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Slit-lamp photography and videography with high magnifications

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Abstract

Purpose—To demonstrate the use of the slit-lamp photography and videography with extremely high magnifications for visualizing structures of the anterior segment of the eye.

Methods—A Canon 60D digital camera with Movie Crop Function was adapted into a Nikon FS-2 slit-lamp to capture still images and video clips of the structures of the anterior segment of the eye. Images obtained using the slit-lamp were tested for spatial resolution. The cornea of human eyes was imaged with the slit-lamp and the structures were compared with the pictures captured using the ultra-high resolution optical coherence tomography (UHR-OCT). The central thickness of the corneal epithelium and total cornea was obtained using the slit-lamp and the results were compared with the thickness obtained using UHR-OCT.

Results—High-quality ocular images and higher spatial resolutions were obtained by using the slit-lamp with extremely high magnifications and Movie Crop Function, rather than the traditional slit-lamp. The structures and characteristics of the cornea, such as the normal epithelium, abnormal epithelium of corneal intraepithelial neoplasia, LASIK interface, and contact lenses, were clearly visualized using this device. These features were confirmed by comparing the obtained images with those acquired using UHR-OCT. Moreover, the tear film debris on the ocular surface and the corneal nerve in the anterior corneal stroma were also visualized. The

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thicknesses of the corneal epithelium and total cornea were similar to that measured using UHR-OCT ($P < 0.05$).

Conclusions—We demonstrated that the slit-lamp photography and videography with extremely high magnifications allows better visualization of the anterior segment structures of the eye, especially of the epithelium, when compared with the traditional slit-lamp.

Keywords

slit lamp biomicroscopy; videography; high magnification; ultra high optical coherence topography; corneal epithelium thickness

Introduction

The slit-lamp is probably the most convenient and essential tool in every ophthalmological office.¹ It was invented more than a hundred years ago with the intension of visualizing the anterior segment of the eye. This device has been rapidly evolving with the acquisition of many features that allow an ample array of uses. Two great examples are the microscopic visualization of the retina, which has been possible since the introduction of powerful converging lenses, and measurement of the human corneal thickness with the slit-lamp, which began with the development of pachymetry.² More recently, Gellrich et al introduced the concept of “videography with the slit-lamp”.¹ The main purpose of this model was to provide an imaging alternative for most ophthalmic pathologies, including ocular surface findings. However, little has been explored about the usage of unique functionalities of some cameras to boost image magnification of the anterior structures of the eye.

The corneal surface is covered by a non-keratinized stratified squamous epithelium. This allows the cornea to function as both, a transmitter and refractor of light, and as a barrier for the entry of pathogens to the cornea. The measurement of corneal epithelium thickness provides important information for assessing corneal function, especially in patients who have been exposed to cataract surgery, in contact lens wearers and, for diagnosing diseases like keratoconus or dry eye syndrome.³⁻⁶ With the attachment of the pachometer to the slit-lamp, epithelial thickness dimensions could be a convenient measurement to obtain during routine clinical visits. However, the relative difficulty for visualizing the epithelium, mainly due to the low magnification of the traditional slit-lamp, makes pachymetric measurements of the corneal layers markedly troublesome. The goal of this study was to demonstrate the use of the slit-lamp photography and videography with extremely high magnifications for visualizing the anterior structures of the eye in more details.

Methods

The integration of a digital camera to the slit-lamp biomicroscopy has been previously described.⁷ Briefly, a digital camera (Canon 60D, Canon Inc., Melville, NY) was attached to a traditional slit-lamp (Nikon FS-2, Nikon Inc., Melville, NY). This modification is conveniently easy, since it is accomplished with the incorporation of an adapter ring in to the slit-lamp, which permits the conversion of the Nikon mount to the Canon mount of the camera. The total cost of the adaptation was about \$700 in addition to the Nikon slit-lamp.

