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Chapter 3

# *In Vivo* Biopsy of the Human Cornea

Akira Kobayashi, Hideaki Yokogawa and Kazuhisa Sugiyama

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/55657

## 1. Introduction

The human cornea is the transparent, dome-shaped tissue that covers anterior segment of the eye. It consists of five thin layers: epithelium, Bowman's layer, stroma, Descemet's membrane and endothelium. Due to its transparency, real-time *in vivo* confocal microscopic observation of the normal and diseased cornea was developed since the early 1990s. [1,2] Since histologic-like images are obtained by the device, it is called "painless biopsy" and/or "*in vivo* biopsy". In this chapter, clinical application of *in vivo* laser confocal microscopy is demonstrated.

## 2. *In vivo* laser confocal microscopy

In 2005, cornea specific *in vivo* laser confocal microscopy (Heidelberg Retina Tomograph 2 Rostock Cornea Module, HRT2-RCM, Heidelberg Engineering GmbH, Dossenheim, Germany) has become available (Figure 1).[3,4] It has permitted detailed *in vivo* layer-by-layer observations of corneal microstructure with an axial resolution of nearly 1 μm. [4]

Before examination, written informed consents are obtained from all patients; this includes possible consequences of this device such as superficial punctate keratopathy. A large drop of contact gel (Comfort Gel ophthalmic ointment®, Bosch & Lomb, GmbH, Berlin, German) is applied on the front surface of the microscope lens and ensuring no air bubbles had formed, a Tomo-cap® (Heidelberg Engineering GmbH, Dossenheim, Germany) is mounted on the holder to cover the microscope lens. Then, the center and peripheral cornea were examined layer by layer by *in vivo* laser confocal microscopy.

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**Figure 1.** Heidelberg Retina Tomograph 2 Rostock Cornea Module The device uses a 60× water-immersion objective lens (Olympus Europa GmbH, Hamburg, Germany) and utilizes a 670-nm diode laser as the light source with the area of observation 400μm square section.

## 3. Normal human cornea and conjunctiva

*In vivo* laser confocal microscopy enables to visualize normal human cornea layer by layer: superficial epithelial cells, basal epithelial cells, Bowman's layer with nerves, Bowman's layer with K-structures (Kobayashi-structures), stroma and endothelial cells (Figure 2).[5,6] K-structures are fibrous structures with a diameter of 5 to 15 μm, and are considered to be anterior collagen fiber bundles running at the posterior surface of Bowman's layer. [5] Mapping of the K-structure revealed that it showed mosaic pattern which is completely different from the whirl pattern of corneal nerves. [6]

In addition, conjuctival cells and meibomian glands are able to visualize *in vivo* (Figure 3).[7, 8]

## 4. Corneal infections

*Acanthamoeba* is a ubiquitous, free-living amoeba found in water (swimming pool, hot tubs, tap water, contact lens solutions), air and soil, but *Acanthamoeba* keratitis is a relatively newly recognized entity: the first case was reported in 1974.[9] As the use of soft contact lenses increased in the early 1980s, the incidence of reported *Acanthamoeba* keratitis increased dramatically. *Acanthamoeba* keratitis is relatively uncommon but is a potentially blinding corneal infection. Clinical diagnosis is very difficult, especially in the early phase of the disease; it is often misdiagnosed and treated as a herpes simplex infection.[10] A definite diagnosis is made by confirmation of *Acanthamoeba* in corneal lesions with direct examination, corneal

