Chapter

Corneal Imaging Techniques for Dry Eye Disease

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Abstract

Dry eye disease (DED) is a common ocular disorder affecting millions worldwide. It is characterized by reduced tear production and/or increased tear evaporation, leading to ocular discomfort and impaired vision. Corneal imaging techniques are valuable tools for diagnosing and monitoring DED, as they can provide objective and quantitative information on the structure and function of the ocular surface and the tear film. This chapter will review the principles and applications of various corneal imaging techniques for DED, such as Slit-Lamp Biomicroscopy, Fluorescein CorneoGraphy, In Vivo Confocal Microscopy, Optical Coherence Tomography, Lipid Layer Interferometry, Topography, and Fluorophotometry. The advantages and limitations of each technique are discussed, as well as their potential role in future research and clinical practice, such as monitoring treatment efficacy and guiding personalized treatment approaches.

Keywords: corneal imaging, dry eye imaging biomarkers, dry eye disease, meibomian gland dysfunction, tear film evaluation

1. Introduction

DED is a widespread ocular ailment that impacts a significant number of individuals across the world. The condition is defined by a decrease in tear production, greater evaporation of tears, or changes in tear composition. Consequently, individuals may experience eye discomfort, vision abnormalities, and damage to the surface of their eyes. Corneal imaging methods are valuable resources for diagnosing and monitoring DED, as they can offer precise and quantifiable assessments of the structure and function of the cornea. Below we will elaborate on some of the most widely used corneal imaging techniques for DED in greater detail.

2. Slit-lamp biomicroscopy

Slit-lamp biomicroscopy (**Figure 1**) is a technique that allows the examination of the anterior and posterior segments of the eye using a high-intensity light source and a magnifying lens. It is a vital implement for diagnosing and managing various ocular conditions, such as DED, corneal injuries, corneal dystrophy, and cataracts.

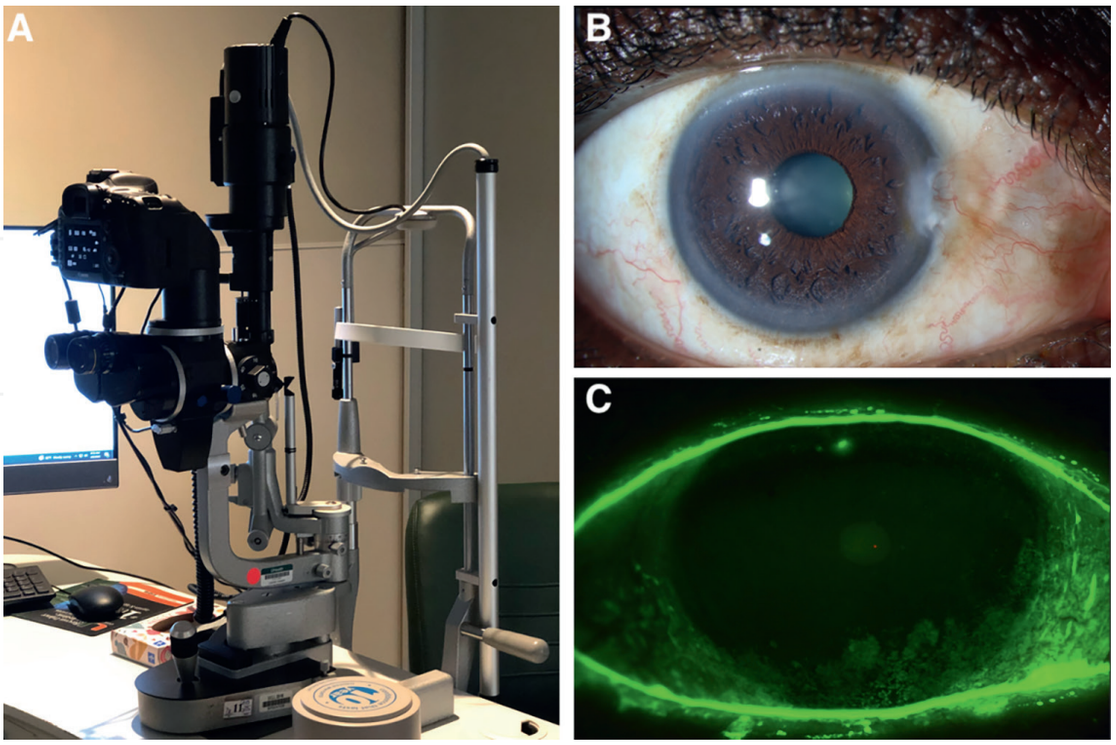

Figure 1.
(A) Slit-lamp biomicroscopy, a binocular microscope equipped with an adjustable light source, lenses for varying magnifications, and light filters. It allows examination of both the anterior and posterior segments of the eye, as well as performing diagnostic procedures. (B) Color photo of the anterior segment of the right eye in a patient with nasal pterygium, conjunctival melanosis, and gerontoxon. (C) Photo of the anterior segment of the eye with a cobalt-blue filter in a patient with fluorescein staining demonstrating superior and inferior corneal epithelial erosions.

2.1 Principles and applications

It consists of a binocular microscope and a light source that can be adjusted in width, height, angle, and intensity. The slit lamp can be combined with various accessories, such as lenses, filters, tonometers, and cameras, to perform additional diagnostic procedures [1]. This method can help to assess the severity of DED by measuring the tear film break-up time (TBUT), the tear meniscus height (TMH), and the presence of corneal and conjunctival staining as well as the underlying causes, such as meibomian gland dysfunction (MGD), blepharitis, or ocular surface inflammation [2].

Slit-lamp biomicroscopy is especially useful for studying contact lens wearers and DED patients who may have altered ocular surface microcirculation due to their conditions. It can reveal subtle changes in the blood flow and vessel density of the conjunctiva that may indicate the severity and progression of DED or the effects of contact lens wear [3].

2.2 Advantages and limitations

- It is noninvasive method.
- It can help in the early detection of DED.
- It provides high-resolution images of the ocular structures and microvasculature such as TMH.

- It allows the manipulation of illumination and magnification, facilitating the observation of different features of DED such as TBUT.

- It can help in monitoring disease progression.

While it is a valuable tool in the diagnosis of DED, it has some limitations that should be considered. Firstly, it is a subjective and qualitative method that relies heavily on the examiner's expertise and experience. Additionally, it may not provide a comprehensive understanding of the underlying causes, severity, or progression of DED. For instance, it cannot measure crucial indicators of DED such as tear osmolarity, tear production, tear evaporation rate, or inflammatory markers.

2.3 Potential role in future research and clinical practice

Researchers can use this technique to evaluate the accuracy and reliability of new diagnostic tools for DED, such as imaging technologies or tear film biomarkers. By comparing the results of these new tools with those obtained from traditional slit-lamp examinations, researchers can determine their usefulness in clinical practice. It can also be used to assess the effectiveness of treatments for DED, such as anti-inflammatory medications, artificial tears, and punctal occlusion. This technique allows them to measure changes in tear film quality, corneal staining, and other parameters, providing a reliable means of evaluating treatment efficacy for improving ocular surface health.

