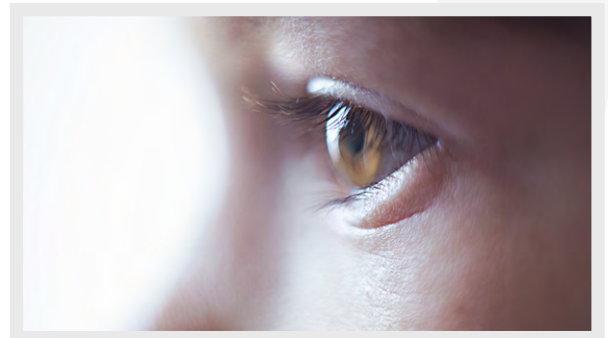


# In-vivo Corneal Imaging

## High speed UHR-OCT reaches the cellular level

*Recent increases in the speed and resolution of ultrahigh resolution OCT are opening up new possibilities for in-vivo, non-contact imaging of the cellular structure of biological tissue at image acquisition rates high enough to suppress significantly image artifacts arising from involuntary eye motion. A spectral domain OCT system designed for corneal imaging was reported recently that combines  $\sim 1.5 \mu\text{m}$  axial resolution with image acquisition rate of 250,000 lines/second. The speed and resolution achieved with this system makes it possible to observe and measure non-invasively many structural features of the human cornea that are of clinical and diagnostic interest: the cellular structure of the corneal epithelium, sub-basal corneal nerves & keratocytes in the corneal stroma, and even nuclei of the endothelial cells at the posterior cornea. With this level of detail, clinicians may someday have far more information to preserve the health of this 'window into the eye'!*

Pathologies of the cornea are the fourth leading cause of world blindness, just behind cataract, glaucoma, and age-related macular degeneration<sup>1</sup>. The challenge is that the earliest signs of corneal diseases often occur at the cellular level of corneal tissue, or in the corneal nerve structure. Such changes cannot be observed with current clinical imaging systems such as slit lamp microscopes, which are the current gold standard for clinical diagnostics of corneal diseases. *In-vivo* confocal microscopy (IVCM) has the necessary resolution to visualize corneal cells, however, it has limited field of view and scanning speed, and requires physical contact of the IVCM imaging probe with corneal tissue. This causes discomfort to patients and may result in inadvertent inflammation of the corneal tissue. The ability to observe and quantify the cellular changes of the cornea using a non-contact imaging method would allow for earlier diagnostics of the disease and provide a safer, more comfortable examination procedure.



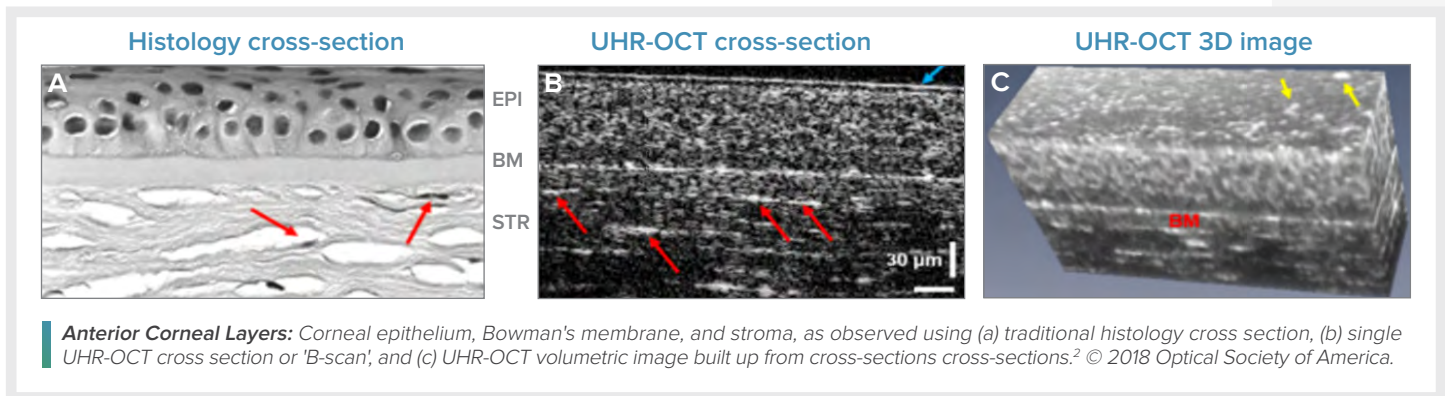
Optical coherence tomography (OCT) has been progressing in its ability to provide *in-vivo* corneal imaging over the past decade, revealing many of the structures key to corneal pathologies. Yet clear volumetric images of the cornea at the cellular level have remained just out of reach due to imaging artifacts originating from fast, involuntary eye motion. Swept-source OCT (SS-OCT) has the speed, though lacks the  $\sim 1 \mu\text{m}$  resolution necessary to image the corneal cellular structure. Spectral domain OCT (SD-OCT) meets the resolution requirements. However, as the image acquisition rate is determined by the camera speed, SD-OCT was limited to  $<100 \text{ kHz}$  until recently.

