

Evaluation of Corneal Oedema – Tools we Have and Those Under Investigation

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DOI: <https://doi.org/10.17925/EOR.2019.13.2.76>

Corneal oedema is a common sign of acute or chronic corneal disease resulting from various aetiologies such as corneal endothelial dystrophy, prior surgery such as cataract surgery, metabolic disorders, toxicity or hypoxia. Measurement of central corneal thickness (CCT) is an objective and surrogate assessment for the evaluation of corneal oedema. The commonly used tools for the CCT measurements include ultrasonic pachymetry, anterior segment optical coherence tomography, specular microscopy and corneal tomography. Terahertz and mid-infrared technology have emerged recently for their applications on corneas. Instead of measuring CCT, they detect the changes of corneal water concentrations because their light wave spectrum is sensitive to water. The utility of these imaging systems as an adjuvant tool for the evaluation of corneal oedema, will be discussed in this article.

Keywords

Corneal edema, corneal disease, corneal endothelial dystrophy, central corneal thickness, CCT, Terahertz, mid-infrared technology

Disclosure: Yu-Chi Liu, Lin Ke and Jodhbir S Mehta have nothing to disclose in relation to this article.

Review Process: Double-blind peer review

Authorship: All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship of this manuscript, take responsibility for the integrity of the work as a whole, and have given final approval to the version to be published.

Compliance with Ethics: This study involves a review of the literature and did not involve any studies with human or animal subjects performed by any of the authors.

Received: 25 October 2019

Accepted: 9 January 2020

Citation: *European Ophthalmic Review*, 2019;13(2):76–80

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Support: No funding was received in the publication of this article.

The cornea is a transparent and avascular tissue. The optical transparency, which is directly related to the state of corneal hydration, is essential for high resolution of vision. Corneal hydration is controlled by (1) factors that draw water into the cornea, including the intraocular pressure and swelling pressure from stromal glycosaminoglycans;^{1,2} and (2) factors that prevent the flow of water into the cornea, i.e., the intact corneal epithelial and endothelial barriers. The functioning corneal metabolic endothelial pump, which is linked to an ion-transport system, is controlled by temperature-dependent enzymes such as Na⁺, K⁺-ATPase.¹ Corneal hydration varies from the anterior to posterior of corneas, with increasing saturation to the endothelium. When the endothelial cells are impaired and the number of damaged endothelia exceeds the threshold necessary to maintain the functioning of the cornea, fluid leaks into the corneal stroma, resulting in corneal oedema. Folds in Descemet's membrane are usually first seen when corneal thickness increases by 10% or more.¹ As the corneal oedema progresses, the oedema extends from the stromal space to epithelial layer, leading to epithelial oedema and bullae. When epithelial oedema occurs, the corneal thickness usually has exceeded 700 µm.¹

Corneal oedema results from various aetiologies (*Table 1*), including endothelial dystrophy; previous surgery, such as cataract surgery or glaucoma filtering surgery; mechanical trauma; metabolic disorders; toxicity or hypoxia.^{1,3,4}

Evaluation of corneal oedema

Clinically, the diagnostic workup for corneal oedema starts with slit lamp examination, in which an oblique and thin light beam on oblique viewing of the optical section of the corneas can be used to estimate the corneal thickness. Measurement of central corneal thickness (CCT) is an indirect and surrogate measure for the evaluation of corneal hydration status. Ultrasound pachymetry has been used for more than 30 years and is one of the most commonly used methods for measuring CCT, by estimating the time difference between echoes of ultrasound waves reflected from the anterior and posterior surface of the cornea.⁵ However, its reliability can be limited due to the susceptibility to confounding factors such as topical anaesthesia, and requiring individual user skill to place the probe as perpendicularly as possible on the central corneal apex. Moreover, it requires cornea–probe contact.⁶ The use of topical anaesthesia has been reported to cause cornea thickening up to 31 µm.⁷ These limitations have led to the introduction of several optical technologies that offer the advantages of a non-contact technique and objective determination of the centre of the cornea. A non-invasive and non-contact tool is especially preferred in patients who have undergone refractive surgery or corneal transplantation to avoid complications that are related to the measuring, such as corneal epithelial abrasions, laser *in situ* keratomileusis (LASIK) flap displacement, or potential exposure of corneal infection due to the probe contact.

