incorporation of fixation monitoring, enhanced signal-strength control, and image registration, are anticipated to further improve visualization. Intracanalicular structures are inherently more difficult to visualize in preoperative eyes, for Schlemm canal appears slitlike in the majority of individuals and becomes increasingly difficult to identify with increasing age¹⁵ and in glaucoma.¹⁶ Nonetheless, with appropriate magnification,

these structures were identifiable in a substantial proportion of preoperative eyes; specifically, SIV was identifiable in 40% and HCF in 60% of the preoperative eyes.

There are several limitations to our study. The first limitation is that angle scans were confined to the 3- and 9-o'clock meridians of the eye, as the eyelids obstruct visualization of the canal in other regions. This confines our study to the horizontal meridian. In spite of this, many structures were seen in the canal. Future studies should explore adjacent clock hours and incorporate both meridional and coronal views to obtain a more complete understanding of canal architecture. There may be selection bias based on images that were excluded because of poor quality of acquisition. This is a study limitation that reflects the technological limitations of OCT because of a loss of clarity from scattering, birefringence, and polarization at the corneoscleral limbus. Accordingly, the authors provided the reasons for exclusion in every image noted in Supplementary Tables S4 and S5. Another limitation involves the inability to scan the exact same canal segment across sessions, reflecting current technological constraints in anterior segment registration. Finally, the relatively small number of eyes undergoing the iVENT procedure reflects the retrospective nature of the study with a requisite need for both preoperative and postoperative images. In addition, it is possible that wound healing in the canal or dilation of the canal post iVENT may hinder interpretation of the valvelike structures, as noted in the Supplemental Tables S4-S7.

Another limitation of this study relates to image grading and reproducibility. Structural identification was performed

using a predefined grading framework and expert consensus rather than a masked, multigrader reproducibility analysis. Although images were independently reviewed by experienced clinical and laboratory investigators, formal inter- or intra-observer agreement statistics were not assessed. This reflects the exploratory and comparative nature of the study, which aimed to establish anatomical correspondence between in vivo OCT findings and well-characterized ex vivo structures rather than to estimate population-level prevalence. Future studies with larger cohorts, standardized acquisition protocols, and masked multigrader designs will be important to further quantify reproducibility, particularly for subtle intracanalicular features.

The published mean diameter of SC from a histologic perspective is 264 ± 55 μm .¹⁷ Our histologic analysis of SC diameter was very close at 268.2 ± 62.3 μm . The preparation associated with SEM causes tissue shrinkage. Studies vary in the degree of shrinkage, from 22% to 37%. Considering tissue preparation shrinkage, there was an overall general agreement of in vivo measurements to known AOS histologic measurements. Our study measured an ex vivo SC OCT diameter of 360.3 ± 83.6 μm , similar to an in vivo OCT study that measured in 19 eyes a mean of 347.2 ± 42.3 μm .¹⁸ Our in vivo preoperative and postoperative HD-OCT angle measurements of SC were longer (≈ 450 μm) than SEM and OCT ex vivo image measurements. This difference likely arises from measurement variations between swept-source and spectral-domain systems, as well as inherent differences in imaging geometry and refractive correction. The detailed optical physics underlying these differences is beyond the scope of this discussion but warrants future investigation.

This is the first in vivo OCT visualization of structures located inside Schlemm canal following a minimally invasive method of ab interno trabeculotomy that minimizes disruption of the intracanalicular structures, supporting evidence for decades of ex vivo investigations into motion-dependent synchronous aqueous outflow. Our preliminary results, although not a perfect comparison between ex vivo and in vivo measurements, reveal that clinicians and researchers have the opportunity to use HD-OCT angle imaging to conduct more detailed investigations of HCFs, CVC, SIV, and their relation to CCOs. Such studies will enhance our understanding of the anatomy, physiology, and pathophysiology of the human aqueous outflow system, MIGS outcomes, and improve our future surgical designs of glaucoma surgery.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Ronald L. Fellman: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization. **Steven Padilla:** Writing – review & editing, Methodology, Data curation. **Avery Liu:** Writing – review & editing, Methodology, Data curation. **Matthew Holliman:** Writing – review & editing, Methodology, Data curation. **Chen Xin:** Writing – review & editing, Methodology. **Elizabeth Martin:** Methodology. **Davinder Grover:** Writing – review & editing. **Ruikang K. Wang:** Writing – review & editing, Methodology, Conceptualization.