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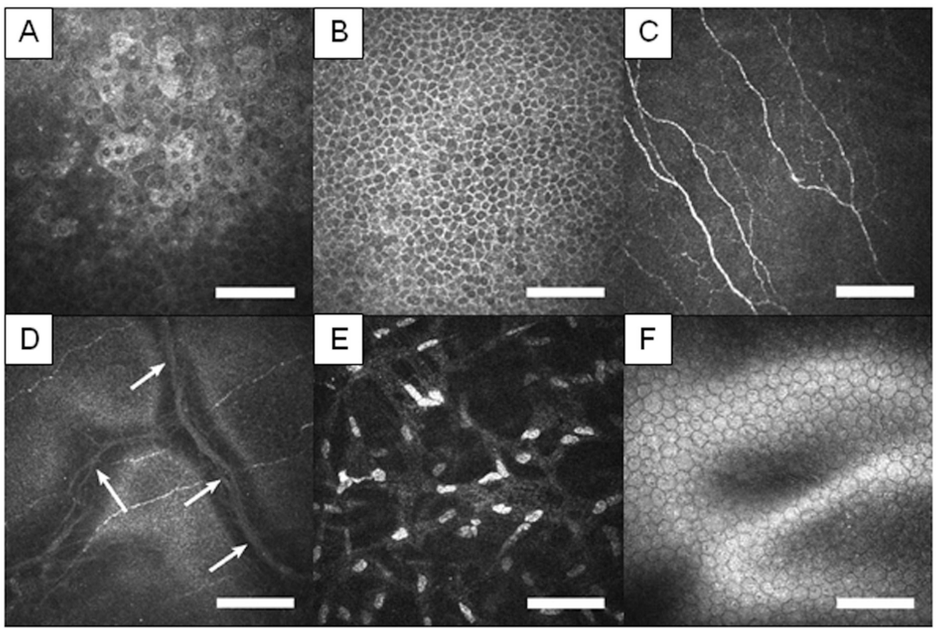



**Figure 2.** Normal human cornea. A. Superficial epithelial cells. B. Basal epithelial cells. C. Bowman's layer with nerves. D. Bowman's layer with K-structures (arrows). E. Stroma. F. Endothelial cells. (Bar=100μm)

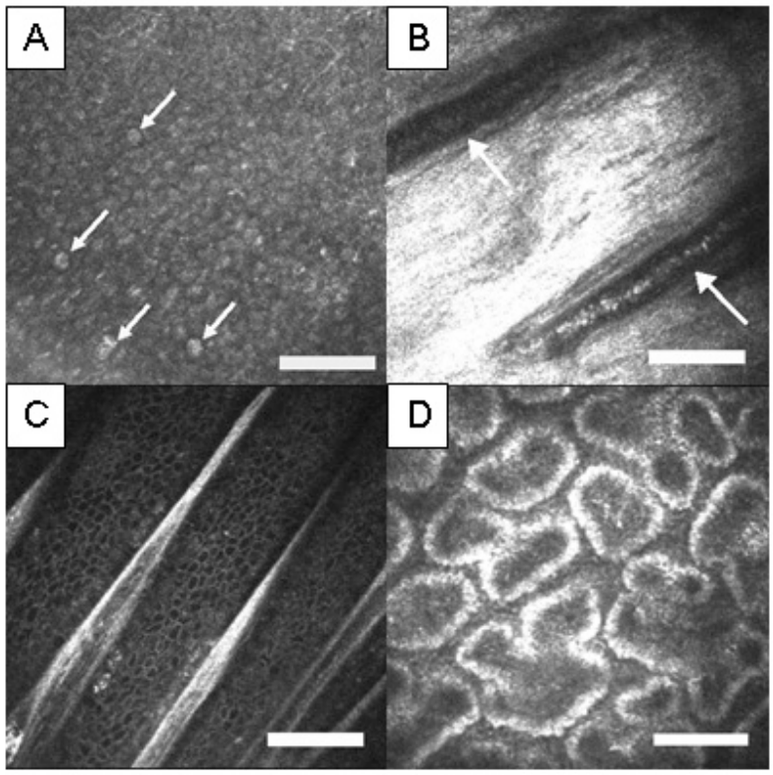



**Figure 3.** Normal human conjunctiva and meibomian glands. A. Conjuctival epithelium with Goblet cells (arrows). B. Sub-conjunctival fibrous tissue with conjunctival vessls (arrows). D. Palisade of Vogt of corneal limbus. D. Meibomian gland of the upper tarsus. (Bar=100μm)