In clinical practice, it is used to assess the severity of corneal damage or DED by applying corneal grading scales. Some of the most commonly used corneal grading scales are the National Eye Institute/Industry Workshop (NEI) grading scale, the Oxford grading scale, and the Van Bijsterveld grading scale [4]. All three scales use a four-point grading system but differ in the number and location of regions evaluated, the type of dye used for the conjunctiva, and the illumination and magnification settings of the slit-lamp biomicroscopy [5].

This method can reveal signs of DED such as conjunctival hyperemia, corneal staining, tear film instability, and MGD [6]. Different filters can be used to enhance the visualization of these signs. For example, a cobalt-blue filter can be used with fluorescein dye to detect corneal epithelial defects or measure TBUT [7].

Overall, slit-lamp biomicroscopy is a valuable and versatile equipment in the diagnosis and management of DED, as well as other ocular conditions. Its ability to provide high magnification and illumination of the anterior and posterior segments of the eye allows for a detailed examination of its structures, function, and abnormalities. However, it should be used in conjunction with other diagnostic tools to accurately diagnose and manage DED. Furthermore, it has potential roles in future research to develop and evaluate new diagnostic tools and treatments for DED and investigate its underlying mechanisms.

3. In vivo confocal microscopy

In vivo confocal microscopy (IVCM) (**Figure 2**) is a noninvasive imaging technique that allows high-resolution and real-time visualization of cellular and subcellular structures of the cornea and other ocular tissues in living eyes. IVCM can provide information about the morphology, density, distribution, and function of various corneal structures, such as epithelial cells, immune cells, nerves, keratocytes, endothelial cells, and meibomian glands [8].

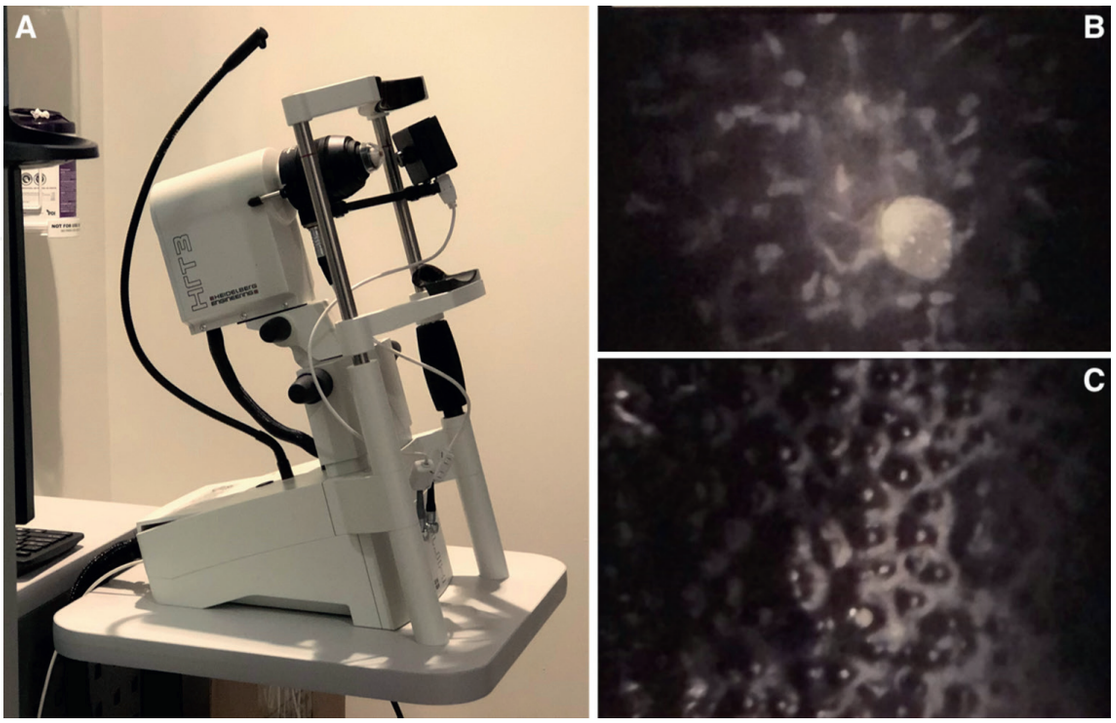

Figure 2.
(A) In vivo confocal microscopy is a noninvasive imaging technique that allows high-resolution, real-time visualization of cellular and subcellular ocular structures. (B) Corneal stromal opacities in a patient with fleck dystrophy. (C) Corneal endothelium with guttae in a patient with advanced Fuchs dystrophy.

3.1 Principles and applications

IVCM works by directing a laser beam onto a specific area, allowing for the detection of cellular structures through the reflection of light from a single focal plane [9]. By scanning different depths of tissue sequentially, it can generate high-resolution images that resemble histological sections. The microscope is able to capture these images of cells at different levels of depth, thus providing a noninvasive way to monitor changes in cells over time [8].

IVCM has various applications, such as studying corneal physiology and pathology, diagnosing infectious and inflammatory diseases, monitoring treatment efficacy, and guiding personalized treatment approaches [8, 10]. It can measure the density, morphology, branching pattern, tortuosity, reflectivity, length, and diameter of subbasal nerve plexus (SNP), which are altered in DED due to neurosensory abnormalities. SNP parameters can also correlate with clinical signs and symptoms of DED [11]. It can also quantify the density, size, shape, and activation state of dendritic cells (DCs), which are increased in DED due to inflammation. DCs parameters can also correlate with clinical signs and symptoms of DED [12]. IVCM can assess the morphology, density, and function of meibomian glands, which are impaired in evaporative dry eye (EDE) due to MGD. Meibomian gland parameters can also correlate with clinical signs and symptoms of EDE [13]. IVCM can diagnose DED more accurately by identifying specific features such as epithelial irregularity, microcysts, dendritic cells, nerve fiber loss, keratocyte activation, and meibomian gland dropout [8, 14]. Moreover, it can help monitor the progression of DED over time and evaluate the response to different treatments [15]. For example, it can show the effects of artificial tears on epithelial integrity, the effects of cyclosporine on inflammatory cell

infiltration, or the effects of thermal pulsation therapy on meibomian gland function [16–18]. It can also assist in the diagnosis and management of corneal and conjunctival diseases, as well as the monitoring of postsurgical outcomes [19–21].

