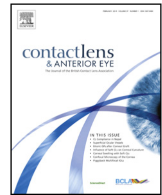
Contact Lens & Anterior Eye 37 (2014) 44–48

Contents lists available at ScienceDirect

Contact Lens & Anterior Eye

journal homepage: www.elsevier.com/locate/clae



Non-contact laser-scanning confocal microscopy of the human cornea in vivo

Nicola Pritchard, Katie Edwards, Nathan Efron *

Institute of Health and Biomedical Innovation, and School of Optometry and Vision Science, Queensland University of Technology, Kelvin Grove, Queensland, Australia

ARTICLE INFO

Article history:
Received 13 March 2013
Received in revised form 22 April 2013
Accepted 4 July 2013

Keywords:
Cornea
Non-contact laser scanning confocal microscopy
Tear film

ABSTRACT

Purpose: To investigate the utility of using non-contact laser-scanning confocal microscopy (NC-LSCM), compared with the more conventional contact laser-scanning confocal microscopy (C-LSCM), for examining corneal substructures in vivo.
Methods: An attempt was made to capture representative images from the tear film and all layers of the cornea of a healthy, 35 year old female, using both NC-LSCM and C-LSCM, on separate days.
Results: Using NC-LSCM, good quality images were obtained of the tear film, stroma, and a section of endothelium, but the corneal depth of the images of these various substructures could not be ascertained. Using C-LSCM, good quality, full-field images were obtained of the epithelium, subbasal nerve plexus, stroma, and endothelium, and the corneal depth of each of the captured images could be ascertained.
Conclusions: NC-LSCM may find general use for clinical examination of the tear film, stroma and endothelium, with the caveat that the depth of stromal images cannot be determined when using this technique. This technique also facilitates image capture of oblique sections of multiple corneal layers. The inability to clearly and consistently image thin corneal substructures – such as the tear film, subbasal nerve plexus and endothelium – is a key limitation of NC-LSCM.
© 2013 British Contact Lens Association. Published by Elsevier Ltd. All rights reserved.

1. Introduction

The Heidelberg laser-scanning confocal microscope (LSCM), with Rostock Corneal Module (Heidelberg Engineering GmbH, Heidelberg, Germany), was developed in 2002 [1], and became commercially available soon thereafter. Over the past decade, the utility of this instrument for examining the cornea in health and disease has been extensively researched. Specific applications include examining the impact of contact lens wear [2], refractive surgery [1], collagen cross-linking [3] and keratoplasty [4] on corneal morphology; diagnosing keratitis [5]; assessing keratoconus [6] and various other corneal dystrophies [7]; and examining the corneal subbasal nerve plexus in patients with systemic diseases such as diabetic neuropathy [8,9] and Fabry Disease [10].

The LSCM enables the tissues of the cornea to be examined at a cellular level. Whereas the maximum optical magnification available with a slit lamp biomicroscope is around 40×, magnification of about 500× is possible with a LSCM [2]. As a result, corneal substructures that are beyond the resolution of the slit lamp biomicroscope – such as subbasal corneal nerves and stromal keratocytes – can be readily imaged in vivo. However, a disadvantage of the LSCM compared with the slit lamp biomicroscope is that operation of the LSCM involves full contact with the anterior surface of the cornea, requiring prior instillation of a topical anaesthetic [2].

Heidelberg Engineering has developed a prototype lens which can be inserted into the Rostock Corneal Module to allow non-contact imaging of the cornea. If proven to be clinically viable, non-contact imaging has the obvious advantages of reducing patient apprehension, obviating the need for anaesthetic, and simplifying and expediting the examination process. Here we report a pilot study on the clinical utility of this device. Consideration is given to the advantages and disadvantages of performing non-contact LSCM (NC-LSCM) versus contact LSCM (C-LSCM), and representative images obtained using these techniques are displayed and evaluated.

2. Methods

In this pilot study, both C-LSCM and NC-LSCM were performed, on different days, on a healthy 35 year old female with no history of systemic disease, ocular disease or injury, or contact lens wear. The participant gave informed consent to be examined for this investigation.

* Corresponding author at: Institute of Health and Biomedical Innovation, Queensland University of Technology, 60 Musk Avenue, Kelvin Grove, Queensland 4059, Australia. Tel.: +61 7 3138 6401; fax: +61 7 3319 6974.
E-mail address: n.efron@qut.edu.au (N. Efron).

1367-0484/$ – see front matter © 2013 British Contact Lens Association. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.clae.2013.07.002

Fig. 1. Rostock Corneal Module with non-contact lens assembly positioned for corneal imaging.

2.1. Contact LSCM

C-LSCM was performed using the Heidelberg Retinal Tomograph (HRT3) with Rostock Corneal Module. For C-LSCM the Rostock Corneal Module was fitted with a Zeiss lens, which has a magnification of 63× and 0.95 numerical aperture. This instrument uses a 670 nm red wavelength diode laser light source. Images generated with the C-LSCM display *en face* sections of tissue of dimensions 400 μm × 400 μm. A large drop of a highly viscous eye gel (GenTeal eye drops, Novartis, North Ryde, NSW, Australia) was placed between the microscope objective and a disposable Perspex cap (TomoCap™) that covered the objective. The eye under examination was anesthetised with a drop of 0.4% benoxinate hydrochloride (Chauvin Pharmaceuticals, Chefaro, UK). Viscotears (Novartis, North Ryde, NSW, Australia) was applied to the eye immediately prior to imaging; this served as both a physical 'cushion' and as an optical coupling medium between the TomoCap and the cornea. The participant was asked to fixate on a near target with one eye while the central cornea of the contralateral eye was examined. Images were captured from all layers of the cornea by using the *z*-axis drive knob to advance the focal plane from the anterior to posterior corneal surface. All captured images were saved digitally.