biopsy or with culture; however, these methods are invasive, time-consuming, and are not always routinely available. The invasive methods are often postponed until there is a high index of suspicion for the disease and when there has been no response to treatments for bacterial, viral and/or fungal keratitis. [10] The usefulness of *in vivo* white-light confocal microscopy in diagnosis and monitoring for improvement of *Acanthamoeba* keratitis has been reported. [11] *In vivo* laser confocal microscopy has also been shown to be useful in the early diagnosis of *Acanthamoeba* keratitis (Figure 4 A-C).[12-15] It is also reported that fungal hyphae can be well visualized by *in vivo* laser confocal microscopy.[16,17]

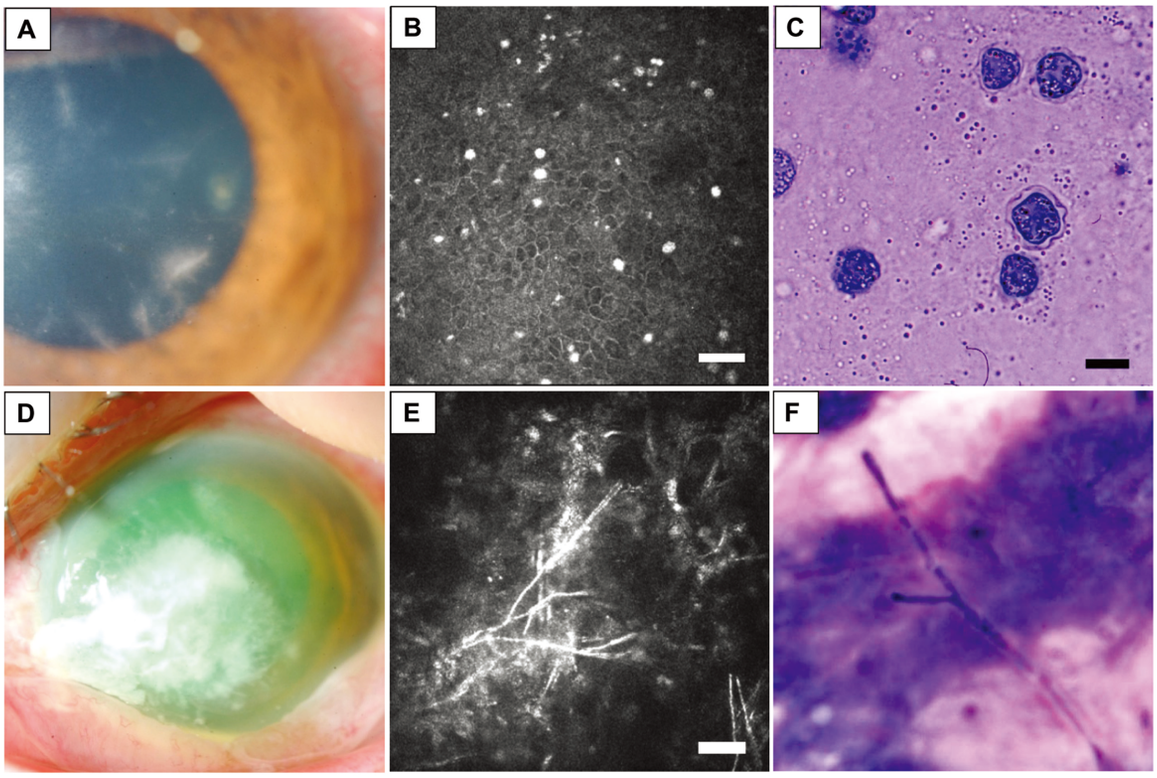



**Figure 4.** Corneal infections. A. Slit-lamp photograph of the cornea with *Acanthamoeba* keratitis. Subepithelial opacities and numerous radial keratoneuritis lesions were observed. B. In the epithelial basal cell layer, numerous highly reflective, high-contrast round-shaped particles 10-20μm in diameter suggestive of *Acanthamoeba* cysts were detected by *in vivo* laser confocal microscopy. (Bar=50μm) C. Direct examination of the epithelial scraping with Parker ink-potassium hydroxide revealed Acanthamoeba cysts. Note that the cysts have double walls with characteristic wrinkled outer wall. (Bar=10μm) D. Slit-lamp photograph of the cornea with *Aspergillus* keratitis. Severe corneal