Sub-micrometer axial resolution OCT for \textit{in-vivo} imaging of the cellular structure of healthy and keratoconic human corneas

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Abstract: Corneal degenerative conditions such as keratoconus (KC) cause progressive damage to the anterior corneal tissue and eventually severely compromise visual acuity. The ability to visualize corneal tissue damage \textit{in-vivo} at cellular or sub-cellular level at different stages of development of KC and other corneal diseases, can aid the early diagnostics as well as the development of more effective treatment approaches for various corneal pathologies, including keratoconus. Here, we present the optical design of an optical coherence tomography system that can achieve 0.95 µm axial resolution in biological tissue and provide test results for the system’s spatial resolution and sensitivity. Corneal images acquired \textit{in-vivo} with this system from healthy and keratoconic human subjects reveal the cellular and sub-cellular structure of the corneal epithelium, as well as the normal and abnormal structure of the Bowman’s membrane and the anterior corneal stroma.

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1. Introduction

Keratoconus (KC) is a degenerative eye disorder that causes structural changes in the cornea resulting in opaqueness (scarring), thinning, appearance of stress lines due to stretching of the corneal tissue and shape alterations in the cornea (changes of the inner and outer curvatures of the cornea) [1]. These morphological changes cause significant visual distortions, such as formation of multiple images (monocular polyopia), streaking, sensitivity to light and loss of night vision [2]. Keratoconus, a bilateral ectasia of the cornea, affects approximately 0.1% of the general population, ranging in age from early teens to mid-thirties, with vision deterioration reducing significantly the patient’s ability to drive a car or read a normal print [3]. It is most commonly an isolated condition, however it can occur alongside other conditions among which are atopic disease and eye rubbing [4]. Keratoconus induces morbidity in the affected population, since comfort with contact lenses, the most common method of early treatment, is very difficult to achieve due to the multifactorial nature of this condition. Surgical treatment options are costly and frequently result in scarring and corneal shape distortion that can affect negatively the patient’s visual acuity [5]. Although keratoconus is a well investigated condition, there are still remaining questions about the very early stages of its development, when morphological changes in the cornea are limited to the cellular level. Other questions pertain to how the human KC cornea responds to surgical or pharmaceutical intervention and how the healing process occurs at a cellular level. By answering these questions, a better understanding of the corneal response to treatment can be achieved, which in turn can lead to optimization of existing or development of novel therapeutic approaches.
Optical coherence tomography (OCT) is a biomedical imaging modality capable of generating cross-sectional and volumetric images of biological tissue both ex-vivo and in-vivo with micrometer scale resolution and up to several millimeters penetration depth below the tissue surface [6,7]. Over the past 15 years advancements in femtosecond and supercontinuum laser technologies have allowed development of OCT systems with < 2 µm or even < 1 µm axial resolution in biological tissue. Some of these systems are Time Domain (TD-OCT) with A-scan rate of < 4 kHz [8,9] which prevents the acquisition of volumetric OCT images in clinical environment due to significant motion artefacts and in some cases, for example in ophthalmology, due to maximum permissible exposure limit of biological tissue. More recently, Spectral Domain OCT (SD-OCT) systems with close to 1 µm axial resolution in biological tissue and data acquisition rates up to 70 kHz were reported [10–15]. Full Field OCT, which utilizes incoherent white light sources, offers the advantage of isotropic 1 µm resolution in biological tissue [16,17], however the ophthalmic applications of FF-OCT are currently limited to mostly ex-vivo studies of the human and animal retina and cornea. Recently, a dispersion-cancelled quantum OCT system that can generate 0.54 µm axial resolution in free space was reported [18]. However, the demand for very high optical imaging power prevents any biomedical applications of this technology.