3.2 Advantages and limitations

It has several advantages over conventional histology because of its noninvasive nature, such as providing real-time images without tissue processing or staining, eliminating the need for tissue biopsy, reducing the risk of infection and scarring. It can detect subtle changes in the ocular surface that may not be visible with slit-lamp examination or staining techniques. For example, it can reveal epithelial cell loss, microcysts, basal cell density reduction, keratocyte activation, nerve fiber alterations, inflammatory cell infiltration, vascularization, and fibrosis in DED patients [8, 22]. IVCM can quantify these changes using objective parameters such as cell density, cell size, cell shape index, nerve fiber length, nerve fiber density, nerve branch density, nerve tortuosity, meibomian gland acinar unit density, meibomian gland acinar unit diameter, meibomian gland fibrosis grade, etc., which can help monitor disease progression and treatment response [20, 23]. It can also differentiate between different types and subtypes of DED based on their distinct ocular surface features. For example, it can help distinguish between the aqueous-deficient dry eye (ADDE) and EDE, as well as between obstructive MGD and nonobstructive MGD [24].

IVCM also allows for repeated imaging with quantitative analysis of morphological parameters making it an excellent tool for monitoring treatment efficacy and disease progression. Additionally, it can be used to assess eye conditions at the cellular level, providing detailed information on disease mechanisms and aiding in developing personalized treatment approaches.

However, it has some limitations, such as limited penetration depth, variability in image quality and interpretation, lack of standardized protocols and criteria for diagnosis, and potential risks of corneal damage or infection from contact probes [8]. It has a small field of view (~400×400 micrometers), which may not represent the whole ocular surface area or capture regional variations. Therefore, multiple images from different locations are needed to obtain a comprehensive assessment [25]. It also lacks population-based norms or standardized criteria for normal or abnormal findings.

Therefore, the interpretation and quantification of IVCM images may vary depending on the operator's experience or software used. Moreover, the correlation between IVCM parameters and clinical symptoms or signs is not always consistent or linear. It is still an expensive and relatively rare device that is not widely available or accessible, limiting its use in clinical practice [26].

3.3 Potential role in future research and clinical practice

The diagnosis and management of DED can be challenging, as no single test can reliably assess the severity and etiology of the condition [27]. Furthermore, there may be a discrepancy between clinical signs and symptoms of DED, as some patients may have significant ocular surface damage without experiencing discomfort, while others may report severe discomfort with minimal signs [28].

The following findings suggest that IVCM is an important tool that has a potential role in future research and clinical practice of DED, as it can provide valuable insights into the pathophysiology, diagnosis, classification, and treatment response of its different subtypes:

- It has been shown that patients with ADDE have reduced density and increased tortuosity of corneal subbasal nerves compared to healthy controls, while patients with EDE have increased density and decreased tortuosity of these nerves compared to ADDE patients [24].

- It has also shown that patients with MGD have altered morphology and function of meibomian glands compared to healthy controls, such as decreased acinar density, increased acinar size variability, increased ductal dilation, etc. These changes correlate with clinical signs such as meibum quality, meibum expressibility, etc. [29].

- It has demonstrated that various treatments for DED can improve ocular surface parameters such as corneal epithelial cell density, corneal nerve density and morphology, meibomian gland morphology and function, etc. [30].

- It can be used to evaluate the corneal and conjunctival epithelium, monitor graft survival after transplantation, and evaluate the effectiveness of topical medications [31].

In summary, IVCM is a noninvasive imaging technique that allows for the visualization of living cells and tissues in situ. Although it has limitations, it can provide detailed cellular information that boosts our understanding of ocular surface diseases and improves eye care management for further research and clinical practice [32].

4. Optical coherence tomography

Optical coherence tomography (OCT) (**Figure 3**) is a noninvasive imaging technique that uses low-coherence light to capture micrometer-resolution, two- and

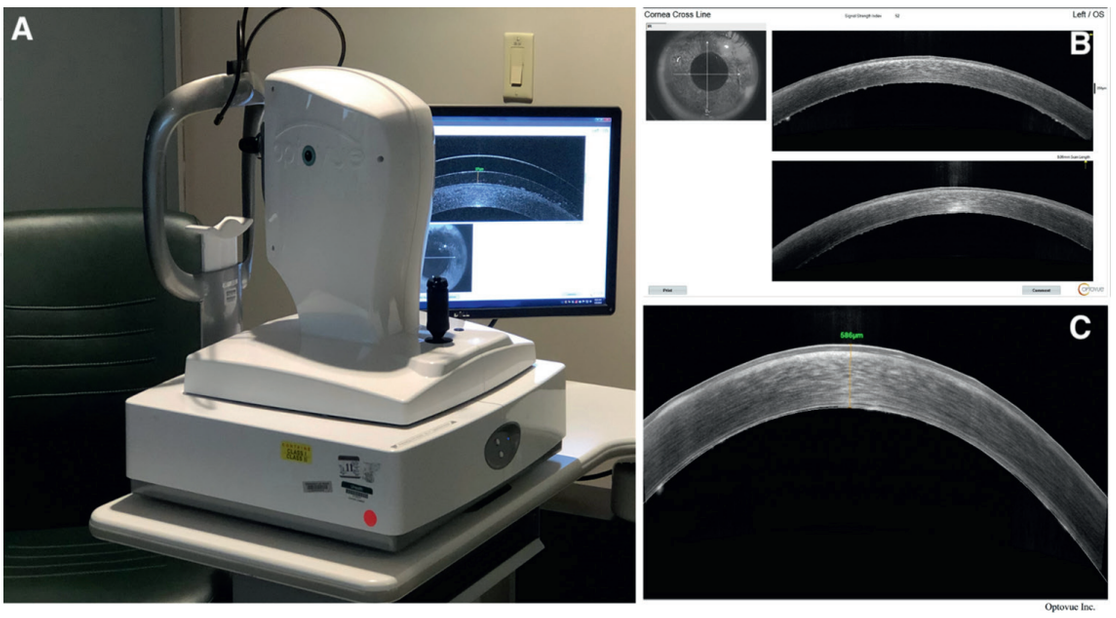

Figure 3.
(A) Optical coherence tomography is a noninvasive imaging technique that uses low-coherence light to capture micron-resolution two- and three-dimensional images creating high-resolution images of biological tissues. (B) Anterior segment optical coherence tomography in a healthy patient. (C) Pachymetry module (corneal thickness).

three-dimensional images creating high-resolution images of biological tissues. It is effectively 'optical ultrasound', imaging reflections from within tissue to provide cross-sectional images. OCT has become vital equipment in medical diagnosis and research due to its ability to provide detailed information about tissue microstructure and function. It is widely used for medical imaging and industrial nondestructive testing (NDT).

4.1 Principles and applications

OCT is based on the principle of low-coherence interferometry, which measures the interference pattern of two coherent light beams. The wavelength used is around 1300 nm to minimize energy absorption in the light beam caused by protein, water, hemoglobin, and lipids. The physics principle that allows the filtering of scattered light is optical coherence [33, 34].

It exploits the fact that coherent light can be split into two beams by a beam splitter: one beam (the reference beam) travels along a known path length and reflects off a mirror, while the other beam (the sample beam) travels through the tissue of interest and reflects off various structures within it [35]. The two beams are then recombined by another beam splitter and detected by a photodetector [36]. The interference pattern produced by the recombined beams depends on the difference in optical path length between them [37]. By varying the position of the reference mirror, different depths within the tissue can be scanned and imaged [38].

