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A simple and non-contact optical imaging probe for evaluation of corneal diseases

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Non-contact imaging techniques are preferred in ophthalmology. Corneal disease is one of the leading causes of blindness worldwide, and a possible way of detection is by analyzing the shape and optical quality of the cornea. Here, a simple and cost-effective, non-contact optical probe system is proposed and illustrated. The probe possesses high spatial resolutions and is non-dependent on coupling medium, which are significant for a clinician and patient friendly investigation. These parameters are crucial, when considering an imaging system for the objective diagnosis and management of corneal diseases. The imaging of the cornea is performed on *ex vivo* porcine samples and subsequently on small laboratory animals, *in vivo*. The clinical significance of the proposed study is validated by performing imaging of the New Zealand white rabbit's cornea infected with *Pseudomonas*. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4929684>]

I. INTRODUCTION

Corneal disease is one of the leading causes of blindness worldwide, together with cataract and glaucoma.^{1,2} It has a complicated epidemiology which includes an extensive variation of infectious and inflammatory eye diseases such as viral, bacterial, or fungal keratitis that result in corneal scarring, eventually leading to functional blindness. The risk factors associated with corneal inflammation include daily activities such as the wearing of contact lens, conditions of the eye such as eyelid misalignment, ocular surface diseases, ocular trauma, previous ocular surgery, corneal sutures, application of topical steroids, and even diseases that are not related to the eye such as diabetes mellitus.³ Its severity can vary from a moderate, self-limiting condition to a vision-threatening complication. Surgical intervention after corneal blindness has developed moderate success rate, where graft rejections and other post-operative complications pose a great challenge.^{4,5} Similar to any other diseases, prevention is therefore the most effective and successful method in reducing the prevalence of blindness. A study conducted in a population of Central African Republic by Schwartz *et al.* in 1997 estimated that up to 90% of all blindness was in fact curable or avoidable.⁶

The visualization capabilities of the various corneal imaging techniques can be broadly classified into photographic and optical tomographic methods as shown in Table I. Each of these reported imaging modalities contributes to the diagnosis, prognosis, and management of corneal diseases, and has distinct benefits and drawbacks in obtaining reliable and repeatable measurements. For example, the *in vivo* laser scanning confocal microscopy (LSCM)^{7–12} is the most advanced confocal system that provides resolution of up to the cellular

level, allowing vision researchers and clinicians to image the entire corneal layers and structures, classify the disease state, and hence evaluate the treatment response without mechanically sectioning of the cornea. However, there is a risk of epithelial injury and the introduction of artefacts as a result of the appplanation. In the case of the ultrasound biomicroscopy (UBM),^{13,14} despite being the standard reference for corneal pachymetry, and its ability to image the anterior segment through edematous or scarred corneal tissue, it is impractical in many clinical situations because of the contact nature of the procedure, which makes it unsuitable for post-operative eyes and patients with prior ocular injuries. Moreover, image acquisition is time consuming and this further creates discomfort to the patient. The supine positioning of the patient during image acquisition may also cause distortion to the eye anatomy, especially the angle configuration.¹⁵ All these indicate that UBM is neither standardized nor in agreement with other non-contact methodologies. In addition, both of these contact procedures require a skilled and experienced operator in order to obtain high quality images and to minimize the risk of corneal abrasions and infections. The anterior segment optical coherence tomography (AS-OCT)^{16–19} is advantageous over the UBM because of its higher spatial resolution and speed of image acquisition, ability for standardization of scans, non-contact nature, and minimal requirements for expertise. Deep tissue imaging and imaging through corneal opacities are not possible with light as an analysis medium. While the AS-OCT is unable to characterize and identify the infectious agent in the case of an inflammatory response, it is able to detect small changes in the microarchitecture of the cornea, and it is possible to monitor treatment response.

Different optical probe imaging configurations have been investigated for their miniaturized size and flexibility for various disease diagnostic applications previously.^{20–24} This paper in this context introduces a potential non-contact and

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TABLE I. Corneal imaging tools used in clinical ophthalmology.

