In Vivo Confocal Microscopic Corneal Images in health and disease with an emphasis on extracting features and visual signatures for corneal diseases: A review study

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ABSTRACT

There is an evolution in the demands of modern ophthalmology from descriptive findings to assessment of cellular level changes by using in vivo confocal microscopy. Confocal microscopy, by producing grey-scale images, enables a microstructural insight into the in vivo cornea in both health and disease, including epithelial changes, stromal degenerative or dystrophic diseases, endothelial pathologies, and corneal deposits and infections. Ophthalmologists use acquired confocal corneal images to identify health and disease states and then to diagnose which type of disease is affecting the cornea. This paper presents the main features of the healthy confocal corneal layers, and reviews the most common corneal diseases. It identifies the visual signature of each disease in the affected layer and extracts the main features of this disease in terms of intensity, certain regular shapes with both their size and diffusion, and some specific region of interest. These features will lead towards the development of a complete automatic corneal diagnostic system which predicts abnormalities in the confocal corneal data sets.

Keywords: confocal microscopy, corneal images, corneal diseases, corneal layers, feature extraction, visual signature.

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1. Introduction

Corneal confocal microscopy is a non-invasive technique for studying the cellular structure of corneal physiology and disease. It offers non-invasive visualization of the living tissues and provides grey-scale images with greatly increased resolutions over light bio-microscopy and bio-cytology which have both been very rewarding approaches to improve definitions of disease phenotypes [1]. This ability to provide high resolution images of all layers in the living cornea has resulted in new discoveries in corneal pathology at the cellular level [2]; it has been used in the detection and management of pathologic and infectious conditions, corneal dystrophies, monitoring contact lens induced corneal changes, and for pre and post-surgical evaluation following laser vision correction (PRK, LASIK and LASEK) [3].

The normal cornea is avascular, and in histological cross-section, it consists of five distinct layers. The corneal epithelium lies at the anterior side and is about 50 µm thick at the central corneal region. Bowman's layer is under the epithelium; it is 10-16 µm thick and is acellular except for the nerves which permeate it. The basal lamina of the epithelium is located on Bowman's layer where the latter in turn separates the epithelium from the stroma. The stromal region is about 450 µm in thickness and contains large nerves, stromal keratocytes, and orthogonal layers of collagen fibres. Descemet's membrane lies posterior to the stroma. It is an acellular layer and about 15-20 µm in thickness. The limiting layer on the posterior side of the cornea is a single layer of corneal endothelial cells [4].

Each corneal disease demonstrates significant qualitative and/or quantitative alterations in the corneal layers in the eye of patient. In other words, any abnormality occurring in the cornea has its own signs relative to the involved layer. Qualitative changes relate to alterations in the morphology of the corneal layer, while quantitative changes relate to measurements such as cell diameter, cell density, nerve length, total number of nerves within a frame, keratocytes density, number of cells within a frame, corneal reflectivity, and corneal thickness [5]. In vivo confocal microscopy is rapidly becoming a useful method to evaluate the morphological characteristics of corneal abnormalities at the histological level and may be helpful in diagnosis, determination of progression, and understanding the pathophysiology of disease [2].

In ophthalmology, reliable and early diagnosis usually depends on the recognition of minute changes in normal structures. To the patient, the functional consequences of such pathological alterations may be the only aspect of concern; however, ophthalmologists look for morphologic criteria that are essential in defining health and disease [1]. We are working in collaboration with a group of clinical specialists and ophthalmologists to achieve more accurate diagnostics of corneal diseases which will help in developing our vision of an automatic diagnosis system, for improving the standards of patient care.

In this paper we list the most common corneal pathologies, classified regarding to the International Committee for Classification of Corneal Dystrophies (IC3D classification) and we summarise the diagnosis of corneal diseases as they appear in corneal layers with an emphasis on the visual signs of each disease (qualitative changes). We analyse the images as well, to extract their features in terms of intensity, region of interest, and contained shapes with both their sizes and diffusion.

Our aim is to work towards the development of an automatic corneal diagnostic system which can distinguish between normal and abnormal cornea using the features and the visual signatures that are extracted from data sets of corneal images from confocal microscopes.

This paper is organised as follows. In Section 2, the development of confocal microscopy is summarised. In Section 3, a summary of corneal layers in health is presented. A summary of corneal layers in disease is presented in Section 4. This section displays the most common corneal diseases classified according to the level chiefly affected using the IC3D classification. The final section contains our conclusions and suggestions for future work.

2. Development of confocal microscopy

This review covers many research papers, review papers, and case studies from the past two decades which worked with confocal microscope images of the cornea. We include a subsection to cover the history of, and the key people involved in, the development of confocal microscopy. The images in this review were produced by several types of confocal microscopy: Tandem scanning confocal microscopy (TSCM), Slit scanning confocal microscopy (SSCM), and Laser scanning confocal microscopy (LSCM). For that reason, a subsection is included to indicate the differences between these three techniques and what distinguishes each type from the others.

2.1 Historical overview of confocal microscopy

The confocal microscope was originally developed by Marvin Minsky in the mid-1950s. The constructed instrument, which was called the "double focusing stage scanning microscope", was patented in 1957 and focussed the light on a small area of tissue [6, 7].

The illumination and observation pathways have a common focal point, and because of that; this principle is termed “confocal” [6]. After Minsky's work, M. David Egger and Mojmir Petran created a multiple-beam confocal microscope in the late 1960s for examining unstained brain sections and ganglion cells. Egger continued in this arena and developed the first mechanically scanned confocal laser microscope. In 1973, he published the first recognizable images of cells produced by this technique [7]. Because of the advances in computer and laser technology coupled to new algorithms for digital manipulation of images, during the late 1970s and the 1980s, a growing interest in confocal microscopy developed [7].