Currently, the Nikon slit-lamp is not available in the US market. The cost of a photography slit-lamp depends on the maker. For example, a Haag-Streit slit-lamp with photography capability would cost about \$45,000. Obviously, adding a camera into an existing slit-lamp would not be costly. According to the camera manufacturer, the Canon EOS 60D comes with an imaging sensor of 22.3 mm $\times$ 14.9 mm, which allows acquiring a maximum resolution of 5,184 $\times$ 3,456 pixels ($\sim$ 17.9 megapixels). The pixel dimension on the camera sensor is 4.3 μ m $\times$ 4.3 μ m. This camera has a special feature called Movie Crop Function, which provides an additional $\sim$ 7 $\times$ optical magnification on top of the 30 $\times$ intrinsically on the slit-lamp. The combination of both magnifiers results in an extremely high magnifications up to $\sim$ 210 $\times$.⁷ A resolution test card (U. S. Air Force 1951, Edmund Optics, Inc, Barrington, NJ) was used to test the spatial resolution displayed on a high definition monitor (Samsung, model UN22D5003BF, Samsung Electronics America Inc., Ridgefield Park, NJ) with a High-Definition Multimedia Interface (HDMI) connection (Fig. 1).⁸

This study was approved by the institutional review board (IRB) for human research of the University of Miami. The consent form was signed by each subject who was treated accordingly to tenets of the Declaration of Helsinki. Eleven subjects (6 males and 5 females) without systemic diseases were enrolled in this study. To standardize the procedure, imaging acquisition was performed only in right eye. Among the recruited subjects, nine were classified as normal eyes with no previous eye surgery and ocular diseases, and three as special cases: five- year post-LASIK, contact lens wearing, and corneal Intraepithelial Neoplasia (CIN) patients, confirmed by pathology.

To acquire the images, we first aligned the eye marker on the headrest to the lateral canthus of the eye; then, the subject was given an external fixation target to diminish eye movement. The photographer viewed the eye through the eyepieces (to ensure clear demarcation of the area of interest while using slit-lamp's magnification) to sharply focus on the area of interest with the magnifications of the slit-lamp. When pictures were acquired with traditional magnification, subjects were instructed to look at the examiner's right ear. Photography protocol: Full view of the eye, ISO 400, shutter speed 1/60, 10 $\times$; Cornea view, ISO 400, shutter speed 1/30, 16 $\times$; Iris view, ISO 400, shutter speed 1/30, 30 $\times$. With the Movie Crop Function, the photographer focused the area of interest through the monitor. During the recording of the corneal image video, a green filter was used in the path of the slit-lamp illumination light to obtain the better contrast of images. The angle between the eyepieces holder and light source was set at 45 degrees to minimize the glare that the light source might cause. Video clips with 640 $\times$ 480 pixels were recorded at the speed of 60 frames per second with the Movie Crop Function. Videography protocol is listed in the following table: was listed as below: cornea, ISO 1000, shutter speed 1/60, 70 $\times$; corneal epithelium, ISO 1000, shutter speed 1/60, 112 $\times$; corneal nerve and tear film, ISO 1000, shutter speed 1/60, 210 $\times$. Each magnification scale was recorded for at least 1 to 2 seconds and the total time of the procedure was about 3 to 5 minutes.

The same settings (with the Movie Crop Function at 112 $\times$) were used to image the central cornea epithelium of the human subjects. To compare the slit-lamp images, we used our custom ultra-high resolution optical coherence tomography (UHR-OCT) to image the central cornea of the same group of subjects. UHR-OCT setup has a three-module super

luminescent diode (SLD) light source with a broad bandwidth of 100 nm and a central wavelength of 840 nm. The calibrated axial resolution of the system was $\sim 4 \mu\text{m}$ in air, corresponding to $\sim 3 \mu\text{m}$ in the water or tissue with a refractive index of 1.33. The power of the sample light was lowered to $750 \mu\text{W}$ by adjusting the source power with a fiber-based pigtail style attenuator to ensure that the light intensity delivered to the eye was safe. We have previously published the advantages of using this device for imaging the anterior segment of the eye.⁹