ulcer was observed. E. In the stormal layer, numerous highly reflective, high-contrast branching filaments suggestive of *Aspergillus* hyphae were detected by *in vivo* laser confocal microscopy. (Bar=50μm) F. Direct examination of the epithelial scraping revealed *Aspergillus* hyphae

## 5. Corneal dystrophies

*In vivo* laser confocal microscopy are proven useful to visualize pathological changes of corneal dystrophies *in vivo* (Figure 5) [18-20]. It is also useful to differentially diagnose confusing

corneal dystrophies. Previously, considerable confusion exists clinically in distinguishing between Thiel-Behnke and Reis-Bücklers corneal dystrophy (Figure 5A, 5C). However, using *in vivo* laser confocal microscopy, unique and characteristic confocal images are readily obtained at the levels of Bowman's layer; relatively highly reflective deposits in Thiel-Behnke dystrophy but extremely highly reflective deposits in Reis-Bücklers corneal dystrophy. The deposits in Thiel-Behnke corneal dystrophy had round-shaped edges with dark shadows, whereas the deposits in Reis-Bücklers corneal dystrophy did not have round-shaped edges but consisted of highly reflective small granular materials without any shadows (Figure 5B, 5D)[18]. It is also possible to differentially diagnose with corneal stromal dystrophies including Avellino (Figure 5E, 5F), lattice (Figure 5G, 5H), macular (Figure 5I, 5J) and Schnyder dystrophy (Figure 5K, 5L).[19, 20]