There are different types of OCT systems based on how they vary the reference path length and how they detect the interference signal. The most common types are time-domain OCT (TD-OCT), frequency-domain OCT (FD-OCT), and swept-source OCT (SS-OCT) [38, 39].

- TD-OCT uses a moving reference mirror to scan different depths within the tissue sequentially. The interference signal is detected as a function of time by a single photodetector [40].

- FD-OCT uses a fixed reference mirror and measures the interference signal as a function of wavelength by using an array of photodetectors or a spectrometer. This allows simultaneous acquisition of all depths within the tissue without mechanical scanning [41].

- SS-OCT uses a tunable laser source that sweeps across different wavelengths rapidly. The interference signal is detected as a function of time by a single photodetector synchronized with the laser sweep [42].

FD-OCT and SS-OCT offer higher speed, sensitivity, and resolution than TD-OCT because they avoid mechanical scanning and use more efficient detection methods [43].

OCT has many applications in biomedical imaging due to its ability to provide high-resolution cross-sectional images of tissue microstructure without requiring invasive procedures or contrast agents [38]. It can provide valuable information about the corneal epithelial thickness (CET), which reflects the health and integrity of the ocular surface [44]. In addition, OCT can image different layers of the cornea, anterior and posterior chamber, etc., providing valuable information for diagnosing and monitoring diseases such as keratoconus, corneal disorders, glaucoma, diabetic retinopathy, age-related macular degeneration, etc. [45].

4.2 Advantages and limitations

One of the primary advantages is its noninvasive nature, which allows for repeat imaging of tissues. Additionally, OCT can provide real-time imaging, which can aid in surgical procedures, reducing the chances of error because it is a computerized procedure [46]. It has several advantages over other imaging modalities; some of these advantages are:

- High resolution: it can achieve submicrometer axial resolution and micrometer lateral resolution in tissue, which allows visualization of cellular-level features that are not accessible by other techniques [47].

- High speed: it can acquire images at rates up to hundreds of frames per second or even megahertz range with FD-OCT methods, which enables real-time feedback during interventions or dynamic studies [38].

- Noninvasiveness: it does not require ionizing radiation or contrast agents that may pose health risks or cause allergic reactions. It also does not require physical contact with the sample except for intravascular applications [48].

- Versatility: it can be integrated with various endoscopic devices or surgical instruments to enable minimally invasive imaging in different anatomical locations, for example, some devices can combine OCT with other modalities like confocal scanning laser ophthalmoscopy (cSLO) or indocyanine green angiography (ICG) [49].

However, it also has some limitations; for instance, it may not capture subtle changes in CET that occur in the mild or early stages of DED. Moreover, OCT measurements of CET may vary depending on the device type, scanning protocol, calibration method, and image processing algorithm [50].

Some other limitations are:

- It is expensive and not always covered by insurance.

- It requires patient cooperation and operator skill.

- It may produce image artifacts and distortions due to eye movements, blinking, or scanning errors.

- It is sensitive to tissue optical properties, and tissues with different optical properties may not be imaged with the same resolution.

- It has a limited penetration depth due to multiple scattering effects within tissue, and OCT typically has a penetration depth ranging from 1 to 3 mm depending on tissue type, which restricts its ability to image deeper tissues [45, 51, 52].

4.3 Potential role in future research and clinical practice

Recently, OCT has also shown potential for diagnosing and managing DED [53]. It can measure parameters such as TMH, CET, and meibomian gland morphology,

which are related to DED severity and symptoms. By integrating OCT-derived data into a new scoring method, researchers have proposed a more objective and accurate way of assessing DED [54]. Therefore, it may play an important role in future DED research and clinical practice by providing novel insights into its pathophysiology, diagnosis, and treatment [55].

One area where OCT is being used is in monitoring treatment efficacy [56]. For example, it can be used to monitor changes in tissue microstructure and function following treatment for diseases such as cancer, and it can also be used to measure retinal nerve fiber layer thickness, macular thickness, choroidal thickness, optic nerve head parameters, etc., which can reflect the progression or regression of various eye diseases [57, 58]. Additionally, it can be used to guide personalized treatment approaches by providing information about tissue structure and function [59].

Another area where it has potential is developing new imaging agents. Researchers are exploring using OCT in combination with contrast agents to improve image contrast and sensitivity, which could allow for earlier detection of diseases [60].

In conclusion, OCT is a very resourceful method with various clinical and research applications. While it has some limitations, its noninvasive nature and high-resolution images make it an attractive option for diagnosing and monitoring eye diseases such as DED.

5. Lipid layer interferometry

Lipid layer interferometry (LLI) (**Figure 4**) is a technique that uses light interference patterns to measure the thickness, stability, and quality of the lipid layer in vivo. LLI is a promising tool for diagnosing and managing DED caused by MGD. It can provide objective and quantitative information about the lipid layer quality and guide treatment decisions based on individual patient needs.

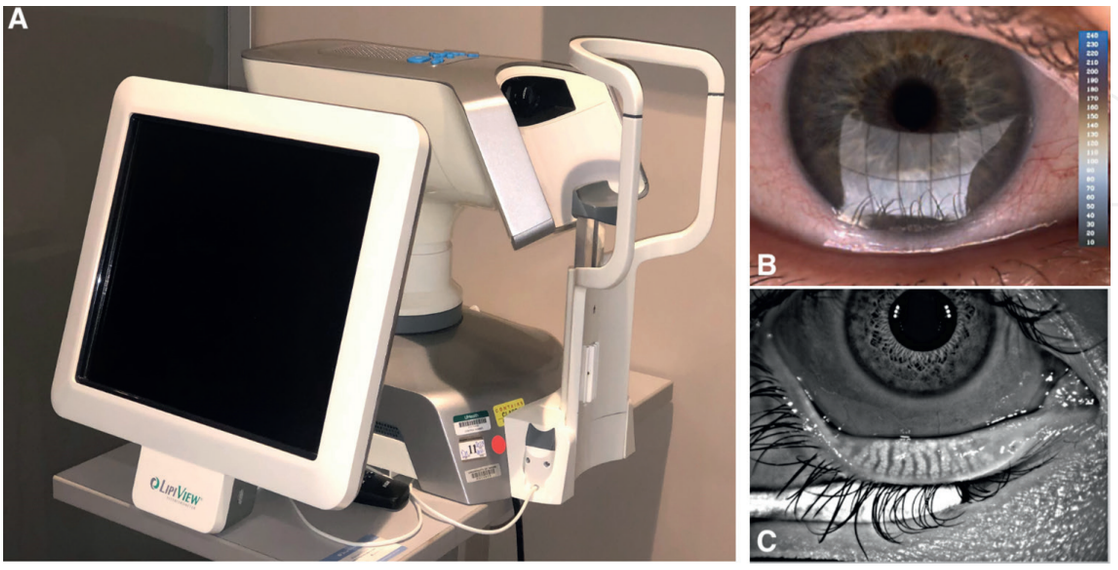

Figure 4.
(A) Lipid layer interferometry is a technique that uses light interference patterns to measure the thickness, stability, and quality of the lipid layer in vivo. (B) Tear film image. (C) Meibomian gland imaging in a patient with dry eye syndrome.

