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REVIEW

CORRELATIONS BETWEEN CONFOCAL MICROSCOPY AND HISTOLOGICAL ASPECTS OF NORMAL CORNEA

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Abstract

The evaluation of the cornea through confocal microscopy is a revolutionary non-invasive technique, which provides the ophthalmologist the histological and cytological in vivo images of the cornea, similar to those obtained through conventional histochemical methods. The current paper tries to prove and justify the similarity between the histological section model and the images obtained through confocal scanning, reflecting our experience with HRT II Cornea module.
Keywords: histological section, confocal microscopy, cornea

Introduction

Confocal microscopy is an increasingly used technique for corneal evaluation at a cellular level that provides images comparable to ex vivo histological sections. The method is non-invasive and generates in vivo images suitable for the evaluation of physiological and pathological modifications on both the ocular surface and the deep cornea.

Principle

The first description of confocal microscopy is related to Marvin Minsky's studies in 1955 upon nervous cells and neural networks. He proposed that the illumination and observation systems should share the same focal point (from which the name "confocal" microscopy derived) [**1**].

Confocal microscopy presupposes that a certain tissue spot is illuminated by a narrow light band and the image is recovered by a probe located within the same plane ("confocal"). By using this principle, a very high-resolution image can be obtained; however, the raw image does not provide sufficient data since the examined field is not wide enough. In order to overcome this technical challenge, special probes that can simultaneously generate thousands of luminous bands were created; afterwards the light bands are collected through the confocal principle in order to produce an image of both high resolution and dimensions [**2**].

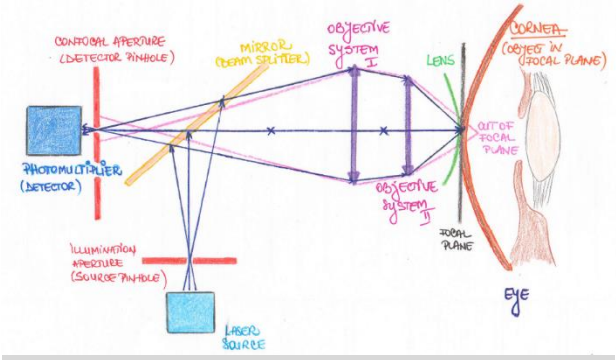

Fig. 1 Schematic representation of the principle of confocality (schematically drawing by Bogdana Tabacaru, MD)

Unlike conventional microscopy where tissue samples are collected, prepared, and then visualized in transverse sections, confocal microscopy offers coronal (frontal) section images, which are parallel to the examined surface [**3**].

Confocal microscopy in the evaluation of ocular surface

Confocal microscopy offers the possibility of a detailed evaluation of the normal and pathological ocular surface, in vivo, at a cellular level: tarsal and palpebral conjunctiva, central and peripheral cornea, tear film and the eyelids [**4**].

The clinical involvement of confocal microscopy includes [**5,6**]:

- pre and post surgical evaluation in refractive surgery and lamellar and penetrating keratoplasty;
- evaluation of patients with corneal ectasia and their post cross-linking follow-up;
- diagnosis and follow-up of corneal and conjunctival infections, corneal dystrophies, corneal, palpebral or conjunctival tumors;
- examination of corneal nerves in ocular pathology, in postsurgical follow-up and in systemic diseases;
- follow-up of contact lenses wearing;
- postsurgical follow-up of the filtration bleb in glaucoma surgery.

Current corneal confocal imaging systems

Several confocal imaging microscopes are commercially available nowadays and include the following: Confoscan P4 (Tomey Corporation, Cambridge, MA, USA), Confoscan 4 (Nidek Technologies, Japan) and probably the most advanced one, which is a laser corneal confocal microscope (Heidelberg Retina Tomograph II Rostock Corneal Module (HRTII)) (Heidelberg, Germany). Our paper reflects the experience of using HRTII Rostock Corneal Module for two weeks in assessing morphological aspects and variations in different corneal evaluations of the normal eyes.