When used for corneal imaging, high resolution OCT is able to visualize in-vivo, identify and in some occasions even measure the thickness of some of the major corneal layers such as the epithelium (EPT), the basement (Bowman’s) membrane (BM), the stroma (S) and the Descemet’s – endothelial complex (DEC) [10,19], image ex-vivo the cellular structure of different corneal layers [20], visualize in-vivo physiological changes in the cornea [22], as well as quantify the tear film thickness [23]. Recently, our research group reported the use of a sub-micrometer axial resolution SD-OCT system to visualize for the first time in-vivo the corneal pre-Descemet’s layer (referred to as the “Dua” layer in some publications), which in the past has been identified and measured only in ex-vivo human corneal preparations [24]. The Descemet’s membrane and the corneal endothelium were also clearly identified in cross-sectional OCT images in that study, and the thickness of all 3 posterior corneal layers was measured in-vivo in healthy subjects.

Here we describe in detail the optical design of a fibreoptic SD-OCT system, that was built to sustain a spectrum with a 3 dB bandwidth of ~275 nm (~380 nm at 10 dB), centered at ~785 nm, in order to achieve 0.95 µm axial resolution in biological tissue, sufficient to visualize the tissue cellular and sub-cellular structure. The system utilizes a new imaging probe to achieve sufficiently high lateral resolution for imaging individual cells in the corneal epithelium in-vivo and without contact with the corneal surface. Both test data of the system’s performance and images acquired in-vivo with it from the corneas of healthy and KC human subjects that show the cellular structure of the cornea, are reported.

2. Methods

2.1 OCT system design

The SD-OCT system is based on a fiber-optic Michelson interferometer (Fig. 1). The core of the interferometer is a 90/10 broad bandwidth, spliced fiber coupler (Gould Fiberoptics, USA). The fiber output of a supercontinuum laser (SuperK, NKT Photonics, Denmark) is connected to a custom filter unit (Fig. 1, CF) comprised of a series of bandpass and neutral density filters (NDF), designed to select a portion from the laser output with ~380 nm spectral bandwidth, centered at ~785 nm and reduce the input optical power to ~20 mW. The reference arm of the SD-OCT system is comprised of a fiber collimator (achromat doublet, f = 10 mm, Thorlabs, USA), an NDF, a beam expander comprised of 2 achromat doublets (f = 40 mm and f = 80 mm, Thorlabs, USA), a focusing lens (f = 10 mm, Thorlabs, USA) and a silver mirror mounted on a miniature manual translation stage (Edmund Optics, USA). A custom dispersion compensation unit (Fig. 1, DC) comprised of 2 BK7 prisms (Edmund
Optics, USA) mounted on miniature translation stages (Edmund Optics, USA) is placed between the beam expander and the focusing lens to allow for passive, manual, hardware dispersion compensation by varying the overlap between the prisms. All components in the reference arm of the interferometer within the rectangle marked with the dashed line in Fig. 1 are mounted on a 12” manual translation stage (Edmund Optics, USA). The sample arm of the SD-OCT system is comprised of a fiber collimator (achromat doublet, f = 10 mm, Thorlabs, USA), a pair of galvanometric scanners (Cambridge Technologies, USA), a beam expander comprised of 2 achromat doublets (f = 40 mm and f = 80 mm, Thorlabs, USA) and a 10x microscope objective. The detection end of the OCT system is comprised of a customized commercially available spectrometer (Cobra, Wasatch Photonics, USA) and an 8192 pixel CMOS camera (Piranha NH-80-08K40, Teledyne Dalsa, Canada) with 34 kHz readout rate. The filtered portion of the NKT spectrum (~380 nm at 10 dB) is spread over 4600 pixels of the camera to achieve spectrometer resolution of ~0.083 nm. Fiberoptic polarization controllers are used in both arms of the system to optimize the shape and magnitude of system’s point-spread-function (PSF). For in-vivo imaging of the human cornea, the optical power of the imaging beam incident on the corneal surface was set to ~700 µW, which is significantly lower than the maximum permissible exposure power as specified by the ANSI (American National Standards Institute) standard [25].