5.1 Principles and applications

LLI works by shining a beam of polarized light onto the tear film and capturing the reflected light with a camera—the reflected light forms an interference pattern that depends on the thickness and uniformity of the lipid layer. The interference pattern can be analyzed using software algorithms to calculate the lipid layer thickness (LLT) in nanometers (nm) or interferometric color units (ICU), which are close but not exactly equivalent to nm, and classify their appearance into different grades [61]. A normal LLT ranges from 80 to 120 nm (or ICU), while a thin LLT is below 60 nm (or ICU). A thick LLT above 100 nm (or ICU) may indicate excessive meibum production or altered tear film dynamics [42]. Different thicknesses produce different colors according to a specific scale. For example, white indicates a very thin LLT (<20 nm), while yellow indicates a thick LLT (>100 nm) [62].

LLI can provide quantitative and qualitative information about the lipid layer in DED patients, which can help diagnose and monitor their condition [61]. The lipid layer prevents excessive evaporation of tears and provides lubrication for blinking [63].

One of the devices that use LLI is LipiView II (Johnson & Johnson Vision), which measures the LLT between blinks and gives a numerical value in ICU units close to nanometers [64]. LipiView II also captures images of the lipid layer spread (LLS), which reflects how well the lipid layer covers and smooths out after each blink. A normal LLS is considered to be uniform and continuous [64, 65]. Several studies have shown that LLI can be useful for assessing DED severity and treatment response. For example, a study by Yunji Lee Et al. found that DED patients had significantly lower LLT and LLS than healthy controls and that LLT correlated with TBUT and ocular surface staining scores. They also found that DED patients with thick LLT (>100 ICU) had different characteristics than those with thin LLT (<100 ICU), such as higher TBUT, lower osmolarity, and lower inflammatory markers [66].

5.2 Advantages and limitations

LLI provides a noninvasive, real-time, and quantitative measurement of the thickness and quality of the lipid layer, enabling the study of various interventions effects on it and ocular surface health [67, 68]. LLI has also been shown to have good reproducibility and sensitivity in detecting changes in the lipid layer [68]. Additionally, LLI has been used to evaluate DED and MGD [69–72].

In summary:

- Noninvasive and real-time method for quantitatively measuring the thickness and quality of the lipid layer in the tear film.
- Good reproducibility and sensitivity in detecting changes in the lipid layer.
- Enables the study of various interventions' effects on the lipid layer and ocular surface health.
- Useful for evaluating DED, contact lens wear, and meibomian gland dysfunction.

- Noninvasive and does not require contact with the eye or the instillation of any dye or drops.

- Provides quantitative and objective measurements of LLT that can be compared over time or between groups.

- Can detect subtle changes in LLT that may not be visible with other techniques.

- Reveals spatial variations in LLT across different regions of the eye.

However, it also has some limitations and challenges:

- The technique may have a limited ability to discriminate between thin lipid layers, as measurements may be affected by the instrument's sensitivity and resolution [71]. Secondly, LLI may not provide a complete picture of the lipid layer structure, as it only provides measurements of the LLT and quality, not its composition or structure [73].

- Some researchers have suggested that LLI measurements may be influenced by environmental factors such as temperature and humidity, which may affect the behavior of the lipid layer [74, 75].

- Finally, LLI measurements may be affected by the ocular surface curvature, refractive errors, blinking artifacts, or variations in the tear film during the measurement process [71].

5.3 Potential role in future research and clinical practice

One potential application is the evaluation of therapeutic interventions, such as topical lipid-based treatments, in improving the LLT and quality. Studies have shown that LLI can detect subtle changes in the lipid layer that may not be visible with other techniques, making it a useful tool in the assessment of DED [76]. In addition, the use of LLI may help in the identification of patients who are more likely to benefit from certain treatments, as well as to monitor the response to therapy [77].

Another potential application is in the assessment of MGD. Studies have shown a correlation between MGD severity and the quality of the lipid layer [78]. LLI may therefore be useful in the diagnosis and monitoring of MGD and in evaluating the effectiveness of treatments targeted at improving meibomian gland function [79]. LLI has been used to evaluate the effects of contact lenses on the LLT and quality [80]. In addition, it may be useful in the assessment of contact lens materials and designs and in developing new contact lens solutions aimed at improving the lipid layer and reducing symptoms of dryness and discomfort [81].

In conclusion, LLI is a promising tool for diagnosing and managing DED caused by MGD. It provides a noninvasive, real-time, and quantitative measurement of the thickness and quality of the lipid layer. LLI has been shown to have good reproducibility and sensitivity in detecting changes in the lipid layer, making it a valuable tool for understanding its role in the tear film and its impact on ocular surface health. However, LLI has some limitations and challenges and should be used in conjunction with other diagnostic methods to obtain a comprehensive evaluation of DED etiology and severity.

6. Topography

Topography (**Figure 5**) is a corneal imaging technique that provides detailed 3D maps of the cornea's shape and curvature. It detects corneal diseases and irregular corneal conditions, such as swelling, scarring, abrasions, deformities, and irregular astigmatisms [82]. It can also monitor the progression of DED and co-existing eye diseases, such as pterygium. It is an essential tool for refractive surgery candidates with DED, as it can measure CET and assess suitability for surgery [83].

6.1 Principles and applications

Corneal topography uses three principles: Placido disc reflection, Scanning slit technology, or Scheimpflug photography. It works by projecting a luminous object onto the cornea and analyzing its reflection.

There are different methods of corneal topography, such as the Placido method, slit scanning technique, and Schiempflug method [84–86]. The Placido method uses concentric rings of light to create a pattern on the cornea that can be captured by a camera [87]. The slit scanning technique uses a rotating slit beam to scan the cornea at different depths [86]. The Schiempflug method uses a rotating camera and light source that are tilted at an angle to capture images of both the anterior and posterior surfaces of the cornea [86]. Corneal topography can provide valuable information about the quality of vision and guide surgical planning for procedures such as laser refractive surgery, cataract surgery, and corneal transplantation [85]. This method can provide useful information for diagnosing and monitoring corneal diseases, planning refractive surgery, fitting contact lenses, evaluating postoperative outcomes, etc. It can be used to characterize the shape of the cornea, specifically, the anterior