Funding/Support: This study received no funding. **Financial Disclosures:** The authors indicate no financial support or conflicts of interest. All authors attest that they meet the current ICMJE criteria for authorship.

Acknowledgment: The authors acknowledge the lifetime work of Dr Murray Johnstone, a mentor and teacher to many of us regarding the anatomy and physiology of the aqueous outflow system. Murray recently passed away, but his passion for imparting knowledge remains steadfast and his ex vivo laboratory work regarding valve-like structures in Schlemm canal is the backbone for this manuscript. The authors thank Tania Bolton of Glaucoma Associates of Texas, for her expertise in determining best methods for acquiring HD OCT angle images.

REFERENCES

1. Rohen J, Rentsch F. Electron microscopic studies on the structure of the outer wall of Schlemm's canal, its outflow channels and age changes. *Albrecht Von Graefes Arch Klin Exp Ophthalmol.* 1969;177:1–17.
2. Van Buskirk EM. Anatomic correlates of changing aqueous outflow facility in excised human eyes. *Invest Ophthalmol Vis Sci.* 1982;22:626–632.
3. Lütjen-Drecoll E. Structural factors influencing outflow facility and its changeability under drugs. A study in *Macaca arcoides*. *Invest Ophthalmol Vis Sci.* 1972;12:280–294.
4. Johnstone MA, Grant WM. Pressure-dependent changes in structures of the aqueous outflow system of human and monkey eyes. *Am J Ophthalmol.* 1973;75:365–383.
5. Bentley MD, Hann CR, Fautsch MP. Anatomic variation of human collector channel orifices. *Invest Ophthalmol Vis Sci.* 2016;57:1153–1159.
6. Johnstone M, Xin C, Martin E, Wang R. Trabecular meshwork movement controls distal valves and chambers: new glaucoma medical and surgical targets. *J Clin Med.* 2023;12:6599.
7. Hariri S, Johnstone M, Jiang, et al. Platform to investigate aqueous outflow system structure and pressure-dependent motion using high-resolution spectral domain optical coherence tomography. *J Biomed Optics.* 2014;19:106013.
8. Xin C, Wang RK, Song S, et al. Aqueous outflow regulation: optical coherence tomography implicates pressure-dependent tissue motion. *Exp Eye Res.* 2017;158:171–186.
9. Johnstone M, Xin C, Acott T, et al. Valve-like outflow system behavior with motion slowing in glaucoma eyes: findings using a minimally invasive glaucoma surgery-MIGS-like plat-

- form and optical coherence tomography imaging. *Front Med (Lausanne)*. 2022;9:815866.
10. Smit BA, Johnstone MA. Effects of viscoelastic injection into Schlemm's canal in primate and human eyes. Potential relevance to viscocanalostomy. *Ophthalmology*. 2002;109:786–792.
 11. Martin EA, Johnstone MA. A novel technique identifies valve-like pathways entering and exiting Schlemm's canal in *Macaca nemestrina* primates with similarities to human pathways. *Front Cell Dev Biol*. 2022;10:868029.
 12. Johnstone MA. A new model describes an aqueous outflow pump and explores causes of pump failure in glaucoma. In: Grehn F, Stamper R, eds. *Glaucoma: Essentials in Ophthalmology*. Berlin Heidelberg: Springer; 2006:3–34.
 13. Johnstone MA. Pressure-dependent changes in configuration of the endothelial tubules of Schlemm's canal. *Am J Ophthalmol*. 1974;78:630–638.
 14. Xin C, Song S, Johnstone M, et al. Quantification of pulse-dependent trabecular meshwork motion in normal humans using phase-sensitive OCT. *Invest Ophthalmol Vis Sci*. 2018;59:3675–3681.
 15. Chen Z, Sun-Mu Li J, Liu S, et al. Effect of age on the morphologies of the human Schlemm's canal and trabecular meshwork measured with swept-source optical coherence tomography. *Eye*. 2018;32:1621–1628.
 16. Jong J, Xu J, Wei A, et al. Spectral-domain optical coherence tomographic assessment of Schlemm's canal in Chinese subjects with primary open angle glaucoma. *Ophthalmol*. 2013;120:709–715.
 17. 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.
 18. Usui T, Tomidokoro A, Mishima K, et al. Identification of Schlemm's canal and its surrounding tissues by anterior segment Fourier domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2011;52:6934–6939.