Several investigators translated the practical laser scanning confocal microscope designs into working instrument. Dutch physicist G. Fred Brakenhoff developed a scanning confocal microscope in 1979, while at almost the same time; Colin Sheppard contributed to the technique with a theory of image formation. Tony Wilson, Brad Amos, and John White nurtured the concept and later (in the late-1980s) demonstrated the utility of confocal imaging in the examination of fluorescent biological specimens [7].

2.2. Types of confocal microscopy

2.2.1.Tandem scanning confocal microscopy.

The first real-time TSCM was developed by Petran and Hadravsky utilising point illumination and detection. The basic part of the system was modified by Nipkow who invented a spinning disc composed of sets of conjugate pinholes arranged in spirals. After a specimen is scanned in parallel, a two dimensional image is generated [1, 8]. To eliminate most of the scattered light, the pinholes need to be as small as possible. However, this results in very low light throughput (only 0.25-1% of light reaches the cornea), which necessitates a high intensity light source and a low light level camera to enable image acquisition [8].

This original microscope in its clinical version was produced by the Tandem Scanning Corporation (Reston, VA, USA) and later by the Advanced Scanning Corporation (New Orleans, LA, USA), and is no longer in production [1].

2.2.2. Slit scanning confocal microscopy.

An alternative to point scanning, the SSCM was first developed by Thaer for observing the in vivo cornea. This involves illumination from a slit of scanned over the back focal plane of the microscope objective [8], which allows all points on the axis of the slit to be scanned at the same time and consequently greatly reduces scanning times over the TSCM [1, 8]. The slit illumination also allows a greater light throughput than the TSCM and thus a lower intensity light source may be used, allowing longer examination times [8]. Contrast with the SSCM is greater in comparison to the TSCM, which provides clearer images of the stroma and enables imaging of the low reflecting layer of wing cells in the epithelium [8]. The slit height may be adjustable, which would allow the user to control the thickness of the optical section and the amount of light throughput. However, the microscope is only truly confocal in the axis perpendicular to the slit and provides lower axial and transverse resolution than the TSCM [1, 8].

There are Commercial sources that provide slit scanning in vivo confocal microscopes such as Tomey Corporation (Cambridge, MA, USA), Nidek Technologies (Gamagori, Japan) and Helmut Hund (Wetzlar, Germany) [1].

2.2.3 Laser scanning confocal microscopy.

The LSCM was developed by Webb, it employ a coherent laser as a high intensity light source. The laser beam is scanned using a set of galvanometer scanning mirrors, which provides fast scanning over the xy plane. The reflected light refocused by the microscope objective is rescanned by the galvanometer scanning mirrors, and imaged onto a pinhole aperture located in front of a photomultiplier [1].

One of the well-established in vivo confocal imaging systems used in ophthalmology is the Heidelberg Retina Tomograph (HRT) (Heidelberg Engineering, Heidelberg, Germany). HRT is a device that was designed to acquire and evaluate topographic measurements of the optic nerve head to detect glaucomatous damage using a 670 µm diode laser. The University of Rostock (Germany) modified the HRT to use a detachable objective system called the 'Rostock Cornea Module' (RCM). This converts it into a high-resolution LSCM for the visualisation of the anterior segment of the eye [1, 8].

Optical sections of only a few micrometres can be imaged with a high contrast and precisely measured because of the high-depth resolution [1]. LSCM provides greater contrast than the TSCM or SSCM [8].

2.3. Performance comparison between different confocal technologies

Niederer and McGhee [8] mention that limited data are available for comparisons of performance between the different types of in vivo confocal microscopy.

A comparison between keratocyte density measured with TSCM and SSCM (Confoscan 3) demonstrated that, providing the effective depth of the sample volume was taken into consideration, comparable measurements of keratocyte cell density were achievable between the two devices [9].

A further qualitative study compared the SSCM (Confoscan 2) and LSCM (HRT II RCM) appearance of the central cornea in normal subjects and in subjects with corneal dystrophy. The observed morphology was similar with both devices, but image contrast appeared greater with the LSCM [10].

Endothelial cell density measurement has been compared between SSCM (Confoscan 3) and LSCM (HRT II RCM). All the results of cell density (for patients and normal subjects) that performed with the RCM were higher than those with the Confoscan 3 [11].

The authors of [8, 12] compared measurements of cell density and image contrast with contemporary SSCM (Confoscan 4, NIDEK, Gamagori, Japan) and LSCM (HRT II RCM). At the levels of the basal epithelium and the endothelium, they observed good agreement between the two devices for cell density measurement. Mid stromal keratocyte density (measured in cells/mm^2) was approximately double with SSCM compared to LSCM, presumably due to differences in optical section thickness. They observed as well that the contrast at the level of the mid stroma was greater with the LSCM compared to the SSCM and the contrast levels were consistent throughout the image, whereas there was loss of image quality towards the edges of the SSCM image. Poor agreement was observed in measurement of sub-basal nerve plexus density, possibly due to differences in image contrast, with values measured with LSCM higher than those measured with SSCM.

A study [13], observed that sub-basal nerve plexus density measured with SSCM was two to three times higher than density measured with TSCM.

3. Corneal layers in health

3.1. Epithelium

3.1.1. Superficial epithelial cells.

Superficial epithelial cells present a polygonal cell pattern, reflecting nuclei with bright illuminated cytoplasm, and perinuclear dark halos. In general, the size of the cell is up to 50 µm in diameter and about 5 µm in thickness in Fig. 1.(a) [1, 8]. Broadly speaking, superficial epithelial cells have light cell boundaries and bright visible nuclei in Fig. 1.(b) [14, 15, 16].

3.1.2. Wing cells.

The epithelial intermediate layer (wing cells), forms a regular mosaic with sharp and reflecting cellular borders. The sizes of wing cells (which are regular in form) are about 20 µm. They can also be subdivided into upper (Fig. 2.(a)) and lower (Fig. 2.(b)) wing cells; the latter are smaller [1]. These cells, which can probably only be imaged with a scanning slit IVCM [15], are identified by their bright cell borders and a bright cell nucleus, and unlike superficial epithelial cells, have no dark oval ring around the nucleus [8]. They are characterised by bright cell borders and a bright cell nucleus with few organelles and the nuclei are usually not visible [3].