Very high-frequency (VHF) digital ultrasound was introduced in 1993 and has gradually improved both in precision and in area of acquisition.⁸ It produces three-dimensional thickness maps of the

Table 1: Aetiology of corneal oedema

Early age onset
Congenital glaucoma
Dystrophy: congenital hereditary endothelial dystrophy, posterior polymorphous corneal dystrophy
Intraocular inflammation
Birth trauma
Late age onset
Acute angle-closure glaucoma
Dystrophy: Fuchs' dystrophy, posterior polymorphous corneal dystrophy
Hypotony
Hypoxia, such as prolonged use of contact lens
Intraocular inflammation
Iridocorneal endothelial syndrome
Infectious keratitis
Hydrops of keratoconus
Surgical trauma
Toxic, such as silicone oil, chemotherapy, amantadine, chlorhexidine
Metabolic disorders, such as myxedema, hypercholesterolemia

cornea over a larger, 10-mm diameter central zone. The improved signal-to-noise ratio when compared to conventional ultrasound biomicroscopy allows visualisation of internal corneal lamellar interfaces. Repeatability of corneal thickness measurements using VHF digital ultrasound has been reported to be 1.68 μm .^{8,9}

Specular microscopy is a non-contact method that assesses not only corneal endothelium morphology but also CCT. The CCT is derived based on the reflection of light waves from the corneal anterior and posterior surfaces. Computerised corneal topographers such as a Placido disk-based topographers, reflect images of multiple concentric circles that can be digitally captured and analysis performed by computer software. Although modern topography devices are able to map a large part of the anterior segment, a complete pachymetric evaluation is not possible without information on the posterior corneal surface.¹ Contrary to topography, corneal tomography evaluates the whole cornea by obtaining information from both the anterior and posterior corneal surfaces. Slit-scanning elevation topography like Orbscan IIz (Bausch & Lomb, Rochester, NY, USA) is a projective technique that measures the triangulation between the reference slit beam surface and the reflected beam captured by a camera. It combines a three-dimensional scanning slit beam system with an added advanced Placido disk attachment. Slits are projected sequentially on the cornea during image acquisition to create an overlapping pattern of scanning slits, and the final image is represented as a three-dimensional topographic map including a pachymetry map of the entire corneal surface.¹⁰ The Pentacam® (Oculus Optikgerate GmbH, Wetzlar, Germany) is an imaging system based on a capture method called Scheimpflug imagery, in which a single rotating Scheimpflug camera measures 25,000 elevation points and gives a three-dimensional scanning representation of the shape of the corneal surface including the measurement of corneal thickness.¹¹ There are another three Scheimpflug-based devices, such as Galilei (Ziemer, Port, Switzerland), are available.¹⁰ Besides corneal topography and tomography, anterior segment optical coherence tomography (ASOCT) is based on low-coherence interferometry and is also non-contact. The ASOCT generates two- or three-dimensional tomographic images by measuring the echo time delay of light backscattered from tissue structures.¹² The

speed and scanning resolution have improved over the past decades. In time-domain optical coherence tomography (OCT), the system acquires approximately 400 A-scans per second using six radial slices oriented 30 degrees apart, while in spectral-domain OCT, the scanning rate is approximately 20,000–40,000 scans per second, minimising unwanted motion artefact and enhancing the resolution.¹² The RTVue® (Optovue, Inc., Fremont, CA, USA), for example, provides a rapid imaging that overcomes eye movement velocity and ensures 5 μm resolution and high-magnification imaging of the cornea within 0.04 seconds.⁶ The high measurement velocity is achieved with a stationary reference mirror, and it collects signals from the entire range of interest and analyses data using the spectral interferogram and rapid Fourier transformation.¹³ Besides spectral-domain OCT, swept-source OCT is also categorised as Fourier-domain OCT. Its light source is rapidly swept within the laser, allowing for the use of a point detector and high acquisition rates up to several MHz.¹²

In vivo confocal microscopes can be used to measure corneal thickness with its function 'confocal microscopy through focusing on the tandem scanning confocal microscopes'. Different layers of the cornea reflect light at different intensities, and the intensity profile Z-curve can be analysed by specialised software to measure the distance between any two points on the curve.¹⁴