An SD-OCT system developed by Prof. Bizheva's research group at the University of Waterloo, Canada, changes all that, delivering volumetric images of cornea acquired from a  $0.75 \times 0.75 \text{ mm}$  area in just 2.8 seconds -- fast enough to achieve clear images of the cellular structure of the corneal tissue with minimal eye-motion induced image artifacts. The OCT system's design is based on broadband laser to generate high imaging resolution and a Wasatch Photonics [Cobra-S 800 OCT spectrometer](#) connected to a 250 kHz line scan camera to achieve high speed volumetric imaging. This system design offers  $\sim 1.5 \mu\text{m}$  imaging resolution in corneal tissue, sufficient to observe individual cells, with signal-to-noise ratio roll-off in free space of approximately 10 dB over a scanning range of 1.4 mm.

The performance of the 250 kHz OCT system was tested by imaging the central cornea of healthy, normal subjects aged 20 – 45 years and the OCT images were compared with IVCM images acquired from the same subjects. Images were captured over the full 540-560  $\mu\text{m}$  thickness of the cornea in segments, revealing clinically relevant structures in exquisite detail for every layer.

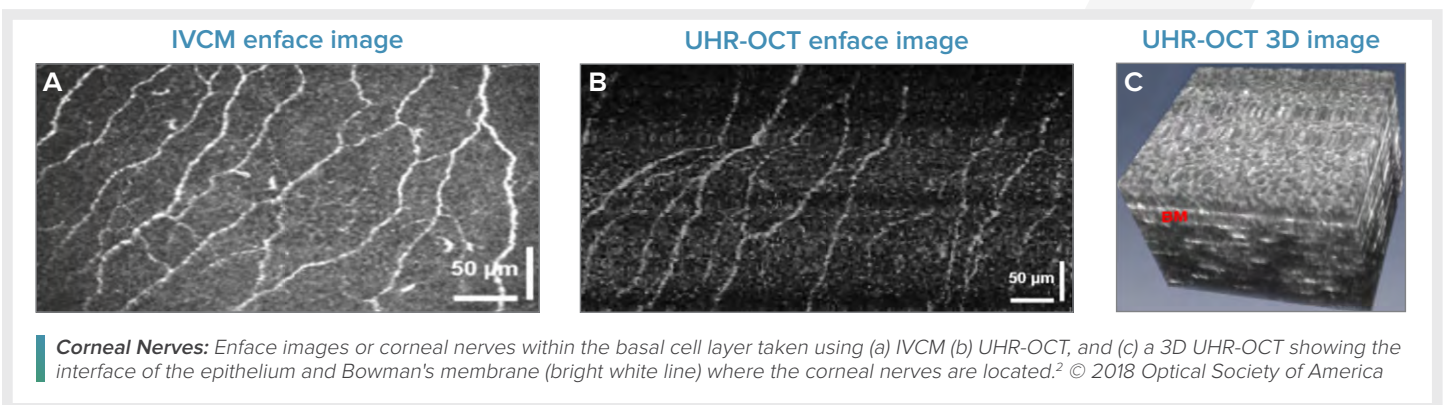
## EPITHELIUM BOUND

The outer layers of the cornea provide the eye's first defense against the elements and infection. Here, high-speed UHR-OCT reveals the cellular structure of the epithelial layer, with a bright white line to delineate the boundary with the Bowman's membrane. Keratocytes, which help to maintain corneal health and facilitate repair, can be seen and counted in the stroma (marked with red arrows). Most incredibly, the tear film is clearly delineated and measurable on the cornea in cross section (blue arrow), and bright spots, likely due to cellular debris and mucin clusters, can be seen in 3D (marked by yellow arrows). This is very promising, as tear film information is lost in histology and IVCM, and has been hard to image using other OCT systems.