Instrument	Scan source	Measuring principle
Optical tomographic method		
AS-OCT ¹⁷⁻¹⁹	SLD 840 nm–1310 nm	Interferometry
Heidelberg Retina Tomograph III Rostock Cornea Module ²⁵	Laser diode at 670 nm	Confocal
Paradigm P60 UBM ¹³	Ultrasound (50 MHz)	Ultrasound
Photographic method		
Pentacam ²⁶	Blue LED 470 nm	Scheimpflug imaging
Orbscan II ²⁶	White flash light	Slit-scanning imaging

non-invasive optical probe system for the high resolution imaging and characterization of the various layers of the cornea.

II. MATERIALS AND METHOD

Each randomly selected eye from each of the forty pigs (*Sus scrofa domestica*) was enucleated from the local abattoir

and used within 6 h of death. The *ex vivo* samples were transported on ice and kept till the investigation was begun to maintain its “freshness.” Each sample was fixed onto a custom eye holder, which was mounted on a translation stage with micrometer accuracy. Extra-ocular tissues such as the conjunctiva and lacrimal gland were removed from the samples. The *in vivo* imaging of the New Zealand white rabbit were conducted in Singapore Eye Research Institute (SERI), in accordance with the guidelines of the National Advisory Committee for Laboratory Animal Research (NACLAR) in Singapore. The rabbit was intramuscularly anesthetized with ketamine HCL (50 mg/kg) and xyaline (10 mg/kg) and eyelid retractors were used to manipulate the animal’s eyelid.

A schematic of the proposed configuration is shown in Figure 1(a), while the photograph of the experimental setup is given in Figure 1(b). The 785 nm diode laser (LBX-785, Oxxius, Wessling, Germany) was used as an illuminating source. The laser beam was coupled into an optical fiber, and the output was collimated using a fiber collimator (NA = 0.25, f = 36.01 mm) before reaching a 45:55 (R:T) pellicle beam splitter. The reflected beam from the beam splitter was used to illuminate the sample surface using a

FIG. 1. (a) Schematic of the proposed experimental setup [inset: rabbit (under general anesthesia) with eyelid retractor] and (b) photograph of the proposed optical imaging probe system.

Mitutoyo infinity-corrected long working distance objective lens (Plan Apo, magnification = 20 $\times$, NA = 0.42, f = 10 mm, working distance = 20 mm). The images from the laser illuminated eye samples were collected through the same objective lens. An infinity-corrected tube lens (f = 200 mm, working distance = 148 mm) was used to refocus the image from the infinity-corrected objective lens onto the PixeLINK CCD camera (Ottawa, Canada). A neutral density filter was placed between the tube lens and the CCD camera in order to control the light intensity. The acquired images were digitally displayed on the computer screen. Depth-sensitive measurements were carried out by moving the objective lens gradually towards the eye at a constant lens speed of 60 $\mu\text{m/s}$, using a translation stage. The first image was captured when the first superficial cells were seen.

III. RESULTS AND DISCUSSION

Porcine eye is chosen as an *ex vivo* animal model in this study because of its similarity in morphology to that of the human eye.^{27–30} It has been used in vision sciences research involving but not limited to glaucoma and corneal transplant studies.^{29–33} The parameters of the porcine eyeball have been covered in a few studies, some of which provide information about the similarities and differences between porcine and human eyes.^{29,34,35} Major differences between the porcine and human eye include the absence of the Bowman's layer in the porcine eye and the corneal thickness. Porcine corneal thickness is found to be twice that of human cornea.³⁶

The New Zealand white rabbit is used as an *in vivo* animal model because of the ease of manipulation and the similarity in corneal size with human.³⁷ Though keratometric readings and elevation values at the anterior and posterior are comparable to human, the corneal thickness and anterior chamber depth values are found to be lower than those in human.³⁸ Similar epithelium, stroma, and endothelium layers were identified in human and rabbit corneas, though the Bowman's and Descemet's layers were not as developed in rabbits.^{39,40}