![Fig. 1. Schematic of the OCT system. CCD-camera; CF-custom filter; CL-collimator; DC - dispersion compensation unit.; FC-fiber coupler; FL-focusing lens; L1 and L2-lenses; M-silver mirror; MO-microscope objective; NDF-neutral density filter; PC-polarization controller; TS-translation stage; VHPG-volumetric holographic phase grating; XY-galvanometric scanners.](image)

2.2 Imaging procedure

This study was approved by the Research Ethics Committee at the University of Waterloo and was carried out in compliance with the tenets of the Declaration of Helsinki. Both healthy and keratoconic male and female subjects, aged 20 to 60 years, were recruited for this study. All subjects passed a slit-lamp biomicroscopy screening and provided written consent for participation in the study. A head band and a fixation target were used to minimize the subject’s head and eye motion. Cross-sectional (1000 x 8192) and volumetric (512 x 512 x 8192) images were acquired from ~1 mm x 1mm and ~2 mm x 2 mm regions of the cornea, located inferiorly with respect to the corneal apex.

2.3 Image processing

Images were generated from the raw OCT data and presented on a logarithmic scale. Because the optical components in the reference and sample arms of the OCT system are made of different types of glass, manual hardware dispersion compensation using the DC unit in the reference arm was not sufficient to generate a perfectly dispersion compensated PSF with a
symmetric shape. Therefore the OCT data was also numerically dispersion compensated up to the 9th order, following a previously published protocol for OCT data processing. Briefly, any dispersion mismatch in the OCT system causes a chirp in the OCT fringe pattern, i.e., a non-linearity in the phase function. To compensate for the residual dispersion mismatch, we computed the phase function from an OCT A-scan, used a polynomial fit up to the 9th order to determine the phase nonlinearity and applied an inverse function to the A-scan to compensate for that non-linearity. In the case of the OCT corneal images, we manually selected an A-scan from a location close to the center of the image. Once the polynomial coefficients were computed, they were applied to all A-scans in the image. No additional image post-processing was used for the OCT images presented in this paper.

Because generation of high lateral OCT resolution by using the 10x microscope objective comes at the expense of severely limited Rayleigh range, sufficiently good image contrast can be achieved only over an axial distance of ~200 µm in corneal tissue. For this reason and since our KC study focuses on imaging the cellular structure of the anterior human cornea, most of the images presented here were cropped to show only the regions of the cornea with good image contrast. The length of scale bars in the cropped images was determined by counting the number of pixels from a selected area in the image and using the pixel height and the spacing between neighboring pixels (A-scans) to convert it to micrometers.

2.4 Histology

For the histological analysis, healthy human corneas were harvested postmortem, while KC corneas were obtained after penetrating keratoplasty. The corneas were stained with hematoxylin-eosin and optical images of the cross-sectional corneal preparations were acquired at different magnification to visualize particular morphological details in the cornea.

3. Results

3.1 OCT system resolution and SNR

Results from the system’s resolution and SNR tests are presented in Fig. 2. Figure 2(A) shows the normalized reference arm (red line) and sample arm (black line) spectra measured at the detection end of the OCT system by using a silver mirror as the test sample, in order to take into account any spectral changes introduced by the optics and fiber optics of the system. The overlap between the two spectra has a 3dB spectral bandwidth of ~250 nm and is centered at 785 nm. To determine the theoretically expected axial point-spread function (PSF) of the SD-OCT system, a FFT was applied to the reference arm spectrum and the resultant PSF is shown in Fig. 2(B) (black line). The OCT axial PSF was also measured experimentally by using a silver mirror as the test sample and the result is shown in Fig. 2(B) (red line). Course dispersion compensation between the two arms of the interferometer was achieved by using the dispersion compensation unit in the reference arm (Fig. 1, DC). Because of the broad spectral bandwidth of the NKT laser and the different types of glass of the lenses used in the reference and sample arms of the system, hardware dispersion compensation alone was not sufficient to generate a symmetric PSF with narrowest full width at half maximum (FWHM) (Fig. 2(C), black line.) Therefore, numerical dispersion compensation up to the 9th order was applied to the measured axial PSF data in order to generate a symmetric PSF with a minimum FWHM (Fig. 2(C), red line). With optimal dispersion compensation, the theoretical and experimentally measured PSF overlap almost perfectly and any small differences are the result of polarization mismatch between the two arms. The FWHM of the PSF was 1.3 µm in free space, which corresponds to ~0.95 µm in biological tissue, assuming an average refractive index of n = 1.38. The PSF shows characteristic sidelobes that are related to the spectral shape of the sample and reference optical beam, which is not Gaussian. Since the presence of PSF sidelobes can generate image artefacts, numerical spectral reshaping can be used to minimize the sidelobes height and correspondingly the associated image artefacts.
However, spectral reshaping also causes broadening of the PSF, which results in lower axial OCT resolution. For this reason, we chose not to apply numerical spectral reshaping to the OCT imaging data presented in this paper.