3.1.3. Basal epithelial cells.

Basal epithelial cells appear as a regular mosaic of dark cell bodies with light, narrow inter-cellular reflecting borders and they have a smaller diameter which is 8-10 µ m (Fig. 3) [1, 8] and without nuclei reflectivity such that cells show only cell borders [1, 14, 15].

3.1.4. Sub-basal nerve plexus (SNP).

The sub-basal nerve plexus (SNP) appears as bright, well-defined long linear nerve fibre bundles, frequently demonstrating branches or anastomosis in Fig. 4.(a) [8, 15]. It is characterised by the appearance of hyper-reflective fibres of lengths that range between 4 and 8 µm, and organised in a vortex pattern in the lower quadrant of the para-central cornea in Fig. 4.(b) [1]. SNP may be identified in Bowman's layer [14].

3.2. Bowman's Membrane

Bowman's membrane (anterior limiting lamina) is an acellular hyper-reflective structure, where SNP may be identified [14, 15]. It appears as an 'amorphous' layer when viewed with confocal microscopy [8]. It consists of randomly arranged collagen fibrils located in between the basal cells and the stroma. This cell free area is 10–16 µm in thick in Fig. 5 [1, 15].

3.3. Stroma

Corneal stroma forms around 80–90% of the whole corneal volume. Stromal keratocytes appear as hyper-reflective cell nuclei, typically forming clusters, with poorly visualised cell processes as appeared in Fig. 6.(a). The corneal nerves enter the cornea in the mid stroma and radiate towards the central cornea, dividing into branches, see Fig. 6.(b), that are thicker than at the sub-epithelial level [8, 14, 15]. The corneal stroma consists of three main histological components: cellular, acellular and neurosensorial. The connective lamellae appear black (that is transparent), whereas the keratocyte nuclei are visible as egg-shaped light reflecting corpuscles. Using cross-section analysis, keratocyte density, which measured in cells/mm^2, is greatest immediately under Bowman's membrane (in the anterior stroma as seen in Fig. 6.(c)) and it is reported to be around 500 - 1300 cells/mm^2. The density declines sharply toward the central stroma (minimum 65 cells/mm^2), and it increases again slightly in the posterior stroma as seen in Fig. 6.(d); it is reported to be around 250 - 900 cells/mm^2 [1, 8, 14]. Keratocyte density decreases with age and appears to

be independent upon sex [1, 14, 15]. In [14] it was mentioned that keratocyte density seemed to decrease 0.45% per year.

3.4. Descemet's Membrane

Descemet's membrane is rather difficult to see under normal circumstances and appears as an acellular layer between the posterior stroma and the endothelium. It is usually indiscernible with confocal microscopy, particularly in younger subjects [8, 14]. Also, nerve plexus is not found [14]. It is thin with thickness about 6–10 μm [1].

3.5. Endothelium

The endothelial cells are located immediately posterior to Descemet's membrane, and are characterised by a regular hexagonal hyper-reflective pattern or regular honeycomb mosaic, surrounded by hypo-reflective borders without obvious nuclei reflections in Fig. 7 [1, 8, 14, 15]. It is easy to recognise this layer because of its clearly identifiable structure [8]. Sometimes, the nuclei of the cells may be visualised [1].

4. Corneal layers in disease

We use the IC3D Classification of the Corneal Dystrophies which was introduced in 2008 [17]. Its goal was to develop a new classification system for corneal dystrophies, integrating the most recent information on clinical description, pathologic examination, and genetic analysis. A template was devised by the IC3D to review the current knowledge of each corneal dystrophy in an organized manner. Each template provides information which includes the name of the dystrophy, alternative names, genetic information (the gene involved and the gene locus of the responsible mutation), onset, signs, symptoms, course, light microscopy, transmission electron microscopy, confocal microscopy and clinical photographs. Each corneal dystrophy is also categorised by the committee based on the level of evidence available to support its existence. The categories are as follows [17]:

Category 1: A well-defined corneal dystrophy in which a gene has been mapped and identified and specific mutations are known.

Category 2: A well-defined corneal dystrophy that has been mapped to one or more specific chromosomal loci, but the gene (or genes) remains to be identified.

Category 3: A well-defined corneal dystrophy that has not yet been mapped to a chromosomal locus.

Category 4: This is reserved for suspected new (or previously documented) corneal dystrophies, although the evidence for it, being a distinct entity, is not yet convincing.

The IC3D classification system is organised anatomically according to which corneal layer is affected. In general, it classified dystrophies into four groups; epithelial and sub-epithelial dystrophies, Bowman layer dystrophies, stromal dystrophies, and Descemet's membrane and endothelial dystrophies. For the purposes of this review article, we will focus only on the organisation that depends on the affected corneal level.

4.1. Epithelial and sub-epithelial

4.1.1. Amiodarone induced keratopathy.

Amiodarone induced keratopathy appears in the corneal epithelial cells. It is characterized by bright intracellular, highly reflective inclusions. In more advanced cases, other corneal layers may be involved: bright micro-dots arise within the anterior and posterior stroma and on the endothelial cell layer. Also, keratocyte density reduces in the anterior stroma [14]. The basal cell layer from a patient during the period of amiodarone ingestion, clearly showing hyper-reflective cell inclusions, is shown in Fig. 8.(a). They are in almost the whole image except the sides and appear as white and adjacent small rounded-shape structures (each one forms almost 0.04% of the image) on high intensity background. During the period of amiodarone ingestion, the anterior stroma as well shows marked increase in the number of micro-dots distributed almost evenly between nuclei of anterior stroma as seen in Fig. 8.(b) [18]. LSCM (HRT II-RCM) was used to capture Fig. 8. (a) and Fig. 8. (b) with fields of view 400×400 pixel.