The resolution of an imaging system is defined as the minimum distance of separation between two closely spaced points, such that they can be distinguished as two entities, rather than one. The effective resolution of the proposed system is similar to the conventional diffraction limit of wide field microscopy. It is bounded by three critical design characteristics and is given by $\frac{\lambda_{ex}}{2n(\sin\theta)}$, where λ_{ex} is the excitation wavelength, n is the refractive index of the imaging medium, and θ is one-half of the angular aperture of the light cone captured by the objective. The term $n(\sin\theta)$ is also known as the numerical aperture (NA) and it gives an indication to the light-gathering ability of the objective lens. The conventional diffraction limit of wide field microscopy can therefore be written as $\frac{\lambda_{ex}}{2NA_{det}}$, where NA_{det} is the numerical aperture of the detection objective. Experimentally, the spatial lateral resolution is determined by the imaging of the U.S. Air Force bar target (USAF1951 chart).⁴¹ The proposed configuration is able to image up to group 7, the highest spatial frequency on the USAF chart. Figure 2 shows the resolution achievable at group 7, element 6. This corresponds to a lateral resolution of about 2.19 μm in air and 1.59 μm in the corneal tissue ($n = 1.376$)⁴² after calculations.

FIG. 2. Measurement of lateral resolution using USAF chart.

No attempts were made to image test targets of higher spatial frequencies due to the limiting magnification and respective NA of the objective lens.⁴³

The most obvious advantage of using near infrared (NIR) illumination over white light is the possibility of examining eye parameters in its most natural and dynamic states, since there is no change in the eye anatomy with respect to lighting conditions. Compared to the longer wavelength that is employed in the various OCT systems, the proposed system has higher scattering in tissue and thus, a lower penetration through scattering ocular structures such as the sclera and iris. In addition, retinal protection is decreased due to the lower absorption by water in ocular media.^{44–46} High power illumination is therefore not desirable in the proposed system, and this sets a constraint to high-speed imaging, which eliminates motion artifacts and reduces examination time. The shorter wavelength of the proposed system however promises a higher theoretical lateral resolution, based on the conventional diffraction limit of wide field microscopy. The optical power of the system is kept within the maximum permissible exposure limit recommended by the American National Standards Institute (ANSI) and other safety standards.^{47–49} Subsequent confocal images of the rabbit also shows that the cells were not damaged due to exposure to laser light at this wavelength and power.

The upright positioning of the sample and non-contact nature of the proposed system also allow viewing of the cornea in its natural and dynamic states. Operation of the proposed system does not require much expertise since there is no requirement for any sample preparation or coupling medium. Unlike the UBM or confocal system, application of this procedure does not expose the patient to risk of corneal abrasion and infections. This procedure is therefore suitable for pre- and post-operative eyes, and for patients with prior ocular injuries. Similar to the current OCTs, visualization of the sulcus is not possible because the posterior layer of the iris is not transparent for the infrared light. Also, the ciliary body will not be entirely

FIG. 3. Digital images of the *ex vivo* porcine cornea captured at different depths, along with a cross section representation of a porcine cornea by AS-OCT.

visible, as the infrared light will be absorbed on its way through the sclera. The proposed system combines the non-contact feature of the OCTs, with the high spatial resolution of the confocal microscope. While the performance of the proposed system is limited in cases of corneal opacities or abnormalities, it is able to provide resolution up to the cellular level, and this allows the characterization and identification of the infectious agent in the case of an inflammatory response. Subsequent treatment response can then be evaluated objectively and this contributes indirectly to the cost-effective factor.

Infinity optics is employed in this proposed optical probe configuration to produce a parallel flux of light rays after passing through the objective. Unlike conventional microscope with fixed tube length, optical components placed in this “infinity space” neither introduce spherical aberration nor modify the objective’s working distance. The parfocality of the system is also maintained with infinity optics. The distance between the infinity-corrected objective lens and tube lens is kept at 150 mm throughout the experiment, which is within the optimal distance recommended by the manufacturer (70 mm–170 mm). Although this distance can be varied, it should be noted that changing this distance will affect the image field diameter. A 45:55 (R:T) pellicle beam splitter is placed in this infinity space to reflect the collimated output from the fiber onto the sample surface via the long working distance infinity-corrected objective lens. The reflected image signal from the sample is collected by the same objective lens in this epi-illumination configuration, before passing through the tube lens onto the CCD camera. The CCD camera is highly sensitive and can detect reflected signals that are several orders smaller than the incident optical power. The amount of optical backscatter changes with respect to the gradient of the