To verify that with this axial resolution the SD-OCT system is able to resolve small details in the axial direction, a pellicle (2 µm thick polymer film with refractive index $n = 1.5$, Thorlabs, USA) was imaged. Figure 2(D) shows two distinct peaks in the OCT axial scan that correspond to the front and rear surfaces of the pellicle. The optical distance between the two peaks is 3 µm which corresponds to a physical thickness of 2 µm considering the refractive index of the polymer.

Results from the system’s sensitivity test as a function of the scanning range are presented in Fig. 2(E). The SNR was measured with ~750 µW optical power of the imaging beam incident on the silver mirror. SNR of 102 dB was measured at a distance of ~100 µm away from the zero delay line. Due to the very broad spectrum and the limited size and number of camera pixels, the SNR roll-off in free space was ~14 dB over a range of 1.5 mm in free space.

Fig. 2. Sample and reference arm spectra measured at the detection end of the system (A). Theoretical (black) and measured (red) axial PSF from a silver coated mirror in the sample arm (B). Hardware (black line) and software (red line) compensated axial PSF (C). Axial scan from a pellicle with 2 µm physical and 3 µm optical thickness ($n = 1.5$) (D). Sensitivity roll-off with imaging depth (E).
Since only ~50% of the entrance aperture of the 10x microscope objective was filled with the imaging beam, the system’s lateral resolution in free space was 2.5 µm, corresponding to 1.8 µm in biological tissue, assuming average refractive index of 1.38 and not taking into account any spherical and chromatic aberrations.

3.2 Corneal images

Figure 3 shows a representative cross-sectional image of the healthy anterior human cornea acquired (A) in-vivo with the OCT system and (B) ex-vivo from a histological preparation of a corneal sample. The cellular structure of the corneal epithelium is clearly visible in Fig. 3(A). Reflective white dots can also be observed inside the cells that most likely correspond to optical reflections from the cellular nuclei. The interface between the corneal epithelium (EPT) and the basement membrane (BM) appears as a thin, highly reflective, almost solid white line, while the interface between the BM and the stroma (STR) is only marked by the presence of keratocytes cells, marked with red arrows in Fig. 3(A). In addition to keratocytes cells, reflections from the thin collagen lamellae in the stroma are also visible in Fig. 3(A) (green arrow). The histological image in Fig. 3(B) shows very similar morphological features in the anterior cornea where keratocytes are marked with red arrows and collagen fibrillae – with green arrows.

Figure 4 shows representative cross-sectional OCT images of a healthy human cornea (A) and the cornea at an early stage of keratoconus (B-D). The image in (A) reveals the normal cellular structure of the epithelium and the low scattering anterior part of the corneal stroma populated with a number of keratocytes (white hyper reflective spots). The thickness of the EPI and BM layers measured from this image was 58 µm and 17 µm respectively, which are typical values for a healthy 25 year old cornea. The OCT image shown in Fig. 4(B) was acquired from a region slightly inferior from the corneal apex from an eye with early signs of KC. The image does not show any structural changes in the EPT and BM layers and the thickness values for those layers (55 µm and 16 µm respectively), also fall into the normal range for healthy corneas. However, the anterior portion of the corneal stroma appears to be not as transparent as the cornea of the healthy human subject. The image shown in Fig. 4(C) was acquired from the same eye at a location slightly inferior-temporal relative to the corneal apex.
and shows clusters of abnormal cells in the basal epithelial layer, marked with the red arrows. In our opinion, most likely this is a cluster of epithelial apoptotic cells, as changes in the shape and size of apoptotic cells causes them to scatter light more strongly compared to normal healthy corneal epithelial cells. A typical histological image of a human cornea with early stage of KC is shown in Fig. 4(D), where the red arrow marks apoptotic cells. Presence of such clusters of apoptotic cells in the corneal epithelium are typical for KC subjects at the moderate and advanced stage of the condition. We have observed similar hyper-reflective cell clusters in the corneal epithelium in many of the KC participants in our imaging study.