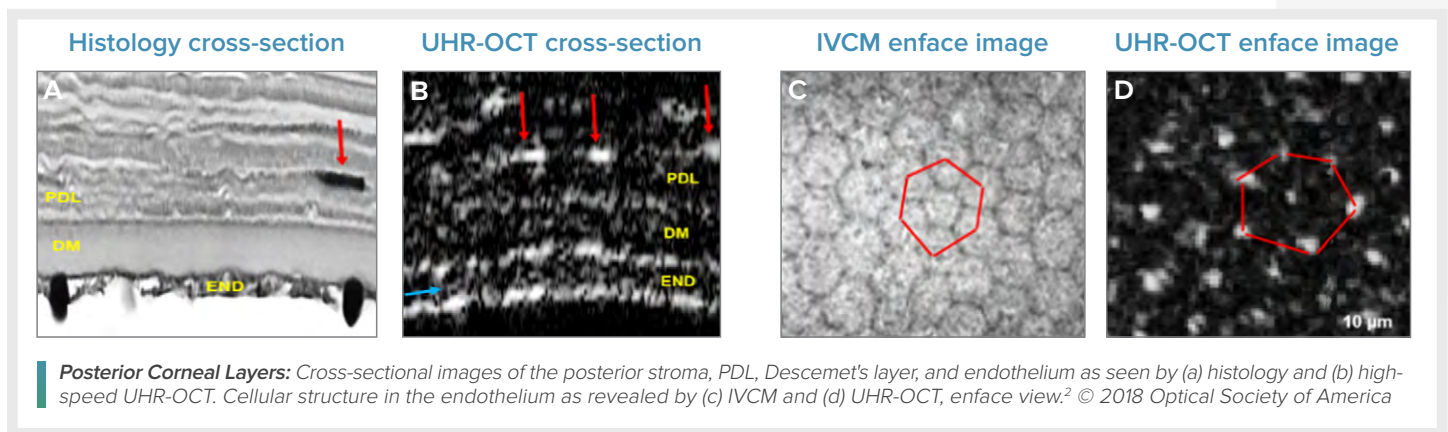
## EVERY LAST NERVE

Some corneal pathologies cause changes in the length, twisting, and density of the nerves located in the basal cell layer of the epithelium, running parallel to its interface with the Bowman's layer. The high spatial resolution and imaging rate of the UHR-OCT system allows these nerves to be imaged *in-vivo*, seen here as long, reflective white lines. The clarity of the nerves as compared to IVCM was sufficient to allow the research group to develop a fully automated algorithm for segmentation in a follow-on study, indicating potential for *in-vivo* diagnostics and monitoring of diseases like diabetic peripheral neuropathy.<sup>3</sup>



## EDGE OF THE ENDOTHELIUM

On the posterior edge of the cornea and just beyond the thick stroma lies the pre-Descemet's layer (PDL) first imaged *in-vivo* by Bizheva's group in 2016, also known as the 'Dua' layer.<sup>4</sup> Below that, the Descemet's layer and endothelium are the final layers in the cornea. The exact locations of these boundaries are important to the precision of keratoplastic laser surgery techniques. Even at this considerable depth, the high speed UHR-OCT system provides sufficient resolution to see each structure clearly, including keratocytes at the boundary between the posterior stroma and PDL (bright white spots, marked here by red arrows). Looking at the corneal endothelium layer enface, it is even possible to see reflections from the nuclei (white spots in the UHR-OCT image), as well as their hexagonal pattern (marked in red). This is quite incredible given that these enface images were generated from 2.8 seconds of data from *in-vivo* scans!



## CONCLUSION

The rapid acquisition rate made possible by this next-generation UHR-OCT system powered by the Cobra-S spectrometer overcomes the traditional limitations of eye motion artifacts. It also provides the detail needed to see many corneal structures which are key in the diagnosis, study, and treatment of corneal disease -- from tear film, corneal nerves, and keratocytes to the PDL layer and endothelial cell structure. Combined with automated analysis, this may someday become a powerful tool for the clinic.

## REFERENCES

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3. Hosseinaee, Zohreh, et al. ["Fully automated corneal nerve segmentation algorithm for corneal nerves analysis from in-vivo UHR-OCT images."](#) Ophthalmic Technologies XXIX. Vol. 10858. International Society for Optics and Photonics, 2019.
4. Bizheva, Kostadinka, et al. ["In vivo imaging and morphometry of the human pre-Descemet's layer and endothelium with ultrahigh-resolution optical coherence tomography."](#) Investigative ophthalmology & visual science 57.6 (2016): 2782-2787.