The measurement of the layer thickness using SHM-SLB was calibrated using a set of polymethyl methacrylate (PMMA) lenses with known thicknesses from 100 mm to 700 mm and refractive index of 1.4927. The similar approach has been documented previously.¹⁰ The true thickness of the PMMA lenses was measured with a mechanical thickness gauge (Vigor Ga175, Q.T.E North America, Inc, Rancho Cucamonga, CA), which had the graduation of 0.01 mm and capacity of 0-10 mm. The set of PMMA lenses was imaged with the slit-lamp using high magnification ($112\times$) and Movie Crop Function. The illumination light was placed at the center of the lens and the slit light was projected perpendicularly to the surface. The camera was placed at the 45 degrees. Video clips were recorded and still images were exported for the video for measuring the apparent thickness. In Adobe Photoshop (Adobe Systems Inc, San Jose, CA), we counted the pixels between two boundaries in the image then converted into the thickness in microns. Only central 1 mm of the image was used to measure the thickness. The regression equation between the true thickness and apparent thickness was analyzed and the coefficient of correlation between instruments in measurements of central PMMA lens was 0.95 (Fig. 2). The regression equation between true thickness of lens and SHM-SLB readings was as follows: $Y=2.3169X-28.201$, where Y is the true thickness of PMMA lens and X is the apparent thickness of the PMMA lens determined with SHM-SLB. The equation was used to estimate the corneal thickness by applying a conversion factor due to different refractive indices. A conversion factor was calculated by using the equation:

$$F=n_{\text{tissue}}/n_{\text{PMMA}}$$

where n_{PMMA} is the refractive index of the PMMA lens at 555 nm ($n_{\text{PMMA}}=1.4927$) and n_{cornea} is the refractive index of the cornea, which is 1.376. Therefore, the conversion factor for total corneal thickness is 0.922. $n_{\text{epithelium}}$ is the refractive index of the 1.4 and the conversion factor for corneal epithelium is 0.938.

The same measurement was conducted in 4 human subjects (1 male and 3 females) without systemic and ocular diseases. Central 1 mm image was used to count the pixels between two boundaries. The corneal epithelial thickness (apparent thickness) was defined as the thickness of the bright band on the surface of cornea with two boundaries. The corneal thickness (apparent thickness) was defined as the distance from the surface of corneal epithelium to the endothelium. After conversion using the regression equation and conversion factors, the thicknesses of the corneal epithelium and total cornea were obtained. To verify the thickness measurement using SHM-SLB, we used the URH-OCT to image the central cornea for measuring the thickness of the corneal epithelium and total cornea. The

corneal images of URH-OCT were processed using our custom developed software (J-OCT-1, version 1.0).¹¹ The central thickness within 1 mm was obtained. The statistical analysis was performed using the SPSS 16.0 software package (SPSS Inc., Chicago, IL). A paired-t test was used to assess the difference between the two measurements of thickness for both corneal epithelium and cornea. All the tests were two-tailed, and $P < 0.05$ was considered statistically significant.

Results

With the different magnification settings in the slit-lamp, high-quality ocular images were successfully obtained. The images with optical magnification from 10×, 16× and 30× (Fig. 3A-C) showed the full view of the eyes. The images with Movie Crop Function and high magnification (70× to 210×, Fig. 3D-F) resulted in enhanced delineation of details of the different structures (e.g. iris).