Figure 5(A) shows a representative cross-sectional corneal image acquired in-vivo from the left eye of a subject with moderate keratoconus. This image was acquired from a 2 mm long lateral scan at a location slightly inferior to the corneal apex with larger separation between neighboring A-scans, in order to provide a wider field of view of the anterior cornea. Therefore the cellular structure of the corneal epithelium is not as clearly visible as in the image in Fig. 4(A)-4(C), which were acquired from a smaller area in the cornea with a denser A-scan pattern. A thin, highly reflective (white) and irregularly shaped line is visible in the
image in Fig. 5(A) (green arrow), separating the corneal epithelium from the stroma, which most likely corresponds to a damaged basement membrane that appears irregular or thickened or sometimes discontinuous. Scarring in the anterior corneal stroma of this eye is moderate and keratocytes are clearly visible in the stroma. However, information from these images is not sufficient to determine the health status of the keratocytes. Highly scattering clusters of cells are also visible in the corneal epithelium, marked with a red arrow. An image of such a cluster of cells was acquired from a smaller region in the cornea with high density A-scan pattern to allow for visualization of individual epithelial cells (Fig. 5(B)). The red arrow in Fig. 5(B) marks the highly scattering feature that most likely corresponds to a cluster of apoptotic epithelial cells. In subjects with moderate stage of KC, damage to the BM membrane such as breakage of the collagen fibrils or folding of sections of the membrane are typical. Such damage causes increased scatter of light and therefore the damaged BM appears as highly scattering, irregularly shaped thickened region in OCT tomograms (Fig. 5(C), green arrow). Such damage is clearly visible in histological images (Fig. 5(D), green arrows).

Figure 6 shows representative cross-sectional OCT images (A, B and D) from the left eye of a subject with advanced stage of keratoconus. All of these images were acquired from a location in the cornea slightly inferior and inferior-temporal relative to the corneal apex. A typical histological image of a postmortem human cornea with advanced stage of KC is shown in Fig. 6(C). The images in Fig. 6 show clearly the cellular structure of the epithelium as well as the EPT basal cell layer, which appears as a darker, well defined layer located directly above the anterior end of the basement membrane (red arrows). The image is Fig. 6(A) shows a local extreme thinning of the corneal epithelium (blue arrow) located directly above the excessive abnormal scarring (fibrosis) of the corneal stroma. The EPI thickness at that location was measured to be ~20 µm. Similar spatially depended stromal scarring and epithelial thinning are observed in Fig. 6(B), where the scarred region in the anterior stroma is separated from the posterior stroma with thin layer of low reflective tissue (green arrows). These features correlate well with histology (Fig. 6(C)). Reflections from keratocytes are visible in the OCT images at locations of the posterior stroma that have not been affected by scarring.
Fig. 6. Representative cross-sectional OCT images acquired from the left eye (A, B and D) of a subject with advanced KC acquired inferior relative to the corneal apex, and a typical histological image of a human cornea with advanced stage of KC (C). The EPT basal cell layer is marked with red arrows (A-C). Extreme thinning for the corneal EPT is visible in (A), marked with the blue arrow. Excessive localized scarring of the anterior corneal stroma appears as highly reflective (white) regions that are separated from the posterior stroma with low reflective regions (B, green arrows). Keratocytes are still visible in the posterior part of the stroma (D, yellow arrows), which appear not to be affected by scarring. The blue arrow in (D) marks reflections from collagen lamellae arranged in a parallel pattern and separating two regions of stromal scarring.

(Fig. 6(A), 6(B) and 6(D), yellow arrows). The image in Fig. 6(D) shows a region in the KC cornea that separates neighboring regions of scarring, where the collagen lamellae appear to be arranged in a parallel pattern, which is typical for the healthy human cornea (blue arrow). The basement membrane is absent in all of the OCT images in Fig. 6.