4.1.2. Advancing wave-like epitheliopathy.

This disease is characterized by the presence of atypical elongated cells with centripetally oriented long axes (needle-shape with each one forming almost 0.3% of the image) that are surrounded by a grey hazy halo. There are hyper-reflective nuclei on darker grey background at the level of the abnormal epithelium as shown in Fig. 9.(a), but confluent hyper-reflective regions are demonstrated at the sup-epithelial level as shown in Fig. 9.(b), where they appear as hazy reflections in the whole image with varying intensity values which become higher at the bottom [19]. TSCM was used to capture Fig. 9. (a) and Fig. 9. (b) with fields of view 475×350 µm.

4.1.3. Epithelial basement membrane dystrophy.

Confocal microscopic images for epithelial basement membrane dystrophy reveals four main features. Firstly, highly reflective irregular material, indicated by arrows in Fig. 10.(a), intermixed with the keratocytes of the anterior stroma and posterior epithelial interface and this area is shown as a reflection of medium values in grey-scale. Secondly, the cells of the basal epithelium have abnormal distended cytoplasm with very reflective nuclei that have grey double-walled aureole (the white circle forms 0.4% of the image), indicated by an arrow in Fig. 10.(b). Thirdly, the cysts are elliptic with size of 50 to 400 µm, indicated by arrows in Fig. 10.(c). The borders of cystic lesions are poorly defined, and there is irregular reflective material within them, as indicated by an asterisk in Fig. 10.(c). A basement membrane bearing some long (at least 300 µm), highly reflective, linear structures that have diffuse posterior borders and well-delineated anterior borders are indicated by arrows in Fig. 10.(d). A non-reflective space appears within this linear lesion and the normal basement membrane [20]. SSCM (Confoscan 2) was used to capture figures of epithelial basement membrane dystrophy features with horizontal field width of 610 µm.

4.1.4. Salzmann's nodular degeneration.

The confocal images of this disease demonstrate adjacent polygonal and irregularly shaped basal epithelial cells with reflective borders and prominent nuclei appearing in each polygonal cell as a small grey dot (forms 0.16% of the image) as shown in Fig. 11.(a). Sub-basal nerve fibres exhibit

an abnormal pattern with increased thickness and absence of branching, indicated by an arrow in Fig. 11.(b). The nerve fibre appears as an undulating sloping line with high intensity. The area surrounding this line is cloudy on both sides. Small bright dots appear on and beside the lower side of the nerve fibre (each forms on average 0.1% of the image). Moreover, there is a presence of reflective cellular elements near these fibres. Fig. 11.(c) shows the nerves in mid-stroma. Their branches are thick and tortuous, with both tracts of granular aspect (indicated by an arrowhead) and highly reflective rectangular segments (each forms 0.5% of the image) along the bundles (indicated by an arrow) [21, 22]. SSCM (Confoscan 4) was used to capture the images of Salzmann's nodular degeneration with field of view 767×575 pixel.

4.1.5. Gelatinous drop-like dystrophy.

The epithelial cells of Gelatinous drop-like dystrophy are hyper-reflective adjacent polygonal shapes with irregular structure and often elongated as shown in Fig. 12.(a). At the level of the Bowman's membrane in Fig. 12.(b), a very small number of sub-basal nerves with increased background intensity can be seen. We can notice as well, some highly reflective small dots spread over the image (each forms 0.08% of the image). Fig. 12.(c) shows large accumulations of brightly reflective amyloid materials beneath the epithelium and within the anterior stroma. Also, the nuclei of keratocytes were poorly identifiable [23]. LSCM (HRT II-RCM) with field of view 400 x 400 pixel was used to capture the images of Gelatinous drop-like dystrophy.

4.1.6. Thygeson epithelial keratitis.

Thygeson keratitis shows highly reflective deposits (50–300 μm in diameter) with cotton-like appearance in the basal epithelial cell layer and Bowman layer, see Fig. 13.(a). These cotton-like deposits accumulate in the middle of the image (each forms on average 0.2% of the image). Highly reflective material appears in Fig. 13.(b) that directly damages epithelial cell connection whilst epithelial cells are swelling and losing polygonal structure, and cell gaps are widening as well. In other words, epithelial cells look like cracked ground. These cells are disconnected and appear as high intensity curvy lines on low intensity background. Small numbers of highly reflective spots (each forms on average 0.33% of the image) appear at the top centre of the image [24, 25]. LSCM (HRT II-RCM) with field of view 400 x 400 pixel was used to capture Fig. 13.(a) and Fig. 13.(b).

4.1.7.Meesmann's dystrophy.

The cystic lesions in this disease, which are 10 to 50 μm in diameter, appear in round, well delineated shapes surrounded by reflective points in the cytoplasm, which probably correspond to cell nuclei, as indicated by arrows in Fig. 14.(a). Above an apparently normal basal membrane, some normal cell nuclei appear as reflective round regions, as indicated by arrowheads in Fig. 14.(a)[20]. Multiple cyst-like changes are seen at the level of the basal epithelium in Fig. 14.(b). They are very similar in size (each forms 3% of the image) and are distributed non-uniformly with white colour while the normal cells have very dark grey colour with unclear borders [8]. Fig. 14.(a) was captured by SSCM (Confoscan 2) with horizontal field width of 610 μm, while Fig. 14.(b) was captured by LSCM (HRT II-RCM) with field of view 400×400 pixel.

4.1.8. Recurrent Erosion Syndrome.

In Fig. 15, superficial polygonal low reflective areas are seen surrounded by bright epithelial cells, and a mucus band appears as a bright line over the epithelial mosaic. The length of each line is approximately equal to the horizontal field width of the image which is 610 µm. SSCM (Confoscan 2) was used to obtain this figure. No epithelial cystic lesions are found by confocal microscopy [20].