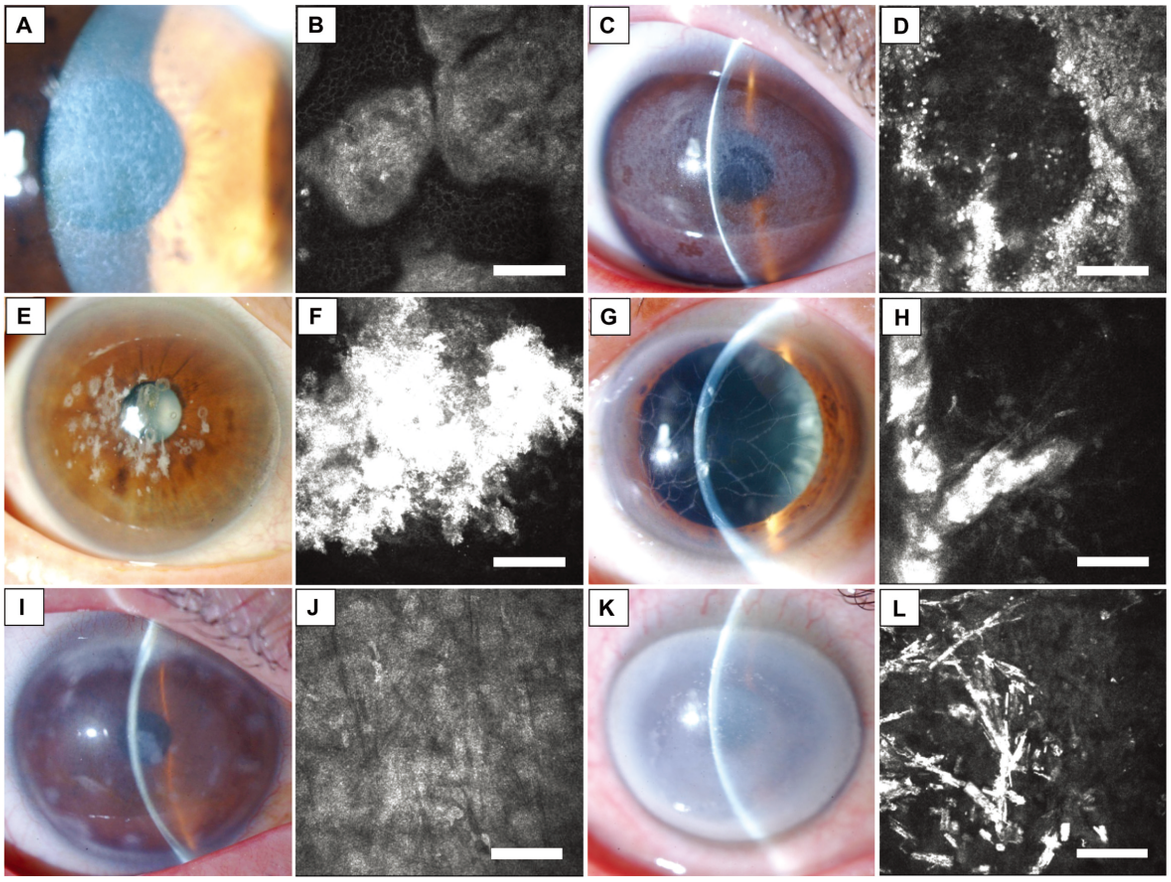



**Figure 5.** Corneal dystrophies. A. Slit-lamp biomicroscopic photograph of Thiel-Behnke corneal dystrophy (*TGFBI* R555Q). Honeycomb-shaped gray opacities were observed at the level of Bowman's layer. B. *In vivo* laser confocal microscopy showed focal deposition of homogeneous reflective materials with round-shaped edges in the basal epithelial layer. All deposits accompanied dark shadows. C. Slit-lamp biomicroscopic photograph of Reis-Bücklers corneal dystrophy (*TGFBI* R124L). Bilateral gray-white, amorphous opacities of various sizes at the level of Bowman's layer were observed. D. *In vivo* laser confocal microscopy showed focal deposition of highly reflective irregular and granular materials in the basal epithelial layer. No deposits accompanied dark shadows. E. Slit-lamp biomicroscopic photograph of Avellino corneal dystrophy (*TGFBI* R124H) showed round gray-white deposits and scattered stellate opacities in the superficial and mid-stroma. F. *In vivo* laser confocal microscopy showed focal deposits of extremely highly reflective material with irregular edges in the stromal layer. G. Slit-lamp biomicroscopic photograph of lattice corneal dystrophy

(*TGFBI* R124C) showed radially oriented thick lattice lines in the stroma. H. *In vivo* laser confocal microscopy showed highly reflective lattice-shaped materials in the stromal layer. I. Slit-lamp biomicroscopic photograph of macular corneal dystrophy (*CHST6* A217T) showed anterior and deep stromal opacities with indistinct borders that extend out to the corneal periphery. Some gray-white discrete deposits could be seen in the stroma. J. *In vivo* laser confocal microscopy showed homogeneous reflective materials with dark striae-like images. Normal keratocytes were not seen. K. Slit-lamp biomicroscopic photograph of Schnyder corenal dystrophy (*UBIAD1* N233H) showed dense anterior stromal disciform opacity with lipoid arcus. L. *In vivo* laser confocal microscopy showed numerous crystals with varying sizes were observed in the sub-epithelial stromal layer.