With a green light filter in the illumination path, the Movie Crop Function, and the high magnification ($\sim 112\times$) features, the cross-sectional images were video-recorded at 60 frames per second. This allowed us to clearly visualize the corneal epithelium (Fig. 4A-C) and the contact lens (Fig. 4D). Moreover, the Bowman's membrane was well defined (Fig. 4A), and the epithelial thickness profile was noticed to increase from mid-periphery to limbus (Fig. 4B). In the post-LASIK eye (Fig. 4C), the interface of corneal flap was evident. The structures and features of the cornea mentioned above were apparently similar to those in the images obtained on the same location of the eyes using UHR-OCT (Fig. 4E-H). By utilizing the highest magnification ($\sim 210\times$), tear film debris (Fig. 5A-B) were apparent on the ocular surface. In addition, corneal nerve network in the stroma were visualized with this setting (Fig. 5C-D).

Photographs obtained from the eye with CIN showed isolated clustered lesions of gray translucent corneal clouding with intervening clear spaces in the nasal cornea of the left eye. However, no clinical evidence of abnormality in the adjacent conjunctiva was noted. The cornea was avascular, and the corneal stroma seemed to be uninvolved (Fig. 6A-D). The UHR-OCT imaging demonstrated thickening and highly increased reflectivity of the corneal epithelial layer, but Bowman's layer and anterior stromal involvement were not evident (Fig. 6E-F). With the Movie Crop Function feature ($\sim 112\times$), picture frames from the clip video were obtained, and compared against the UHR-OCT results. Characteristics of corneal lesion were evidently similar in both, cropped images and UHR-OCT. The thickness of the corneal epithelium in the neoplastic areas was significantly greater than the adjacent normal corneal regions. In addition, the basement membrane is noted to curve under the corneal epithelial lesion, but not under the intact regions (Fig. 6G-H). Compared the average central thicknesses of corneal epithelium and cornea measured by SHM-SLB and URH-OCT, there were no significant differences ($P > 0.05$) (Table 1).

Discussion

Slit-lamp biomicroscopy is the imaging modality of choice used in every ophthalmic clinic to provide *in vivo* "microscopic" evaluation of the structures of the eye. Furthermore, the use

of optical pachymetry based on slit-lamp biomicroscopy was considered an important tool for routine ophthalmic examinations, especially when the thicknesses of the corneal layers were a concern for establishing a diagnosis or for surgical considerations. The poor resolution of images provided by this modality has made it obsolete. Its major reason being the low spatial resolution, which makes visualization of the epithelium extremely difficult.¹² In contrast, high resolution imaging modalities such as confocal microscopy, high frequency ultrasound,¹³ and optical coherence tomography (OCT),^{10, 14} have a much better spatial resolution, which makes visualization of the epithelium significantly easier. However, the advanced imaging modalities also have their limitations. For instance, Confocal microscopy is good for visualizing the enface view of the epithelial cells, but not for measuring the thickness of the epithelial layers. This is because to obtain this measurement, direct contact with the eye is needed. The field of view is quite small.¹⁴ On the other hand, the OCT is a non-contact modality; nonetheless, it needs to be operated by a well-trained imaging technician. Moreover, these imaging instruments are relatively costly, which may prevent its wide use on clinic. Compared to the imaging modalities described above, the incorporation of a simple adaptor and a digital camera are a low-cost route to enhance imaging acquisition. The slit-lamp biomicroscope with high magnifications has demonstrated to be a great alternative to image the cornea. As we demonstrated in a previous paper, the modality could be also used to image the bulbar conjunctival microvasculature.⁷