4.1.9. Acanthamoeba infective keratitis.

Acanthamoeba cysts and trophozoites have a typical structure which allows rapid diagnosis leading to early institution of treatment. The cysts appear as highly reflective, round-shaped particles (10–20 µm in diameter) or ovoid structures, ranging in diameter from 10 to 26 µm within the corneal epithelium and stroma. It is possible to find cysts as single-walled structures, as indicated by arrows in Fig. 16.(a), or conglomerates with double-walled structures, as indicated by arrows in Fig. 16.(b) [1, 8, 15, 26, 27]. Fig. 16.(a) was captured by LSCM with field of view 400×400 pixel, and TSCM was used to capture Fig. 16.(b) with field of view 475×350 µm.

4.1.10.Bacterial keratitis.

Confocal microscope images of bacterial keratitis show typical hyper-reflective defects without recognizable structure, as indicated in Fig. 17.(a). The adjacent epithelium is oedematous [1]. Bacterial keratitis is demonstrated as either distinct needle-like deposits or amorphous deposits at different epithelial depths as shown in Fig. 17.(b) [8]. An increased density of dendritic-like cells within the epithelium, shown in Fig. 17.(c), has also been reported in a patient with bacterial keratitis [8, 28]. The study of [29] used confocal microscopy to identify Nocardia which are filamentous bacteria. The confocal scan of Nocardia in this study revealed multiple, thin, and short filamentous structures that demonstrated right-angled branching within the epithelium as indicated by white arrow in Fig. 17.(d). These filamentous structures were surrounding by round to oval bright structures which representing inflammatory cell infiltration as indicated by black arrow in Fig. 17.(d). Fig. 17.(a), Fig. 17.(b), and Fig. 17.(c) were taken by LSCM (HRT II-RCM) with field of view 400×400 pixel, while Fig. 17.(d) was taken by SSCM (Confoscan 3).

4.1.11.Viral keratitis: herpes simplex virus.

Herpes simplex-virus (HSV) infection is usually followed by an inflammatory response, which can seriously damage all corneal layers [30]. A classification scheme has been proposed for HSV keratitis [30, 31]: infectious epithelial keratitis (IEK), neurotrophic keratopathy, herpetic stromal keratitis (HSK), and endotheliitis. Superficial epithelium layer shows, as indicated in Fig. 18.(a), increase in cell size and hyper-reflectivity while there is a decrease in cell density [30]. In HSV endothelitis [31], pseudoguttata look alike true corneal guttata but are less regularly shaped. As indicated by single arrows in Fig. 18.(b), instead of a round white dot in the centre, they possess a line of high reflection on the border of the elevated dark area. Intercellular gaps are characterized by small black dots at the vertices of endothelial cells and by pronounced intercellular borders as shown in Fig. 18.(b) by double arrows. Some patients with HSV infection have alterations of the

sub-basal nerve plexus. Other patients have no visible sub-basal nerve or reduced nerve density [8, 32]. An SSCM (Confoscan 4) with field of view 460×345 µm was used to take Fig. 18.(a), while Fig. 18.(b) was captured by SSCM (Confoscan 3) with field of view 425×320 µm .

For easier reference to the appearance of epithelial and sup-epithelial diseases, we summarise the visual signature of each disease in Table 1 in appendix A.

4.2. Bowman layer

4.2.1. Reis-Bückler's dystrophy.

Images of patients with Reis-Bückler's dystrophy show focal deposition of homogeneous, reflective material, as indicated by arrows in Fig. 19.(a), interspersed with the basal epithelial cells. This image shows medium intensity branching and undulating lines in the middle on a hazy background. In the left of the image, there is an elongated reflective area that has many values of intensity in grey-scale; the outside boundary is dark grey and the inner side near the boundary is grey and the middle is light grey that approximates to values of white. The region of interest (which forms 20% of the image) located in the right side (almost starts from the centre right and extends to the right top); it is a high intensity area (arrows in the figure) with grey boundary that is interspersed with the basal epithelial cells .Highly reflective material at the level of Bowman's layer is indicated by an asterisk in Fig. 19.(b). This reflective area covers almost the whole image except the sides. Boundaries of the reflective area have lower intensity. We can notice as well, a low intensity patch in the centre left, which forms 2% of the image and another two patches in the right centre and in the top centre (each forms 0.3% of the image). In Fig. 19.(c), showing the anterior stroma [33], asterisk indicates fine diffuse deposits interspersed between keratocyte nuclei which are indicated by arrows.

Another study demonstrates the presence of focal highly reflective and large number of adjacent irregular and granular materials (on average each material forms 0.2% of the image) without dark shadows in the basal epithelial layer, shown in Fig. 19.(d). At the level of Bowman's layer, there is highly reflective small granular material actually replacing Bowman's layer shown in Fig. 19.(e) [34]. SSCM (Confoscan 4) with field of view 767×575 pixel was used to take Fig. 19.(a), Fig. 19.(b), and Fig. 19.(c), while Fig. 19.(d) and Fig. 19.(e) were captured by LSCM with field of view 400×400 pixel.

4.2.2. Thiel-Behnke dystrophy.

This disease shows the presence of deposits with homogenous reflectivity, rounded edges and dark shadows in the basal epithelium, as indicated in Fig. 20.(a). We see these deposits appear as haze of different intensity values in the right third of the image and also in the left third. In the central third, the basal epithelial cells appear clearly with hypo-reflective borders. The edges that separate these three parts have a very low intensity undulating structure but at the level of Bowman's layer. This homogeneous reflective material appears as haze with different values of intensity and there are some diffuse bright irregular spots (each forms on average 0.3% of the image). Bowman's layer

is completely replaced as indicated in Fig. 20.(b) [8, 34]. LSCM with field of view 400×400 pixel was used to take Thiel-Behnke dystrophy images.

For easier reference to the appearance of Bowman layer diseases, we summarise the visual signature of each disease in Table 2 in appendix A.