2.2. Non-contact LSCM

NC-LSCM was also performed using the Heidelberg Retinal Tomograph (HRT3), but the Rostock Corneal Module was modified by inserting a novel prototype non-contact Nikon lens (CF Plan EPI SLWD) which has a magnification of 50× and 0.45 numerical aperture. Images produced with this lens system captured tissue sections of dimensions 500 μm × 500 μm. Since the instrument did not come into contact with the eye, a TomoCap was not fixed to the front of the lens, and no corneal anaesthetic or viscous gel were used. The participant was asked to fixate on a near target with one eye while the central cornea of the contralateral eye was examined. With the front of the lens positioned about 14 mm from the ocular surface (Fig. 1), serial images were captured from as many layers of the cornea as could be visualised, by using the *z*-axis drive knob to advance the focal plane from the anterior to posterior corneal surface. All captured images were saved digitally.

3. Results

Using the NC-LSCM, we were only able to capture images from the pre-corneal tear film, corneal stroma and endothelium. It was not possible to set 'zero' depth at the anterior corneal surface due to normal involuntary eye movements and microsaccades. Consequently, the exact depth of captured images within the cornea could not be determined.

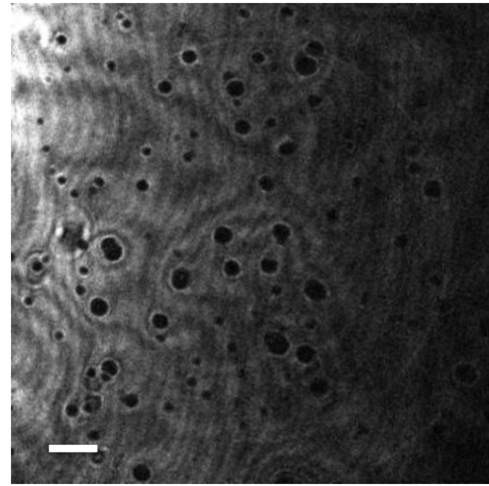


Fig. 2. Tear film imaged using NC-LSCM. Reference bar = 50 μm.

The tear film (Fig. 2) appeared as a series of alternating, light and dark interference fringes. The fringes were generally parallel and ran a slightly curved, vertical course. Interspersed throughout the image were generally round, black spots of various sizes, each with a distinct light border. These are presumed to be air bubbles in the tear film. We were unable to image the tear film using C-LSCM.

Superficial and basal corneal epithelial cells, and the subbasal nerve plexus, could be imaged using C-LSCM (images of these layers are not shown here but have been published previously [2]); however, despite extensive scanning, we were unable to image these corneal layers using NC-LSCM.

An image of the stroma, displaying keratocyte nuclei throughout the image frame, was captured using NC-LSCM (Fig. 3A). Since the depth of images acquired with NC-LSCM cannot be determined, it is unclear whether this image depicts the anterior, mid- or posterior stroma. An image of the anterior stroma (depth 110 μm) captured using C-LSCM is shown in Fig. 3B for comparison. The component features of Fig. 3A and B – keratocyte nuclei – are similar in form; however, they appear slightly larger in the image obtained using C-LSCM (Fig. 3B), primarily as a result of the slightly higher magnification of the Zeiss lens. As well, some of the keratocytes appear brighter.

Fig. 4A shows a bifurcating stromal nerve, which varies in brightness along its length, captured using NC-LSCM. Again, the precise depth of this structure within the cornea is uncertain. Keratocyte nuclei are scattered throughout the image. A stromal nerve imaged using C-LSCM (depth 115 μm) is shown in Fig. 4B. The morphological features of the nerves in Fig. 4A and B are similar, although, as discussed above, the surrounding keratocyte nuclei are slightly larger, and generally brighter, in the image captured using C-LSCM (Fig. 4B).

A diagonal strip of endothelium can be seen in Fig. 5A, with adjacent keratocyte nuclei in the posterior stroma to the top-left. This image was created as a result of non-parallel alignment of the plane of the optical elements in the Rostock Corneal Module and the corneal surface. Specifically, the top-left of the lens would have been angled further away from the cornea than the bottom-right of the lens, resulting in this transverse section image.

A full-field image of the endothelium captured using C-LSCM is shown in Fig. 5B. In both Fig. 5A and B, predominantly 4, 5 or 6-sided endothelial cells are visible, and are all of similar size relative to each other. The cells have a bright reflective surface, with dark borders. Small black spots visible in the centre of most cells may represent nuclei.

Download English Version:

https://daneshyari.com/en/article/2699224

Download Persian Version:

https://daneshyari.com/article/2699224

Daneshyari.com