The implementation of a device with extremely high magnification on the SLB was a considerably easy and cost-effective step. Only a beam splitter and a commercially available camera with the Movie Crop Function were needed. This adaptation drastically boosted the spatial resolution by a factor of 4×, which reached a lateral resolution of ~3 μm on the high definition monitor. The resolution of the modified SLB was much higher than the resolutions obtained from a traditional slit-lamp with other cameras.¹⁵ This boost in resolution is enough for imaging the movement of a cluster of red blood cells.⁷ In the present work, we found that the corneal epithelium was easily visualized and the image quality was similar to the images obtained with our ultra-high resolution OCT. However, the Bowman's membrane was not well demarcated in the slit-lamp images. We demonstrate the detailed structure in the anterior segment by comparing the slit-lamp high magnification images with OCT images. The quality of high magnification slit-lamp photograph is not as good as that obtained with OCT imaging. OCT imaging may not be practical for implementing into traditional slit-lamp for routine ophthalmic examination and teaching. It may be worth noting that real-time visualization of the epithelium was better than the still captured images, which means its use is better suited for office use. The real time imaging with high magnifications showed that the thinnest point of corneal epithelial layer was easily identified at the center of the cornea, and then the thickness of corneal epithelium increased gradually to the mid-periphery and periphery region. Interestingly, features observed in the high-magnification images of the CIN case (abnormally increased corneal epithelium and intact basement membrane) were analogous to the ones seen under the UHR-OCT. This would be very helpful for the practicing ophthalmologist since it would allow looking for more detailed signs of diseases with the same slit-lamp used to perform the rest of the ophthalmic exam.

Real-time recording is a useful feature in the adapted slit-lamp videography. Because the depth of focus was very shallow with the extremely high magnifications, real-time imaging may have helped to bring the structure of interest in focus. With the high speed recording (60 fps), sharply focused images of the corneal layers of interests can be exported for further processing, which may be used for measuring the layer thickness. The boundaries of the epithelium found in the slit-lamp images, could be easily outlined.

We demonstrate the capability of performing pachymetry of the central corneal layers including corneal epithelium and total cornea. As expected, the thickness of the epithelium measured using SHM-SLB was close to the results measured by URH-OCT in the present study and a previously published study.¹¹ Further developments in this field could be the implementation of a pachometer attached to the slit-lamp for gauging the layer thickness.¹⁶ In addition, features like confirming the perpendicularity of the incident beam and the central location of the cornea may be needed for repeatability study and follow up testing.

Other advantages of our approach are the role in telemedicine and teaching. In general, there are several major components in a teleophthalmology system. These consist of image capture, digitization, transmission storage and interpretation.^{17, 18} Capturing the details of the anterior segment of the eye may further improve the usefulness of slit-lamp based teleophthalmology. The implementation of a computer to our current system would be of great help since it will allow the sharing of captured videos with remote parties. The real-time display and viewing to show the OCT image like corneal epithelium is feasible with our approach. The high resolution pictures and videos obtained through this methodology can drastically enhance teaching, since details of the eye would be displayed on the monitor. For example, it would be easier to visualize the movement of contact lenses in situ. Moreover, the real time display could be easily used for direct demonstration of the structural ocular details to patients and their family members.

The cornea is one of the most richly innervated organs. Corneal innervations not only provide sensation, but also play a crucial step in the maintenance of the structures of the eye such as regulating epithelial integrity, epithelial proliferation and wound healing.^{14, 19, 20} The analysis of the density and regeneration speed of corneal nerves is a useful tool for making the diagnosis of neurotrophic keratopathy, and evaluating the recovery status after refractive surgery or keratoplasty.²¹ The imaging of corneal nerve with the super-high magnification slit lamp in the present study demonstrates the capability of viewing the corneal nerves. It may not be practical for detailed analysis unless further hardware and software are developed.

In conclusion, we demonstrated enhanced slit-lamp photography and videography with super-high magnifications for visualizing the anterior segment of the eye, especially for visualizing the epithelium. The implementation of the extremely high magnification was easy and cost-efficient, which can be potentially used in routine eye examination for better visualization of the anterior segment.

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Fig. 1. Spatial resolutions of Movie Crop Function and optical magnifications

The US Air Force 1951 resolution test card (A) was imaged with the optical magnification $\sim 30\times$ and the black and white bars are readable at the element 3 of the group 5 of the resolution card. (B) With the highest magnification of $\sim 210\times$, the element 3 of the group 7 on the resolution card (equivalent $3.11\ \mu\text{m}$) was viewed clearly. (C) The spatial resolution of images as a function of magnification. Note that the spatial resolutions were higher with the Movie Crop Function ($70\times$, $112\times$ and $210\times$).