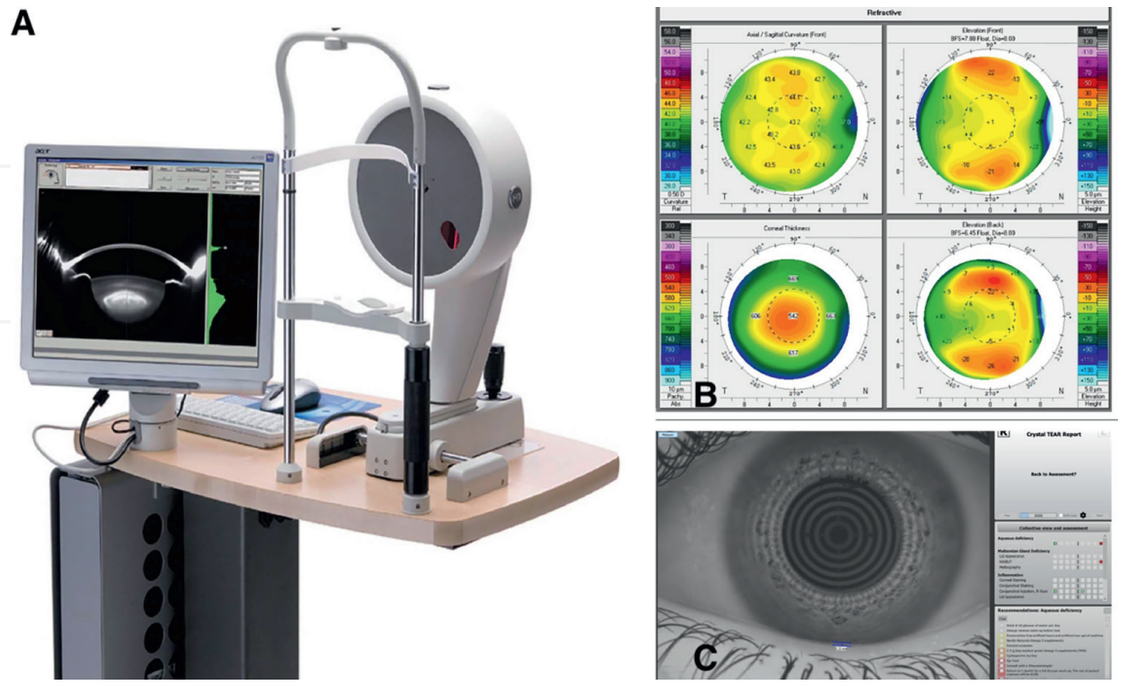

Figure 5.
(A) Corneal topography is an imaging technique of the cornea that provides detailed 3D maps of the shape and curvature of the cornea. (B) Corneal topography in a patient with mild corneal astigmatism. (C) Placido ring illumination and measurement of the tear meniscus.

surface of the cornea. Most corneal topographical systems are based on Placido discs that analyze rings that are reflected off the corneal surface [88]. The posterior corneal surface cannot be characterized using Placido disc technology [88].

Corneal topography is a technique that is also used to measure the thickness of the cornea [89]. It has been used to assess the effect of dry eye treatment on corneal thickness. Precise corneal topography measurements are essential for co-existing eye diseases in DED (DED) patients [90]. The tear film instability and hyper-osmolarity, ocular surface inflammation and damage, and neuro-sensory abnormalities play etiological roles [90]. Studies have been conducted to assess the repeatability of corneal topography measurements in dry eye patients and healthy controls [90, 91].

6.2 Advantages and limitations

- It can detect subtle changes in the corneal surface that may indicate DED, such as swelling, scarring, abrasions, or deformities. These changes can affect vision quality and increase the risk of infection or inflammation [88].

- It can help monitor the progression and severity of DED by measuring parameters such as corneal thickness, curvature, astigmatism, and irregularity. These parameters can reflect the degree of damage to the cornea caused by DED and guide treatment decisions [88, 92, 93].

- It can help evaluate the effectiveness of treatments for DED by showing improvements or worsening in corneal parameters after interventions such as artificial tears, punctal plugs, cyclosporine drops, or surgery.

However, it has some limitations when it comes to assessing DED. Here are some of them:

- It is unable to directly evaluate the quality or quantity of the tear film. Its findings only indicate the effect of DED on the cornea, which means it may not detect mild or early stages of DED that have not yet resulted in significant corneal changes [94].

- It may be influenced by factors other than DED, such as contact lens wear, ocular surgery, allergies, or infections. These factors may cause false-positive or false-negative results when evaluating DED with corneal topography [95].

- It may not be consistent or repeatable in severe cases of DED.

- It may not be covered by insurance for DED diagnosis.

6.3 Potential role in future research and clinical practice

- Developing new algorithms and models to improve the accuracy and reliability of corneal measurements [96, 97].

- Exploring novel parameters and indices to better characterize corneal shape and biomechanics.

- Investigating correlations between corneal topography and other ocular or systemic factors, such as intraocular pressure, tear film quality, diabetes mellitus, etc.

- Evaluating outcomes and complications of emerging surgical techniques or devices that modify corneal shape or function.

- Comparing different modalities or brands of corneal topography devices to establish standards and norms.

- A study found that corneal topography measurements could be used to predict corneal strength and resistance to deformation [98]. These findings suggest that corneal topography may have a valuable role in evaluating ocular biomechanics, which could be useful in diagnosing and monitoring various eye diseases.

It can be useful for several purposes in the clinical practice of DED:

- Screening: It can help identify patients who may have DED or are at risk of developing it by showing signs of irregularity or distortion in the corneal surface. For example, patients with MGD may show increased central corneal steepening due to tear film instability. Patients with Sjögren syndrome (SS) may show decreased central corneal thickness due to chronic inflammation [90].

- Diagnosis: It can help confirm or rule out DED by showing objective evidence of ocular surface damage or alteration due to tear film dysfunction. For example, patients with DED may show reduced TBUT. It can also measure TBUT by capturing images of the cornea at different intervals after blinking and calculating how long it takes for irregularities to appear [95]. Patients with DED may also show increased staining of the cornea with fluorescein dye due to epithelial defects or erosions. It can quantify staining by analyzing the intensity and distribution of fluorescence on the cornea.

- Monitoring: It can help track the progression or improvement of DED by showing changes in the corneal surface over time.

- It can measure CII by comparing different regions of the cornea and calculating how much they deviate from an ideal spherical shape [88, 99]. Patients with DED may also show worsening or improvement in their ocular surface disease index (OSDI). It may correlate OSDI scores with objective parameters such as TBUT, staining, and CII [100].

In conclusion, corneal topography is a valuable diagnostic and treatment tool for various ocular conditions such as DED. It also provides opportunities for advancing knowledge and innovation in ophthalmology. However, alone it is insufficient for diagnosing or treating DED. It must be used in conjunction with other tests and methods, including patient history, symptom assessment, tear film evaluation (e.g., Schirmer test), ocular surface staining (e.g., fluorescein), meibomian gland function (e.g., meibography), and inflammatory markers (e.g., MMP-9).