The primary advantage of laser scanning confocal microscopy is the ability to serially produce images of thin layers from the cornea. Accordingly, the depth of focus for the tandem scanning confocal microscope (TSCM), like Confoscan devices, is of 7–9 μm, and in slit scanning systems, it is of 26 μm, whilst in using the laser confocal microscope, it is of 5–7 μm [**2**]. When examining the conjunctiva and corneoscleral limbus, the major limitation of the white light in-vivo confocal microscope is due to the backscattering of light. However, laser-scanning technology combined with the Rostock Cornea Module (RCM) microscope proved to be less affected by backscatter enabling accurate imaging of the cornea and conjunctiva. The conjunctiva, peripheral cornea and limbus are therefore best examined at the surface and at medium depth by using the HRT II Cornea than with a standard confocal microscope [**2**].

Corneal structure – histological aspect

The cornea is the anterior part of the external layer of the eyeball, a transparent structure that resists to deformation, with a central thickness of approximately 0.5 mm and a diameter of 11.5 mm. It consists of dens conjunctive tissue, flanked by a layer of epithelium on each of its sides [**7,8**].

Classically, the transverse sectioning of a cornea leads to the visualization of a 5-layered structure (from the exterior to the interior): corneal epithelium, Bowman membrane, corneal stroma, Descemet membrane and corneal endothelium [**8-10**].

The anterior surface consists of a non-keratinized stratified squamous epithelium, with a thickness of approximately 50 μm, 3-6 layers of cells in the central zone and 8-10 layers of cells in the peripheral one. The basal cells are polygonal shaped while the superficial ones have a flattened aspect [**7**]. The epithelial cells undergo a continuous division and regenerate as a response to the contact with the surrounding environment [**7**]. An intense mitotic activity can be observed within the cells at the base of the epithelium, ensuring wounds with a remarkable healing capacity. The renewal cycle has a periodicity of about 7 days [**8,9**].

The epithelial surface is permanently maintained humid thanks to the microvilli of the

apical cells that are contained within the precorneal tear film [**7-9**].

The most sensitively innervated part of the eyeball is the corneal epithelium, with branches that sprout from the ophthalmic branch of the trigeminal nerve (thus making it sensible to pain stimulus) [**7-9**].

The Bowman layer (Bowman's membrane), a prominent basal membrane with a mean thickness of 10 μm [**7-9**], that is firmly attached to the epithelium by hemidesmosomes [**11,12**], is under the epithelium. The Bowman layer is also closely attached to the underlying conjunctive tissue [**7**]. This provides the cornea with significant stability, resistance and protection against trauma and bacterial invasion [**8,9**]. This layer is acellular, has no self-regeneration capacity and is composed of condensed intercellular substance and randomly distributed collagen fibers [**8,9,13**].

The central layer of the cornea, also called stroma or substantia propria makes up 90% of the corneal thickness [**9**] and contains 60-70 layers of type I [**7,9**] and V [**9**] collagen fibers. These are uniform in diameter and incorporated in a proteoglycan-rich extracellular matrix. The collagen fibers are regularly distributed within layers, forming bundles which are parallel among themselves and intersect bundles from other layers at an angle of about 90 degrees [**7,8**]. The unique disposition of these bundles reduces the interference of light, ensuring corneal transparency [**7,14**]. The fibers of each layer of the corneal stroma are perfectly parallel and run through the whole length of the cornea. Between the layers of collagen bundles, there are fibrocytes and cytoplasmic extensions of the fibroblasts (named keratocytes), which are flattened and have the aspect of butterfly wings [**8**]. The cells and fibers of the corneal stroma are inserted within a fundamental substance which is rich in glycoproteins, chondroitin and keratin sulphate [**8,9**]. Although the corneal stroma is an avascular structure, some migrated lymphoid cells can sometimes be normally observed [**8**].

The corneal stroma is crossed by myelinated nervous fibers, which run through the epithelium. At the level of the Bowman membrane, the nerves lose their myelin sheath and head for the surface, crossing the intercellular space through the epithelium [**9**].