refractive indices of the various structures in the cornea, whereby a large difference in the refractive indices corresponds to a larger reflectance and hence, a higher contrast.⁵⁰ Figures 3 and 4 show the images of the porcine and rabbit cornea across its entire thickness, respectively, along with their cross-sectional images. The images are captured by moving the objective lens gradually towards the eye, hence moving the focal plane along the optical axis, towards the corneal stroma and endothelium. It should be noted that the physical distance moved by the objective lens does not equate to the change in the focal plane through the cornea. This is because of the fact that cornea having higher refractive index and introduces different (larger) optical path length (OPL) as per the formula, $OPL = nd$, where n is the refractive index of the cornea and d is the physical distance. This implies that for every 60 μm shift in the objective lens, the focal plane is shifted by a distance of 82.56 μm along the optical axis. At a constant lens speed of 60 $\mu\text{m/s}$, it takes approximately 12 s and 5 s, respectively, to cover the entire thickness of the porcine and rabbit corneas. The average thickness of the human cornea is approximately 500 μm ,⁵¹ and it takes an estimated time of 6 s to scan from the epithelium to the endothelium layer. Even though the rabbit was under general anesthesia, its breathing motion caused slight movements between individual frame acquisitions, and this results in significant motion artifacts. The problem of motion artifacts was not as significant in the *ex vivo* porcine model since the eye samples were secured firmly on the custom mount.

It is evident from Figures 3 and 4 that the images have become obscured at the endothelium due to light emanating from regions above and below the focal plane. Unlike the commercial laser confocal microscope system, this proposed

FIG. 4. Digital images of the *in vivo* rabbit cornea captured at different depths, along with a live histological cross section representation of a rabbit cornea by *in vivo* confocal microscopy (IVCM).

system is unable to eliminate the out-of-focus blurs and achieve the optical sectioning. Further processing of the 2D images is required in order to enhance image quality, followed by the development of software for the 3D reconstruction of the cornea across its entire thickness. These research directions will be explored in future.

Two sets of measurements were taken for each sample, and the procedure was repeated on forty different samples, with low variation in contrast. Though the proposed system demonstrates high repeatability, further studies are required to determine whether there are discrepancies with results obtained from other non-contact methodologies. An agreement suggests that the proposed system will complement existing imaging modalities in the assessment and evaluation of corneal diseases, so as to decrease morbidity and improve the effectiveness of subsequent treatment. Another limitation of

the proposed system is that the 2D images cover only an area equivalent to the laser spot size. This means that multiple sections and hence, a longer image acquisition process is necessary in order to image the entire cornea. The average white to white cornea diameter of a healthy adult is ~ 11 mm.⁵² Assuming a laser spot size of ~ 0.4 mm, at least 28 laser spots are needed to scan across the diameter of the cornea. This problem can be easily overcome by introducing a galvanometer and scanning optics. A larger area then can be analyzed by sweeping the focused Gaussian beam in a raster fashion.

The efficacy of the proposed system is further demonstrated by studying the rabbit's cornea infected with *Pseudomonas* as per the approved guidelines. The imaging of the infected eye was performed 10 days after the infection and the results are as shown in Figure 5. *Pseudomonas* multiplies rapidly and crowd out the host tissues, hence disrupting the

FIG. 5. In vivo imaging of (a) a healthy cornea and (b) a cornea infected with *Pseudomonas* using the proposed system.

normal physiology of the eye. The more densely populated epithelium and higher reflectivity of Figure 5(b) can be associated with hallmarks of bacteria keratitis such as loss of corneal transparency, peripheral epithelial edema, and deep stroma abscesses.

IV. CONCLUSION

An optical method to examine and characterize the different layers of the cornea is proposed and illustrated in this study. An initial lab prototype is developed based on a NIR epillumination configuration. The images acquired are believed to show the collagen fibrillar structures as well as the individual cellular structures at a higher resolution that was never seen with current clinical devices. These digital images can be saved into the computer database for further image processing and segmentation to allow clinicians to evaluate and capture the disease progression and treatment response overtime.

It is envisaged that this proposed method can in the long run enable accurate data analysis and subsequent diagnostic procedures of infectious and inflammatory corneal diseases involving but not limited to viral, bacterial, or fungal keratitis.

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