7. Fluorophotometry

Fluorophotometry is a technique that uses a device called a fluorophotometer to measure the fluorescence intensity of a dye called fluorescein after it is applied to the eye. Fluorescein is a yellow-green dye that binds to damaged cells on the corneal surface and emits light when exposed to blue light. It can provide information about various aspects of DED, such as corneal epithelial integrity and permeability, by measuring how much fluorescein penetrates into the cornea and how fast it is eliminated from the eye. It can also yield data concerning tear film stability, tear turnover rate and aqueous humor flow rate [101].

7.1 Principles and applications

It is based on the principle that some molecules (fluorophores) can absorb light at a certain wavelength (excitation) and emit light at a longer wavelength (emission). The intensity of the emitted light depends on several factors, such as the concentration of the fluorophore, the optical properties of the tissue, and the presence of quenchers or enhancers.

Fluorescein is a commonly used fluorophore for ocular fluorometry because it has a high quantum yield (the ratio of photons emitted to photons absorbed), low toxicity, and good solubility in water. Fluorescein has an excitation peak at 490 nm (blue light) and an emission peak at 520 nm (green light) [102].

To perform this technique, a known amount of fluorescein solution is instilled into the eye using a calibrated micropipette or strip. After allowing some time for diffusion and clearance of excess dye from the ocular surface, a fluorometer device is used to scan different regions of interest within the eye [103]. The fluorometer consists of an excitation source (usually a xenon arc lamp), an optical system (including filters or monochromators), a photodetector (usually a photomultiplier tube), and an electronic system for data acquisition and analysis [104]. The fluorometer measures the fluorescence intensity at each point along a scan line across the eye. The fluorescence intensity can be converted into concentration units using calibration curves obtained from standard solutions [105]. The concentration profiles can then be plotted as functions of location within the eye or time after dye instillation.

This method can be used to evaluate several parameters related to DED:

- Corneal epithelial permeability: It can measure this parameter by calculating the rate or extent of fluorescein penetration across the cornea [106]. This can reflect the degree of epithelial injury or dysfunction in DED patients.

- Tear film stability: It can measure this parameter by calculating the decay rate or half-life of fluorescein concentration on the corneal surface [103]. This can reflect the quality and stability of the tear film in DED patients.

- Tear turnover rate: This can reflect tear production and drainage imbalances, which may contribute to ocular surface irritation and inflammation in DED patients [106, 107].

7.2 Advantages and limitations

- It is objective and quantitative: it provides numerical values that can be compared across different individuals or time points [103].

- It is noninvasive: it does not require any contact with the eye surface or any tissue sampling [103].
- It is sensitive: it can detect subtle changes in the corneal epithelium that may not be visible with other methods such as slit-lamp examination or staining [103].
- It is specific: it measures only the permeability of the corneal epithelium, which is directly related to its barrier function and not influenced by other factors such as tear volume, osmolarity, or viscosity [103].
- It is fast and easy: the test can be performed in a few minutes with minimal training required for the operator [103].
- It is safe: the dye used in the test is typically administered in low doses and has a low risk of adverse reactions [103].
- It is reliable: the test has high repeatability, meaning that results can be reproduced consistently over time and across different operators [101, 103].
- It is dynamic: it can be used to monitor changes in corneal permeability over time, providing valuable information about disease progression or response to treatment [103].
- It is predictive: the test can indicate risk factors or prognosis for DED, allowing for early intervention and better management of the disease [103].
- It is responsive: changes in corneal permeability measured by fluorophotometry can reflect the effects of treatment, allowing for more precise and personalized treatment plans [108].

However, it also has some limitations:

- It requires specialized equipment: It is an expensive device that may not be widely available in clinical settings.
- It requires careful calibration: Before each measurement, the device needs to be adjusted according to ambient light conditions, background fluorescence, and patient characteristics such as pupil size, iris color, and refractive error [109].
- It requires standardized protocol: There are many variables that can affect this method such as dye concentration, volume, and instillation method; time interval between instillation and measurement; and number and duration of measurements. These variables need to be controlled and reported for accurate interpretation and comparison of data [110].
- It may cause discomfort: some patients may experience a burning sensation, tearing, or blurred vision after fluorescein instillation; these effects usually subside within minutes but may interfere with patient compliance or cooperation.

7.3 Potential role in future research and clinical practice

It can be used to evaluate the safety of contact lens wear. By measuring the corneal epithelial permeability and tear film thickness, it can detect changes in the ocular surface associated with contact lens wear, which can inform the development of safer and more effective contact lenses [111]. It can also be used to evaluate the efficacy of new drug candidates for DED by measuring the drug penetration through the cornea and the ocular surface, and it can provide insights into the drug's pharmacokinetics and bioavailability, which are crucial factors in determining the efficacy of topical ocular treatments.

Below are a few examples of how clinicians utilize fluorophotometry in the management of DED:

- Management of DED patients: It may be useful clinically because an increased corneal uptake of fluorescein reveals subtle damage to the corneal epithelium [98]. Its measurements of the penetration of fluorescein across the corneal epithelium could be of value in diagnosing or monitoring DED [103]. It has been investigated whether fluorophotometry correlated with previously established DED diagnostic tests and whether it could serve as a novel objective metric to evaluate DED [108].

- Evaluation of treatment effectiveness: It can be used to monitor the effectiveness of treatments aimed at improving tear film stability and corneal integrity, such as artificial tears, punctal occlusion, and topical medications. Measuring the rate of tear turnover and corneal permeability before and after treatment, it can help determine if the treatment is working as intended [103].

- Assessment of contact lens safety: It can be used to assess the safety of contact lens wear by measuring the corneal epithelial permeability and tear film thickness. These measurements can help identify changes in the ocular surface associated with contact lens wear, which can inform decisions about contact lens selection and management [111].

- Research: It is also used in research settings to investigate the underlying causes of DED and to evaluate new treatments. The quantitative measurements provided by fluorophotometry are useful for assessing treatment efficacy and for monitoring changes in tear film stability and corneal integrity over time [103].

In conclusion, fluorophotometry is a promising noninvasive technique that can provide objective and quantitative information about various aspects of DED, including corneal epithelial integrity and permeability, tear film stability, tear turnover rate, aqueous humor flow rate, and blood-retinal barrier integrity. It can be used to monitor changes in corneal permeability over time, evaluate risk factors or prognosis for DED, develop personalized treatment plans, investigate the pathophysiology of DED, and develop new treatments. However, careful calibration and specialized equipment are required before use.

8. Fluorescein corneography

Fluorescein corneography (FCG) (**Figure 6**) is a cutting-edge technique that has been developed to detect punctate epithelial erosions (PEE) with great precision.