The Descemet membrane (the basal membrane of the endothelium) is a homogenous structure; it is very thin (among the thinnest basal membranes within the human body), of around 5-10 μm [**8,9**], and consists of fine collagen type VII [**9**] and VIII [**13**] filaments, which are organized as a hexagonal tridimensional network [**8,9,14**].

The posterior surface of the cornea, improperly named corneal endothelium [**7**] consists of a simple squamous, sometimes cubical epithelium [**8**]. The cells of this layer contain secreting organelles, characteristic for cells implied in active transport and protein synthesis. It is supposed that these cells are responsible for the synthesis and maintenance of the Descemet membrane [**8**]. The intercellular space is impermeable, thus preventing the aqueous humor inflow within the corneal stroma [**9**].

The apical surface of endothelial cells is exposed to the aqueous humor from the anterior chamber [**7**].

The corneal endothelium and epithelium determine the corneal transparency. The cells of both corneal layers have the capacity of transporting sodium, a phenomenon that determines the maintenance of the cornea in a relatively dehydrated state, which associated to the regular disposition of the stromal collagen fibers, provides an increased degree of transparency [**8**].

Recently, H.S. Dua has described the existence of a sixth corneal layer, called the pre-Descemet's stromal layer or the Dua's layer, an acellular structure with a thickness of 6-15 μm, consisting of 5-8 layers of collagen [**15**].

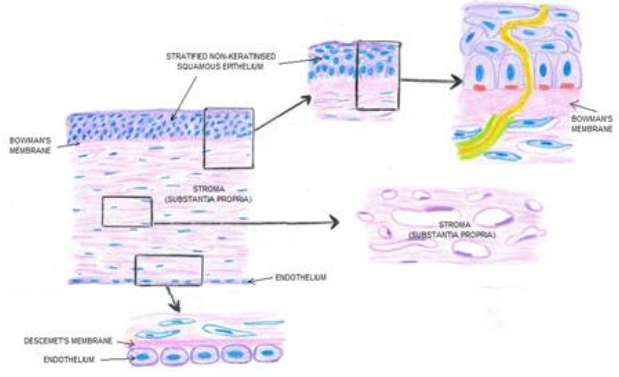

Fig. 2 Histological aspect of the cornea (schematically drawing by Bogdana Tabacaru, MD)

Confocal microscopy views of sections from different depths of the normal cornea

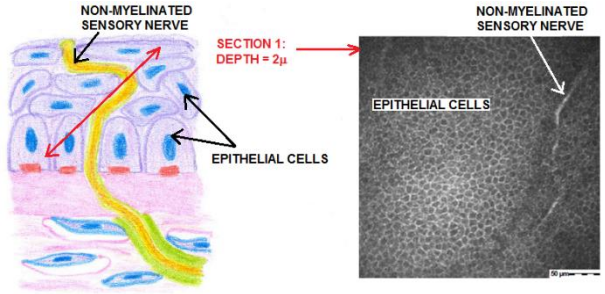

Fig. 3 Histological aspect of the anterior part of the cornea (scheme) and corneal confocal microscopy image at 2μ depth of a normal left eye (schematically drawing by Bogdana Tabacaru, MD)

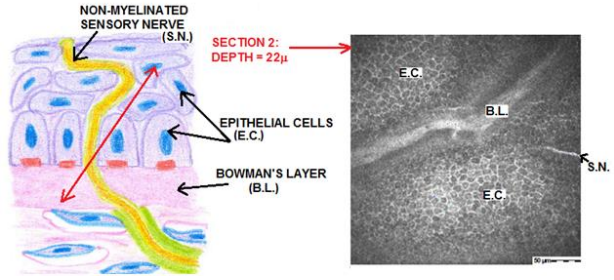

Fig. 4 Histological aspect of the anterior part of the cornea (scheme) and corneal confocal microscopy image at 22μ depth of a normal right eye (schematically drawing by Bogdana Tabacaru, MD)