A vast number of studies have been conducted to investigate the reproducibility, repeatability and interchangeability of these modalities, and several considerations have to be taken into account before making comparisons. It has been reported that specular microscopy had lower CCT readings than those obtained by ultrasound pachymetry, in normal subjects and post-LASIK patients, with the range of difference of 14.4–31.6 μm .^{15–17} There is also a tendency of Scheimpflug tomographers to underestimate the measurement of CCT, compared to ultrasound pachymetry, in normal corneas and patients with keratoconus, with a mean difference of 5–9 μm .^{18–21} In general, optical readings tended to be slightly thinner than ultrasound pachymetry readings.¹⁷ Optical pachymeters, like specular microscopy or Scheimpflug scanning, require clear reflection of the anterior and posterior corneal surfaces to generate a reliable thickness measurement. Histopathologic features, such as the stromal haze or interface debris, the altered organisation of collagen fibres in keratoconus, or the irregularity of endothelium in patients with corneal oedema, affect the corneal optical properties and interrupt the pathway of light rays, while the velocity of ultrasound is less disturbed by media clarity.²² However, the contact technique of ultrasound pachymetry is prone to be confounded by the changes of corneal rigidity, such as corneal oedema or post refractive surgery. The speed of the ultrasound and the point of sound reflection may be changed in these scenarios, which in turn results in the inconsistency of the measurements.^{23,24} Moreover, in ultrasound pachymetry, the definite posterior corneal reflection point is not well understood:²⁵ it can be located in front of corneal Descemet's membrane, or in front of the anterior chamber, and this may also affect the measurements.

When comparing ASOCT with Pentacam or ultrasound pachymetry, Nam et al. showed that the RTVue had better reliability and accuracy.⁶ There are two theoretical reasons that can explain the high reliability of corneal thickness measurement with RTVue. First, RTVue measures the central zone average of the corneal thickness instead of single-point thickness, and the central zone average is more reliable than an individual value. Second, the fast scan speed of RTVue may offer the advantages to minimise the artefact due to eye movements.⁶ In ultrasound pachymetry measurements, the accuracy is examiner dependent, while in ASOCT

Table 2: Tools evaluating cornea oedema

Device	Principles
Measure corneal thickness	
Slit lamp biomicroscopy	<ul style="list-style-type: none"> • Uses an oblique and thin light beam on oblique viewing of the optical section of the corneas to estimate the corneal thickness
Ultrasound pachymetry	<ul style="list-style-type: none"> • Estimation of corneal thickness is based on the speed of sound in the normal cornea (1,640 m/sec) and the time difference between the ultrasound waves reflected from the anterior and posterior surface of the cornea
Specular microscopy	<ul style="list-style-type: none"> • Corneal thickness is derived based on the reflection of light waves from the anterior and posterior corneal surfaces
ASOCT	<ul style="list-style-type: none"> • Based on the emission and reflection of light (low-coherence interferometry), allowing exquisite delineation of the cornea
Corneal tomography	<ul style="list-style-type: none"> • Orbscan II: combines an advanced Placido disk system with slit-scanning technology and derives posterior surface map as well as whole cornea mathematically • Pentacam: uses a rotating Scheimpflug system camera with Placido disk technology to generate a three-dimensional representation of the shape of the cornea • Other devices: TMS-5 (Tomey Corp., Nagoya, Japan), Galilei (Ziemer, Port, Switzerland) and Sirius (CSO, Costruzione Strumenti, Oftalmici, Florence, Italy)
IVCM	<ul style="list-style-type: none"> • Different layers of the cornea reflect light at different intensities, and the intensity profile Z-curve is analysed by specialised software to measure the distance between any two points on the curve
Measure corneal water concentration	
Terahertz scans (under investigation)	<ul style="list-style-type: none"> • THz spectrum is very sensitive to water. The water content of the cornea is derived based on the reflective or absorptive THz signals)
Mid-infrared scans (under investigation)	<ul style="list-style-type: none"> • Mid-infrared radiation has very high sensitivity to the presence of the water molecule • Similar to THz scans, the water concentration is estimated based on the reflected signals from the cornea

ASOCT = anterior segment optical coherence tomography; IVCM = in vivo confocal microscopy.

or Pentacam evaluation, the accuracy depends mainly on the patient’s correct gaze and alignment.