Figure 7 shows representative cross-sectional corneal images acquired in-vivo from the right eye of a keratoconic subject with iatrogenic keratoectasia that presents itself with extreme thinning and steeping of the cornea near the corneal apex. The subject had not been initially diagnosed with KC and therefore had undergone Radial Keratectomy (RK) in the early 1990s in an attempt to reduce significant myopia, which has resulted in significant scarring and further thinning of the stroma. The cross-sectional OCT images in Fig. 7(A) and 7(B) were acquired from a region in the cornea slightly below and to the right relative to the corneal apex in order to avoid image artefacts from direct reflection from the corneal surface. The imaging beam was focused at the most anterior end of the stroma. Because this subject’s cornea is so thin in the imaged region (~250 µm to 300 µm); almost all corneal layers can be observed clearly with good contrast. The cellular structure of the corneal epithelium is clearly visible with distinct white reflective spots visible inside the cells (yellow arrows), that most likely correspond to cellular nuclei. The scar tissue extends into the corneal stroma and appears as irregularly shaped, highly reflective regions, interspaced with regions of low reflectivity (red arrows) likely areas showing the loss of keratocytes. Some scarring of the epithelium is also visible (top left corner of the image in Fig. 7(B)) due to the RK surgery. The images in Fig. 7(C) and 7(D) were acquired with the focus of the imaging beam positioned near
Fig. 7. Representative cross-sectional OCT images of the KC cornea from the right eye of a subject with advanced KC, acquired inferiorly relative to the corneal apex. Images (A) and (B) were acquired with the imaging beam focused on the corneal epithelium and reveal its cellular structure. Yellow arrows mark reflective white spots inside the cells that most likely correspond to cellular nuclei. Images (C) and (D) were acquired with the imaging focusing on the posterior end of the cornea, showing the DM and END layers (blue) arrows. All images show excessive scarring of the stroma (highly reflective regions), alternating with small regions of low reflectivity (red arrows). Green arrows mark the tear film.

the posterior end of the corneal stroma. The image contrast in this case is sufficient to visualize clearly the double line of the endothelial layer (blue arrow in Fig. 7(D)) and the darker band directly above it, corresponding to the DM and PDL. In all images shown in Fig. 7, the tear film is also clearly identified (green arrows).

4. Discussion

Images acquired from healthy and keratoconic subjects with the sub-micrometer axial resolution OCT system demonstrate its ability to image in-vivo the cellular and sub-cellular structure of biological tissue without the need for physical contact with the tissue. The high axial resolution would also permits more precise thickness measurement of the thin layers of the human cornea, such as the basement and Descemet’s membranes and the corneal endothelium, as the precision of thickness layer assessment is directly related to the axial resolution of the OCT system. This advantage of the high resolution OCT was already demonstrated by other research groups that used OCT technology with ~1.2 µm axial OCT resolution in the cornea to measure the thickness of the corneal epithelium and BM [19], and the tear film [23]. Our research group used an earlier version of the sub-micrometer axial resolution OCT system to image in-vivo and quantify with high precision the thickness of the corneal endothelial layer, which is only ~4 µm thick, as well as the Descemet’s membrane and the pre-Descemet’s layer in the posterior healthy human cornea [24].

Visualization of the cellular structure of the human cornea could eventually lead to identification, counting and sizing of both epithelial cells and keratocytes from cross-sectional OCT images, by developing novel automatic OCT image processing algorithms. Previously corneal epithelial cells and keratocytes have only been visualized in histological sections or in confocal microscopy images acquired in-vivo though physical contact with the corneal epithelium. Although SD-OCT systems with 1.1 µm [10] and 1.2 µm [26] were previously reported and used to image the healthy and KC cornea, as well as measure the thickness of the
tear film respectively, none of them were able to image the cellular structure of the corneal epithelium most likely due to insufficient lateral resolution.