Fig. 2.
The measurement of PMMA lens with SHM-SLB and a mechanical dial gauge.

Fig. 3. Ocular images acquired with various magnifications by the Canon 60D camera attached to the Nikon FS-2 slit lamp

Images A-C were acquired with still imaging mode; Images D-F were acquired with video recording when Movie Crop Function was on. (A) Full view of the eyelid, ISO 400, shutter speed 1/60, 10 $\times$; (B) Cornea, ISO 400, shutter speed 1/30, 16 $\times$; (C) Iris, ISO 400, shutter speed 1/30, 30 $\times$; (D) texture of iris, ISO 1000, shutter speed 1/60, 70 $\times$; (E) iris and margin of pupil, ISO 1000, shutter speed 1/60, 112 $\times$; (F) iris, ISO 1000, shutter speed 1/60, 210 $\times$.

Fig. 4. The central corneas in different healthy subjects imaged with slit lamp videography with high magnification and UHR-OCT

The images were extracted from recorded video clips. The central cornea (A) and inferior cornea near limbus (B) were captured in a healthy subject. The central cornea was captured in a post-LASIK eye (C) and an eye wearing a contact lens (D). The epithelium (A-C) and Bowman's membrane (A) were clearly visualized. The contact lens (D) was also clearly seen. The layers and contact lenses appeared similar to those in images (E-H) obtained using ultra-high resolution optical coherence tomography (UHR-OCT). EP: epithelium; BM: Bowman's membrane; FI: LASIK flap interface; CL: contact lens; S: stroma. Bars = 250 μ m.

Fig. 5. Tear film debris and corneal nerve were visualized using slit-lamp videography with high magnification ($\sim 210\times$)

(A) Air bubbles (yellow arrow) and possible tear film debris on the ocular surface (white arrow). Noted that air bubbles have a greater diameter than debris and appeared transparent with diffuse light. (B) With the narrow slit light, the air bubbles extended out to the surface of the tear film (yellow arrow) and appeared to have a strong reflection; the debris were visualized as the low contrast projections of the ocular surface (white arrow). (C-D) The corneal nerve (CN) and branches (white arrow) in the anterior stroma. Bars = 100 μm

Fig. 6. The cornea in CIN case; imaged by slit lamp photography and videography with high magnification and UHR-OCT

(A-B) The clinical images acquired with the Haag-Streit BX-900 slit lamp showed the corneal epithelium lesion (white arrow). Bar=5 mm. (C-D) Initial presentation of the left eye showed the “leopard-spot” corneal epithelial lesion lying in the nasal cornea; and the interface of the lesion was not clear with the traditional magnification. ISO 400, shutter speed 1/60, 10×, Bars = 5 mm. (E-H) Images acquired by UHR-OCT, (E) the thickness of the corneal epithelium was greater than normal region (white arrow). (F) Bowman's layer appeared intact and anterior stroma was not involved (white arrow). (G-H) Images acquired by slit lamp with high magnification, ISO 1000, shutter speed 1/60, 112×. (G) The image showed the thickness of corneal epithelium increasing gradually to the corneal lesion region (white arrow). (H) The basement membrane kept the intact statue (white arrow). EP: epithelium. Bars = 250 μm.

Table 1
Comparison of corneal epithelial and total corneal thicknesses measured with SHM-SLB and UHR-OCT (n = 4)

	SHM-SLB	UHR-OCT	P Value
Epithelial thickness (μm)	54.9 ± 6.9	55.6 ± 3.5	0.35
Corneal thickness (μm)	567.8 ± 22.7	560.2 ± 29.3	0.06

Mean ± SD

SHM-SLB: super high magnification slit lamp biomicroscopy

UHR-OCT: ultra-high resolution optical coherence tomography