In general, the CCT measurements assessed by these instruments had highly satisfactory reproducibility, repeatability and significant linear correlation, but the readings cannot be used interchangeably.¹⁶ Conversion equations should be introduced and considered for clinical comparison of the measurements.

Besides using CCT as a surrogate marker for the severity of corneal oedema, another approach to evaluate corneal oedema is to directly quantify the water content of the cornea, which is an area in which researchers and clinicians have been starting to explore. Table 2 lists the current tools and tools under investigation for the evaluation of corneal oedema.

Terahertz technology

Terahertz (THz) waves are electromagnetic radiation in the frequency range of 0.1–10.0 THz, and 1 THz represents 10¹² Hz. The most effective THz pulses are generated by a femtosecond laser, and the power used for imaging is low at μW range.²⁶ The wavelength that the THz time-domain spectroscopy generates is approximately 30 μm, which is short enough to provide reasonable spatial resolution but long enough to prevent serious loss of signal due to scattering. The penetration depth of 1,000 μm approximately.^{26,27} Recently, THz technology has emerged as a novel, non-invasive and non-contact tool in biomedical fields. As water has a very high dielectric constant and is highly absorptive to THz spectrum, THz waves are therefore extremely sensitive to water content in tissue, allowing its application to a variety of hydration-related diseases or conditions in different fields.²⁸ By sensing the changes of water content in the tissue, THz technology has been used to accurately predict the skin flap failure earlier than clinical diagnosis,²⁹ to early detect teeth that are intact but present with caries lesion,³⁰ to identify early the features of skin cancer in skin biopsy samples,³¹ and to help in the diagnosis of otitis media by early detection of the presence of pus.³²

The application of terahertz scans on corneas

A cornea consists of 78% water by volume, which is the highest of any connective tissue in the body.¹ The high water content of cornea, the homogeneity of corneal stromal tissue, and the relative lack of physiological variations compared to other structures in the body, allow THz imaging to be a promising method for sensing the corneal hydration level.³³ Moreover, the effect of scattering from corneal collagen or extracellular matrix is less in the THz band compared to that in higher-frequency bands like infrared.^{33,34} As the wavelength is non-ionizing, the scanning system has been considered biologically innocuous.

The safety of THz scans has been demonstrated in the *in vitro* and animal studies.^{35–36} *In vitro* experiments demonstrated no significant effects on cell cycle kinetics and no discernible chromosomal DNA damage after exposure to 84.8 mW/cm².^{36,37} Koyama et al. evaluated the *in vitro* cellular effects on human corneal epithelial cells following exposure to 0.12 THz radiation at 5 mW/cm² for 24 hours, and no significant genotoxicity or morphological changes were observed.³⁸ More recently, our group investigated the *in vivo* biological effects of continuous THz exposure with a newer version of scanning systems, in which the power density falls into the μW ranges, using a rabbit model (unpublished data). After continuous exposure of THz waves for 1, 4 and 8 hours (the actual image acquisition time for scanning is only within seconds), the corneas remained clear with no development of corneal haze or lens opacity. The corneal keratocyte activity, corneal endothelium count, and corneal thickness were evaluated using the *in vivo* confocal microscopy and RTvue scans, to examine whether there were negative impacts from the scanning on the corneas. After continuous exposure up to 8 hours, the quantification of stromal keratocyte reflectivity remained at the pre-scan level, indicating the THz waves did not induce activation of keratocytes. There were also no significant changes in the corneal endothelium count and corneal thickness. The corneal temperature, measured by the thermography, and the retinal rod and cone cell-mediated responses presented in the electroretinography, were not affected. Similar safety

profiles were also shown in the histological assessments on the corneas, lens and retinas. Even after continuous scans for 8 hours, no inflammatory cells and stromal fibrotic reaction were observed in the corneas, and no necrosis, gliosis, degeneration of photoreceptors or neurons, retinal detachment, or inflammation reaction were noted in the retinal tissue. A comprehensive immunohistochemistry analysis further confirmed that there was no cellular stress response, tissue fibrogenesis, cell apoptosis or inflammatory reaction induced.