4.3. Stromal

4.3.1. Lattice dystrophy.

In Fig. 21.(a), confocal microscopy reveals the presence in the mid-stroma of unspecified, undulating and thinner string-like structures, apparently interacting with the keratocytes [35, 36]. Also, highly reflective deposits are observed in Fig. 21.(b) under the basal epithelial cells. In Fig. 21.(c), immediately under the basal cells of the epithelium, Bowman's layer is seen to be irregular in thickness, by the differences in grey-scale. This may be due to accumulation of amyloid [36]. Poorly demarcated, hyper-reflective, linear, and branching structures of varying intensity are the main characteristics of amyloid deposition in lattice dystrophy shown in Fig. 21. (d) [8]. Fig. 21.(a), Fig. 21.(b), and Fig. 21.(c) were captured by TSCM (model 165A) with field of view 450×360 µm. LSCM (HRT II-RCM) with field of view 400×400 pixel was used to produce Fig. 21.(d).

A study on lattice dystrophy [33] revealed reflective punctiform structures in the basal epithelial cell layer, indicated as arrows in Fig. 22.(e). In Fig. 22.(f) in the anterior stroma, fine, diffuse, reflective deposits (indicated by asterisk) are interspersed between keratocyte nuclei (indicated by arrows). In Fig. 22.(g), showing the anterior and middle stroma, tubular structure filaments with well-defined edges are indicated by arrowheads interspersed with normal keratocytes indicated by arrows. SSCM (Confoscan 4) with field of view 767×575 pixel was used to take Fig. 22.(e), Fig. 22.(f), and Fig. 22.(g).

4.3.2. Fleck dystrophy.

In fleck dystrophy hyper-reflective and largely intracellular dots with various shapes are distributed throughout the corneal stroma [37, 8, 14]. They consist of spherical matter with diameters of 3 to 5 µm and are sometimes enclosed in cyst-like structures as indicated in Fig. 23 which was taken by SSCM[37, 14].

4.3.3. Granular dystrophy.

In granular dystrophy confocal images, hyper-reflective breadcrumb-like deposits measuring approximately 50 µm in diameter are observed in the basal epithelial cell layer, as indicated by arrows in Fig. 24.(a). In the anterior stroma near the Bowman layer region, reflective diffuse deposits are noticeable in Fig. 24.(b) (indicated by arrows). A nerve fibre without myelin appears as a bright line (indicated by arrowhead) in Fig. 24.(b). In the deep stroma, shown in Fig. 24.(c), keratocyte nuclei are indicated by arrows interspersed with punctiform reflective deposits which are indicated by arrowheads [33]. SSCM (Confoscan 4) with field of view (767×575 pixel) was used to obtain Fig. 24.(a), Fig. 24.(b), and Fig. 24.(c).

4.3.4. Avellino corneal dystrophy.

In the basal epithelial of a Avellino corneal dystrophy patient, focal deposits of highly reflective granular materials without dark shadows are observed, see Fig. 25.(a). At the level of the superficial and middle stroma, Fig. 25.(b) shows clusters of highly reflective granular materials with irregular edges [38]. The images of Avellino corneal dystrophy were captured by LSCM (HRT II-RCM) with field of view 400 × 400 pixel.

4.3.5. Schnyder crystalline corneal dystrophy (SCCD).

A study analysing the corneal morphology in Schnyder dystrophy demonstrated that highly reflective elliptic material, presumably cholesterol or lipid, accumulates inside and around anterior keratocytes which have hypo-reflective irregular structure, indicated by arrows in Fig 26.(a). Also, brightly reflective punctiform deposits indicated by arrows in Fig 26.(b) are associated with the sub-epithelial nerves. Fig. 26.(a) and Fig. 26.(b) were captured by a TSCM (model 165A) with field of view 450×360µm and each image was cropped to 250×170µm. Moreover, crystalline needle-shaped adjacent deposits appear in the anterior stroma [39], and the crystals are needle shaped as shown in Fig. 26.(c), or rectangular [40].

Another study shows that, at the level of Bowman's layer, a small number of sub-epithelial nerves are detectable with high background intensity in Fig. 26.(d), but in other cases these nerves are undetectable [40]. LSCM with field of view 400×400 pixel was used to take Fig. 26.(c) and Fig. 26.(d).

The study of Schnyder dystrophy [41], confirms the presence of large or multiple deposits of brightly reflective crystalline material extending from the anterior to the mid stroma. Some abnormal nerve branches with an irregularly curved appearance are also found in the anterior stroma as shown in Fig. 26.(e) which was taken by SSCM (Confoscan 2). The sub-epithelial nerve plexus are undetectable.

4.3.6. Macular corneal dystrophy.

In the superficial stroma of Macular corneal dystrophy as in Fig. 27.(a), we can see highly reflective deposits without distinct borders. Fig. 27.(b) shows the middle stroma containing homogeneous reflective materials with dark striae-like features [38]. LSCM (HRT II-RCM) with field of view 400×400 pixels was used to capture the images of Macular corneal dystrophy.

4.3.7. Central Cloudy Dystrophy of François.

This disease reveals small highly reflective granules and deposits in the superficial stromal layer as seen in Fig. 28.(a). In the deep stroma adjacent to the corneal endothelial layer, Fig. 28.(b) shows multiple dark acellular striae among extracellular matrices with increased intensities [42]. Fig. 28.(a), and Fig. 28.(b) were taken by SSCM (Confoscan 2).

4.3.8. Pre-Descemet's membrane corneal dystrophy.

Anterior stroma of patients with Pre-Descemet's membrane corneal dystrophy shows normal keratocyte nuclei and a few irregular highly reflective particles as seen in Fig. 29.(a). Posterior stroma in Fig. 29.(b) reveals hyper-reflective and non-homogenous structures with numerous tiny

inclusions (indicated by arrows) [43]. SSCM (Confoscan 2) was used to capture Fig. 29.(a) and Fig. 29. (b).

4.3.9. Posterior amorphous corneal dystrophy.

The confocal microscope images of this disease demonstrate micro-folds and diffuse hyper-reflective sheet-like opacities with spikes extending from the posterior stroma to the stroma immediately adjacent to the endothelial layer (see Fig. 30 which was taken by SSCM (Confoscan 3)) [44].