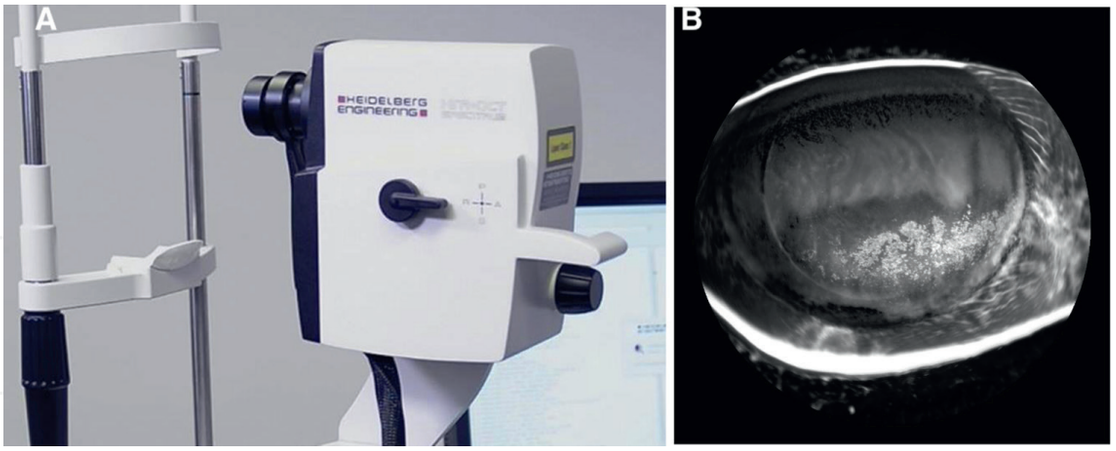

Figure 6.
(A) Fluorescein CorneoGraphy captures corneal epithelium fluorescein staining patterns. (B) Distribution of fluorescein stain in the tear film.

The technique utilizes a repurposed imaging system, the Heidelberg Spectralis II OCT with fluorescein angiography function, to capture the corneal epithelium staining patterns. The OCT-fluorescein angiography (FA) system is highly effective in providing robust detection of PEE in patients with DED [112].

8.1 Principles and applications

The principle behind FCG is to utilize the advanced capabilities of the Spectralis optical coherence tomography (OCT) system with fluorescein angiography (FA) function to visualize corneal epithelium staining patterns. The Spectralis imaging system has advantages over typical imaging with a cobalt-blue filter, as it uses a 490 nm excitation laser that enables superior excitation of fluorescein and more detailed imaging of the corneal surface. Additionally, the device uses an appropriate barrier filter to enable specific imaging of fluorescein emission around 525 nm [112].

To perform FCG, the inferior lid of the imaged eye is gently pulled down, and 2 microliters of 0.25% fluorescein sodium are instilled on the patient's lower tarsal conjunctiva using a micropipette. One minute after fluorescein instillation, an ocular wash using sterile PBS is performed using the same micropipette. This is done by asking the patient to incline their head to the contralateral side of the eye of interest, and 300 μL of sterile PBS is instilled through a micropipette, aiming at the ocular surface from the lateral sides in order to reduce fluorescein pooling and debris/tear film on the surface [112].

To obtain a complete corneal area visualization, the image frame is focused on a midpoint between the lacrimal caruncle and the corneal limbus while maintaining a thorough view of the corneal epithelium from limbus to limbus. The lens is focused on the corneal epithelium in such a way that both the central and peripheral epithelium are clearly visualized in their totality. The sensitivity knob is kept constant during imaging, with minute compensation for maximal clarity [112].

Some applications of FCG include:

- Diagnosis of PEE: It is a highly sensitive and accurate method for detecting PEE, which is a common sign of DED. This technique can provide clinicians with a standardized methodology for robust detection of PEE.

- Evaluation of the effectiveness of DED treatments: It can be used to evaluate the effectiveness of different DED treatments by monitoring changes in corneal staining patterns over time.
- Research studies: It can be utilized in research studies to investigate the pathophysiology of DED and to evaluate the efficacy of potential new treatments.
- Evaluation of corneal epithelial damage: It can be used to evaluate the extent of corneal epithelial damage in patients with other corneal disorders, such as corneal abrasions or infections.
- Screening tool for DED: It can be used as a screening tool for DED, especially in patients with light-colored irises who may not show typical signs of the condition.

8.2 Advantages and limitations

- Ability to focus on all regions of the cornea in one image.
- High contrast visualization.
- High sensitivity in detecting corneal staining.
- Particularly effective for light-colored iris patients.
- Standardized methodology for robust detection of PEE.
- Sensitive, rigorous and reproducible.
- Suitable for clinical and research practices.
- Commercially available.
- Safe and painless.

Nonetheless, it comes with certain constraints:

- Patient compliance for a perfectly centered image: The FCG standard operative procedure requires the patient to be compliant in achieving a perfectly centered image, which can be challenging for some patients.
- Need for external fixating light and second assistant: The OCT device used in the procedure requires an external fixating light to aid patients in centration, and a second assistant is needed to obtain a lid aperture, which may increase the complexity and length of the procedure.
- It may not be covered by insurance for DED diagnosis.

8.3 Potential role in future research and clinical practice

FCG has emerged as a crucial diagnostic tool that plays a pivotal role in unraveling the intricate pathophysiology of DED through its ability to generate high-resolution, real-time images of the corneal epithelium staining patterns, and it enables

researchers to gain valuable insights into its underlying mechanisms. As a result, it holds significant potential for facilitating future research efforts aimed at enhancing our understanding of the etiology and management of DED [112].

Furthermore, it provides a means to obtain intricate images of the corneal epithelium staining patterns, which can be utilized to identify the underlying causes of the condition and unlock new potential treatment options. In addition, it can provide an objective way of assessing the effectiveness of different treatment modalities for DED, including topical eye drops, artificial tears, and other therapies. By monitoring changes in corneal staining patterns over time, FCG can serve as a sensitive and reliable metric to track disease progression and evaluate the response to different treatments. This information can provide clinicians and researchers with valuable insights into the efficacy of different interventions [112].

In clinical practice, it can be used to diagnose and monitor PEE, providing clinicians with a standardized and highly sensitive methodology for detecting PEE. Additionally, it can be used as a screening tool for DED, especially in patients with light-colored irises who may not show typical signs of the condition. Furthermore, FCG can be used to evaluate the effectiveness of different DED treatments by monitoring changes in corneal staining patterns over time [112].

In summary, FCG is an effective technique that has high sensitivity and accuracy in detecting PEE, which is a frequent sign of DED. FCG has considerable potential to aid future research endeavors focused on improving our comprehension of the causes and treatment of DED. Providing detailed images of the corneal epithelium staining patterns, it can help identify the root causes of the condition and pave the way for novel therapeutic interventions.

9. Conclusion

The various corneal imaging techniques available provide valuable diagnostic and management tools for ocular surface conditions, particularly DED. Each technique has its strengths and limitations, and a combination of tools can provide a more complete evaluation of the ocular surface. As technology continues to improve, these imaging techniques hold great potential for advancing our understanding of ocular surface diseases and developing new treatments. In clinical practice, utilizing a variety of imaging techniques can improve the accuracy of diagnosis and individualize treatment plans for patients with ocular surface conditions. Overall, these imaging techniques offer a promising future for improving the care of patients with DED and other ocular surface conditions.

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