The application of THz sensing to the field of ophthalmology was first introduced in 2010.³⁹ Due to a very high dielectric constant and high sensitivity to changes of water content in biological tissue, a pulsed, reflective THz imaging system (300 GHz and 3 THz) can be a potential tool to measure the hydration level of the cornea.³⁹ Bennett and his team reported that there was an approximately linear relationship between THz reflectivity and water concentration, using a porcine eye model.³⁸ The same group further conducted a feasibility study on rabbits and found a positive correlation between the THz reflection point signal and the corneal hydration, as well as the CCT measured by the pachymetry device.^{40,41} Of note, although the changes in the corneal water concentration in their experiment was only a few percent, the THz system was able to detect the difference within this interval with statistical significance.⁴⁰ Lomdina et al. further reported that an 1% decrease in the content of water mass in corneas led to a clearly detectable drop of the THz reflected signal by 13%, indicating good detection sensitivity.⁴² Similarly, our group correlated the THz absorptive signals of human cadaveric corneas with various extents of corneal oedema, with the CCT measured by the RTvue OCT, after adjustments for the absorption coefficient and travelling distance in the tissue. We found a strong correlation when the CCT was <800 μm ($r=-0.77$, $p=0.011$).⁴³

The idea of using THz technology to generate hydration maps for corneas was also proposed,⁴⁰ although some issues have to be overcome. The sensitivity of the mapping system and the reliability of measurements of corneal water contents were first tested in human normal subjects after strengthening the imaging resolution and imaging field.³⁴ Although the feasibility was well demonstrated, numerous practical challenges, relating to patient orientation and movement during the scanning, need to be addressed before more accurate quantitative analysis can be obtained.

One favourable characteristic of THz scanning technology is that it is relatively unaffected by ocular surface roughness, and the light waves can penetrate dry or hazy corneas, allowing the sensing of underlying tissue in a non-contact manner. Its high sensitivity to water content

allows it to be a potential novel device to early diagnose the water gradient changes before corneal thickness has a measurable change by traditional devices like OCT or pachymetry. It also has the potential to monitor subtle corneal hydration changes, such as the progression in Fuchs' dystrophy, the tear film dynamic changes, or the changes of stromal hydration level intraoperatively during refractive surgery such as LASIK. Despite improvements in the system design and encouraging results in the animal studies as well as a pilot clinical study, substantial engineering efforts are required to refine the scanning system to eventually bring the THz technology closer to the medical practice.

Besides THz, a similar concept of the use of mid-infrared laser was also proposed to monitor corneal hydration:²⁷ a dry cornea was expected to reflect a larger amount of laser signal than a hydrated cornea. A strong correlation between the reflected signal and the water content of the cornea was observed in a porcine eye model, and the system had good sensitivity to detect 0.91% changes in the water concentration (by weight %). The mid-infrared system operated in a 3- μm range, corresponding to the water molecules' approximately 3300 cm^{-1} phonon resonance. The high photon energy implies a very shallow penetration, approximately 10 μm .²⁷ Hence the clinical application would be mainly on the cornea surface, such as tear film dynamics.

Summary

Standard and existing evaluation tools for corneal oedema, such as ultrasound pachymetry, specular microscopy, ASOCT, Orbscan II or Pentacam, provide measurements of corneal thickness, a surrogate measure for the severity of corneal oedema. The principles of these devices are different; hence the measurements are not interchangeable, although generally a good correlation among instruments is present. The THz or mid-infrared imaging system has been emerging recently, and it provides the information on the direct water content in corneas. The preliminary data from *ex vivo* and *in vivo* corneas demonstrated a strong correlation between the reflective or absorptive signals and the CCT measured by ASOCT or pachymetry. It has the potential to detect subtle alteration in the hydration level in the cornea before the corneas start to have changes in thickness. However, research on its sensitivity and specificity is required. Generation of a hydration map, like a pachymetric map, for corneas, is also feasible, although many technical challenges have to be overcome. These light-based probing, long-wavelength sourced, and water-sensed based systems can be utilised as independent or adjuvant diagnostic and monitoring tools for the evaluation of corneal oedema. □

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