## 6. Cytomegalovirus corneal endotheliits/uveitis

Corneal endotheliitis, characterized by corneal edema associated with linear keratic precipitates and endothelial dysfunction, may be caused by herpes simplex virus, varicella zoster virus, or other viruses such as mumps. It often leads to irreversible corneal endothelial cell damage and severe visual disturbance. Most recently, cytomegalovirus (CMV) was recognized as a new etiologic factor for corneal endotheliitis [21-24]. Clinical manifestations of CMV endotheliitis are characterized by linear keratic precipitates associated with multiple coin-shaped lesions and local corneal stromal edema with minimal anterior chamber reactions. *In vivo* laser confocal microscopy is able to demonstrate the characteristic owl's eye cells in the corneal endothelial cell layer (Figure 6). Owl's eye cells are typically seen at autopsy or in biopsy specimens from the kidneys, lungs and other organs in cases of congenital or acquired CMV infection. By *in vivo* laser confocal microscopy, owl's eye cells are readily seen *in vivo* as large corneal endothelial cells with an area of high reflection in the nucleus surrounded by a halo of low reflection. Therefore, owl's eye cells may be a useful adjunct for the non-invasive diagnosis of CMV corneal endotheliitis [25].

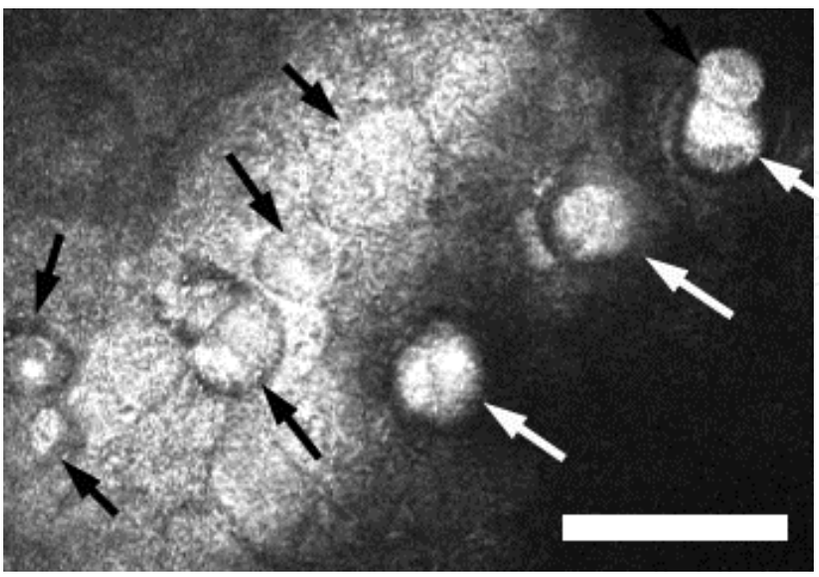


**Figure 6.** Owl's eye cells in cytomegalovirus corneal endotheliitis. I*n vivo* laser confocal microscopic photo showed numerous owl's eye cells (arrows) in the corneal endothelial cell layer. (bar=100μm)

## 7. Post surgical anatomies

*In vivo* laser confocal microscopy is useful for visualization of post keratoplasty anatomies (Figure 7). Descemet's stripping automated endothelial keratoplasty (DSAEK) is a new type of keratoplasty technique in which only posterior portion of the donor cornea is transplanted inside the eye. Since the donor is attached with air, no stitches are required; this enables maintaining much of the structural integrity of the cornea and induces minimal refractive change, suggesting distinct advantages over standard penetrating keratoplasty.[26] *In vivo* laser confocal microscopy analysis identified subclinical corneal abnormality after DSAEK with high resolution; this includes subepithelial haze, donor-recipient interface haze (Figure 8), and interface particles(Figure 8).[27, 28] Quantitative analysis showed that these postoperative hazes and particles decreased significantly over follow-up. [27, 28] The influence of these hazed on vision are still under investigation.