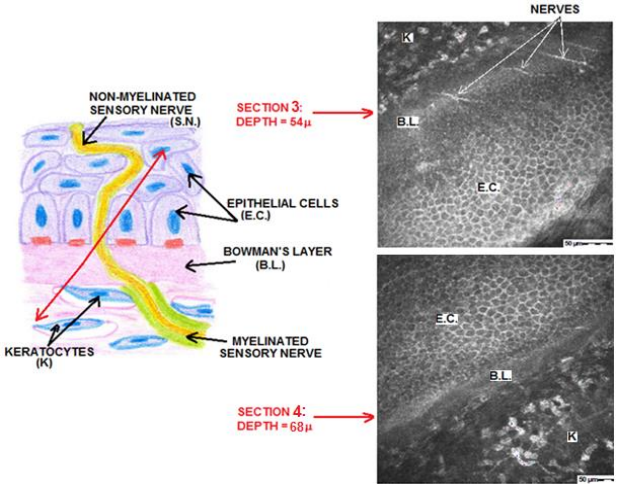

Fig. 5 Histological aspect of the anterior part of the cornea (scheme) and corneal confocal microscopy images at 54μ and 68μ depth of two normal right eyes (schematically drawing by Bogdana Tabacaru, MD)

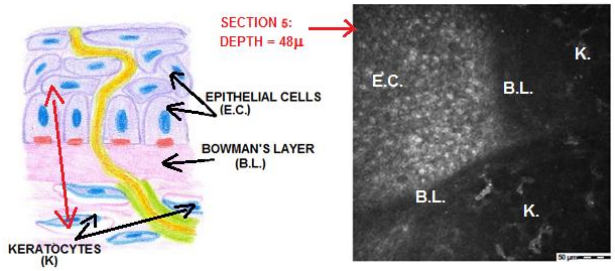

Fig. 6 Histological aspect of the anterior part of the cornea (scheme) and corneal confocal microscopy image at 48μ depth of a normal right eye (schematically drawing by Bogdana Tabacaru, MD)

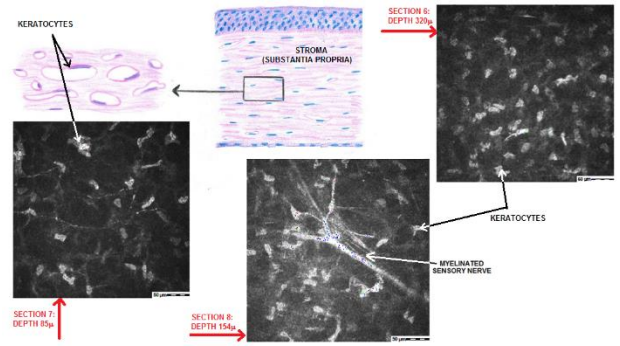

Fig. 7 Histological aspect of the middle part of the cornea (scheme) and corneal confocal microscopy images at 85μ (right eye), 154μ (left eye) and 320μ (right) depths of three normal eyes (schematically drawing by Bogdana Tabacaru, MD)

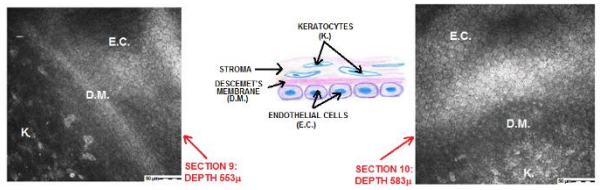

Fig. 8 Histological aspect of the posterior part of the cornea (scheme) and corneal confocal microscopy images at 553μ and 583μ depths of two normal right eyes (schematically drawing by Bogdana Tabacaru, MD)

Conclusions

Confocal microscopy is a modern technique for the evaluation of the corneal structure at a cellular level; it is non-invasive and allows a

detailed examination of all the in vivo corneal layers. The images obtained through confocal microscopy are similar to those of histopathological conventional microscopy and can become useful within the ophthalmology clinic, since they facilitate the diagnosis and follow-up of patients with corneal pathology. Assessing tissue repair following surgical intervention or injury and identifying patients at risk follow progression and measure therapeutic response in a range of neuropathies, but mainly in diabetics, seem to be very attractive issues in the future.

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