Although the current optical design of the sub-micrometer resolution OCT system allows for in-vivo imaging of the cellular and sub-cellular structure of biological tissues, it has some limitations that offer opportunity for future optimization of the system’s performance. For example, the spectrometer spreads the detected spectrum only over 4600 out of the 8192 camera pixels, which limits the system’s scanning range to ~1.1 mm in biological tissue. This short scanning range causes difficulty with involuntary head motion in the imaged subjects that can result in cutting of flipping the OCT B-scan about the zero delay line. A compromise between the cost, size and performance of the spectrometer was made, as spreading > 380 nm spectral bandwidth over a 58 mm long photodiode array while keeping optical aberrations at minimum is technologically very challenging and at this time, prohibitively expensive.

Another issue is the limited camera image acquisition rate compared to the frequency of involuntary ocular motion. The data acquisition rate of the camera used in the current design of the system is fairly slow (34 kHz), compared to fast eye motion, which introduces motion artefacts in the volumetric OCT image stacks. Although motion correction algorithms may work well for certain types of motion artefacts, they may fail to correct for others. Because eye motion combines both translational and rotational displacements, effective compensation of such motion artefacts if very challenging. Axial eye motion causes washout of the interference fringes which results in reduced axial resolution and sensitivity and in turn affects negatively the ability to visualize individual cells in OCT images of the human corneal epithelium. This effect can be observed in some of the images presented in this paper (for example in Fig. 4(C) and Fig. 5(B)). This fringe washout effect is particularly strong in the case of subjects with advanced stage of KC, as their vision is compromised to such an extent to prevent effective use of fixation targets during the OCT imaging session. In the future, upgrading the camera with a faster version would allow for much better visualization of individual cells in cross-sectional OCT images of the corneal epithelium. If the camera frame rate is sufficiently high, 3D visualization of these cells would become feasible as well. To achieve such an improvement without compromising the system’s resolution, scanning range or detection efficiency, an optimal replacement camera would require a linear array of 8192 pixels, tall pixel design (pixel height ≥ 100 µm and < 250 µm), enhanced NIR spectral response and data acquisition rate ≥ 100 kHz. At this time, there are no commercially available cameras that satisfy all of these requirements. Spectrometer redesign to fill in all of the 8192 pixels of the current camera would improve the system’s scanning range by ~80%.

Although in this paper we highlighted one clinical application of the sub-micrometer axial resolution OCT system for in-vivo imaging of healthy and KC corneas, the use of the same system to image other corneal pathologies both in the anterior and posterior cornea is also feasible and does not require any modification of the system’s optical design. Eventually, this OCT system could be combined with an ophthalmic surgical microscope that can aid ophthalmic surgeons during corneal surgeries. By modifying the optical imaging probe, the same system can be used for non-invasive cellular resolution of the human retina. In that case the use of hardware or software adaptive optics will be required to visualize in-vivo individual retinal cells, as the lateral resolution of the unprocessed OCT retinal images is determined by the optics of the eye and the diameter of the optical imaging beam. Imaging of other parts of the human body with the sub-micrometer axial resolution system are also feasible, however, the optical density and scattering of the imaged tissue will pose a limit to the penetration depth at which individual cells can be resolved in the OCT images.

5. Conclusions

In conclusion, we have developed a SD-OCT system operating in the 800 nm spectral range that can provide sub-micrometer axial resolution in biological tissue. When used for in-vivo imaging of healthy and keratoconic corneas, the system is able to visualize the cellular and
sub-cellular structure of corneal tissue in cross-sectional images. The system can easily be applied to imaging other corneal pathologies without the necessary of modification of the optical design. Future advances in the camera technology that can lead to faster silicon based CCD or CMOS cameras with enhanced sensitivity in the NIR, will shorten the image acquisition time and reduce the effect of motion artefacts in volumetric images acquired with the sub-micrometer axial resolution OCT system presented here. Such an improvement in the camera data acquisition rate in combination with motion correction algorithms would eventually allow for volumetric visualization of the cellular structure of corneal tissue, counting of cells and precise cellular size measurement from OCT images acquired \textit{in-vivo}. Better characterization of the corneal tissue morphology at a cellular level can have a significant effect on the early diagnostics and the effectiveness of treatment of corneal degenerative conditions such as keratoconus.

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