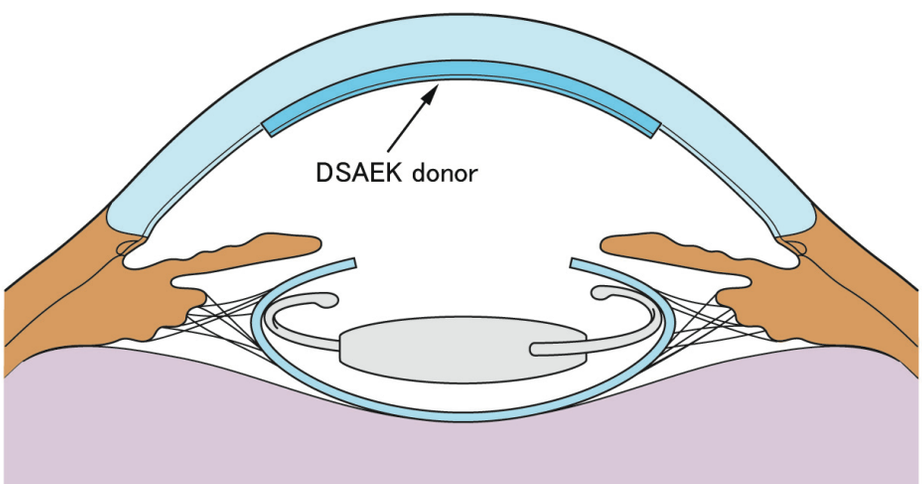



**Figure 7.** Schema of DSAEK. Posterior portion of the donor cornea is transplanted inside the eye.

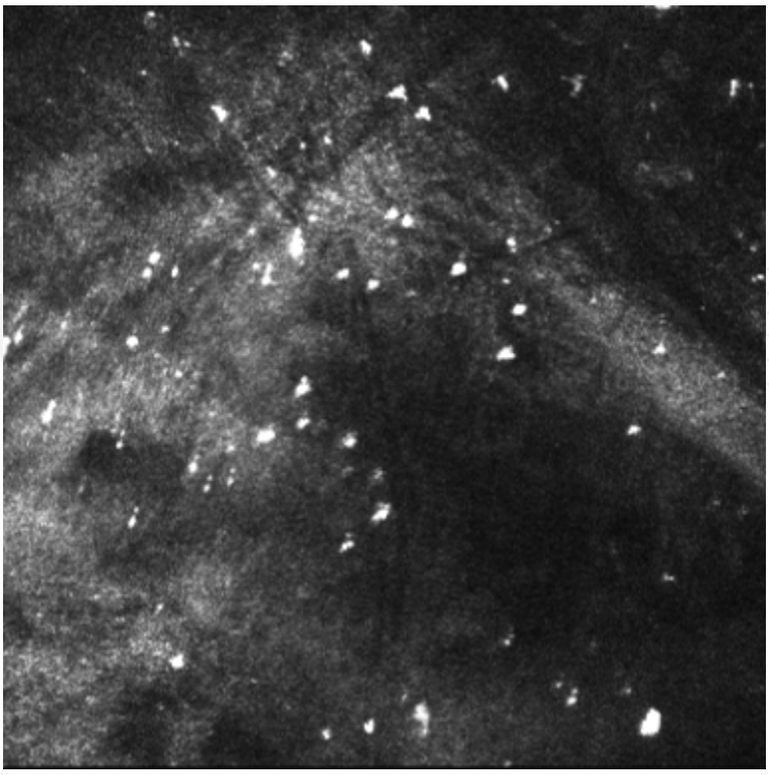


**Figure 8.** *In vivo* laser confocal microscopy of donor-recipient interface after DSAEK. At the level of the donor-recipient interface, highly reflective particles and interface haze were observed(400×400μm).

## 8. Conclusion

*In vivo* laser confocal microscopy is able to identify histologic-like real time optical sectioning of the normal and diseased cornea/conjuctiva with high resolution. It is clinically useful in diagnosing *Acanthamoeba* keratitis, fungal keratitis, cytomegalovirus endotheliitis and corneal dystrophies. It is also useful to observe post surgical corneal anatomies. Further investigation using this device is required to understand in vivo histology of normal and diseased cornea/conjunctiva.

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