4.3.10. Corneal amyloidosis.

Corneal amyloidosis appears as sub-epithelial and anterior stromal grey white deposits. The confocal microscopic show these deposits as intercellular, hyper-reflective, cotton candy-like material, fibrillar amyloid material scattered throughout the anterior stroma as shown in Fig. 31 [14, 45].

4.3.11. Fungal keratitis.

LSCM is able to reveal numerous hyper-reflective elements resembling Fusarium, Aspergillus hyphae or Candida pseudofilaments in the anterior stroma even in the early phase of fungal keratitis [1, 46].

Fusarium solani reveals numerous highly reflective linear elements (hyphae) of lengths that range between 200 and 300 µm and of widths that range between 3 and 5 µm, with branches at 90° angles in the anterior stroma as indicated by white arrows in Fig. 32.(a). Round inflammatory cells are present also in the stroma as indicated by black arrows in Fig. 32.(a) which was taken by LSCM (HRT II-RCM) with field of view 300×300 µm. Aspergillus hyphae have the same characteristics as Fusarium solani hyphae with branches at 45° angles [1, 46].

Candida pseudiphilaments are characterized by numerous high reflective elongated particles which measure 10–40 µm in length and 5–10 µm in width located in the anterior stroma as indicated by white arrows in Fig. 32.(b), which was captured by LSCM (HRT II-RCM) with field of view 400×400 µm [1, 46].

For easier reference to the appearance of stromal diseases, we summarise the visual signatures of each disease in Table 3 in appendix A.

4.4. Descemet's membrane and Endothelial

4.4.1. Fuchs' endothelial dystrophy (Cornea guttata).

Guttae are characteristic of Fuchs' endothelial dystrophy. At the level of the endothelium, corneal guttae appear in Fig. 33.(a) as roundish hypo-reflective areas of varying size with central bright spots and surrounded by hyper-reflective endothelial cells [8, 14, 47, 48]. TSCM (Topcon SP 2000 P) was used to capture Fig. 33.(a). Descemet's membrane displays significant fibrosis in Fig. 33.(b) which was captured by SSCM (Confoscan 2) [47].

4.4.2. Iridocorneal endothelial syndrome (ICE syndrome).
The endothelium in ICE syndrome displays epithelium-like transformation, with prominent bright nuclei with indistinct cell borders[49]. The study [8] confirms that endothelial cells appear as epithelioid-like cells with irregular borders and non-homogenous, diversely shaped nuclei as shown in Fig. 34. It is worth mentioning here that eyes with ICE syndrome also have some degree of iris atrophy [49]. LSCM (HRT II-RCM) was used to capture Fig. 34 with field of view 400×400 µm and this image was cropped to 255×255 µm.

4.4.3. Posterior polymorphous corneal dystrophy (PPCD).
Vesicles are observed at the level of endothelium in PPCD and appear as relatively big, and well-delineated roundish shape lesions (having diagonal pattern) containing endothelial cells with surrounding haze, as shown in Fig. 35.(a) (taken by LSCM (HRT II-RCM) with field of view 400×400 pixel). They appear as a prominent hyper-reflective band lesion at the level of Descemet's membrane, indicated by arrow in Fig. 35.(b) [8, 50].

A case report in [50] observes the appearance of endothelial vesicular lesions in curvilinear pattern with associated endothelial pleomorphism and polymegathism, see Fig. 35.(c). In patients with more advanced posterior polymorphous dystrophy, very prominent corneal nerves at the level of Bowman's membrane are delineated, see Fig. 35.(d). Fig. 35.(b), Fig. 35.(c), and Fig. 35.(d) were taken by SSCM (Confoscan 2).

4.4.4. Brown-McLean syndrome.
In a Brown-McLean syndrome patient, the stromal structure was masked by the oedema, with no clear outline of the stromal cells, see Fig. 36.(a). At the level of the endothelium, pigmentation was observed in Fig. 36.(b) as round, bright, hyper-reflective bodies measuring 5 to 120 µm [51]. The images of Brown-McLean syndrome were taken by SSCM (Confoscan 2).

For easier reference to the appearance of Descemet's membrane and endothelial diseases, we summarise the visual signatures of each disease in Table 4 in appendix A.

5. Conclusions and Future Work

The cornea has three main layers separated by two thin membranes. The epithelium is the outermost layer separated from the central stromal layer by Bowman's membrane. Descemet's membrane separated stromal layer from the innermost endothelial layer. Each layer and membrane has its distinguished histological structure.

Confocal microscopy is a powerful diagnostic technique that allows non-invasive in vivo cellular imaging of all layers of the cornea enabling the clinical investigation of numerous corneal diseases which are extensive and fall under the areas of corneal dystrophies, degenerations, and inflammations. In vivo confocal microscopy is helpful in evaluating the morphological characteristics of corneal abnormalities at the histological level and may be helpful in diagnosis, determination of progression, and understanding the pathophysiology of disease. The ability of

providing high-resolution images of all layers in the living cornea has enabled new discoveries of corneal pathology at the cellular level.

In this review, we summarise the diagnosis of most common corneal diseases which are typically classified by the layer of the cornea that is affected using IC3D classification, and we have focused on presenting the qualitative changes (visual signature) in the structure and morphology of the involved layer and some parameters of quantitative analysis especially the reflectivity.

We detected the regions of interest in corneal diseases and extracted their shape-based and grey level features manually in a step called knowledge base creation, and we categorised these extracted shape-based features of the reviewed diseases into five groups in Table 5 in appendix B. This step is going to help us in investigating the edge and region based segmentation techniques that are able to detect these regions and then to select the most suitable and effective ones. The next stage is to extract features of the segmented regions; these features give a representative of the information that the image has to offer. We are planning to apply statistical texture-based feature extraction techniques that give feature vectors which will be used as input to our classifier aiming to identify the disease that is related to the abnormal cornea. We aim to investigate machine learning approaches to accurately identify the abnormal features of the proposed application.

Put briefly, our aim and future work from this review, as visual computing specialists, is to process the confocal microscopic corneal images, and activate appropriate segmentation and feature extraction algorithms to develop an automatic system that can help the ophthalmologists in detecting abnormal cornea and identifying the disease that is related to this abnormality.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Appendix A

Table 1: The visual signatures of the reviewed epithelial and sub-epithelial diseases.

Epithelial and sub-epithelial diseases	
The disease	**The visual signatures**
Amiodarone-induced keratopathy	– In the basal epithelial cells, white and adjacent inclusions appear that are small and have rounded-shape structures[18].
Advancing wave-like epitheliopathy	– Vertically stretched epithelial cells that have needle-shape and surrounded by a grey hazy halo with high intensity nuclei[19].
Epithelial basement membrane dystrophy	– Large area of light grey colour reflection can be seen with highly reflective nuclei that appear as white circles that have grey double-walled aureole[20]. – Many elliptic cysts with poorly defined borders[20]. – High reflective linear structure that has well-delineated anterior border[20].
Salzmann's nodular degeneration	– Highly reflective adjacent irregular and polygonal shaped basal epithelial cells appear with highly reflective nuclei that appear in each polygonal cell as a small grey dot[22]. – A nerve fibre appears as undulating sloping line with high intensity. Small bright dots appear on and beside the lower side of the nerve fibre[21, 22].
Gelatinous drop-like dystrophy	– Hyper-reflective and adjacent polygonal epithelial cells with irregular structure[23]. – Beneath the epithelium, amyloid materials appear as highly reflective accumulations spread in irregular ways[23].

Thygeson epithelial keratitis	− Highly reflective deposits with cotton-like appearance in the basal epithelial[24]. − Epithelial cells that look like cracked ground. These cells are disconnected and appear as high intensity curvy lines on a low intensity background. Small numbers of highly reflective spots also appear[24, 25].
Meesmann's dystrophy	− Well-delineated rounded shapes lesions surrounded by reflective points in the cytoplasm[20]. − Multiple cyst-like changes which are very similar in size and distributed non-uniformly at the level of the basal epithelium with white colour while the normal cells have very dark grey colour with unclear borders[8].
Recurrent Erosion Syndrome	− A large area of high reflection with two bright white slanted lines over the epithelial mosaic[20].
Acanthamoeba infective keratitis	− Highly reflective elliptic cysts within the corneal epithelium. These cysts could be single-walled structures that are very clear and varying in size, or could be double-walled structures with low intensity and thick border[1, 8, 15].
Bacterial keratitis	− Crowded hyper-reflective dendritic-like cells appear at stroma depths and the background can hardly be seen[8, 26]. − Thin, and short filamentous structures (Nocardia) that demonstrated right-angled branching surrounding by round to oval bright inflammatory cell[29].
Viral keratitis: herpes simplex virus	– Increase in cell size and hyperreflectivity while there is a decrease in cell density in the Superficial epithelium layer[30]. – Pseudoguttata possess a line of high reflection on the border of the elevated dark area, and intercellular gaps appear as small black dots at the vertices of endothelial cells[31].

Table 2: The visual signatures of the reviewed Bowman layer diseases.

Bowman layer diseases	
The disease	**The Visual signatures**
Reis-Bückler's dystrophy	− A high intensity elongated area with grey boundary that is interspersed with the basal epithelial cells[27]. − High reflective small granular materials replaces Bowman's layer. These granular materials group together as one big white area[28].
Thiel-Behnke dystrophy	− Deposits which appear hazy with different intensity values and cover a wide area of the basal epithelium. The edges of these hazy deposits have very low intensity undulating structure[8, 28]. − The Bowman's layer is completely hidden by reflective materials that appear as haze with different values of intensity and with some diffuse bright irregular spots[8, 28].

Table 3: The visual signatures of the reviewed stromal diseases.

Stromal diseases	
The disease	**The visual signatures**
Lattice dystrophy	− In the mid-stroma, small numbers of undulating and thin string-like structures of different lengths and high intensities interacting with the keratocytes which appear as hyper-reflective irregular shapes[29, 30]. − In the anterior and middle stroma, big bright tubular structure with well-defined edges interspersed with normal bright keratocytes. Keratocytes have irregular structures of different sizes[27].
Fleck dystrophy	− High intensity irregular large spots enclosed in a cyst-like structure throughout the stroma. A high intensity spherical area, relatively large, is connected with the cyst-like structure[8, 14].
Granular dystrophy	− High reflective irregular deposits varying in size appeared in the anterior stroma with high intensity curved line[27]. − In the deep stroma, a large number of high intensity small deposits of punctiform structures are dispersed between high reflective rounded and oval shape keratocytes nuclei[27].

Schnyder crystalline corneal dystrophy (SCCD)	– High reflective and small elliptic material accumulated inside and around anterior keratocytes which have hypo-reflective irregular structures[31, 33]. – In the anterior stroma, high intensity and well-delineated needle-shaped adjacent deposits largely bundled together[32]. – In the anterior stroma, some abnormal nerve branches can also be found. The nerve branches appear intersecting in the middle of the reflection area and have an irregularly curved shape[32, 33].
Avellino corneal dystrophy	– At the level of the superficial and middle stroma, a high reflective cloud of granular materials appears that has irregular curvy borders with low intensity[34].
Macular corneal dystrophy	– High reflective accumulation of granules in the superficial stroma[34]. – In mid-stroma, multiple hypo-reflective materials that have striae-like shapes appear. These linear shapes slope vertically and are thick[34].
Central Cloudy Dystrophy of François	– In the superficial stromal layer high reflective granules with irregular size groupings[35]. – Deep stroma appears as a hazy hyper-reflective background with many intersecting, low intensity, and thick lines[35].
Pre-Descemet's membrane corneal dystrophy	– Very small highly reflective dots interacting with normal keratocyte nuclei of anterior stroma[36]. – Posterior stroma shows hyper-reflective vesicles with irregular shapes containing bright granules[36].
Posterior amorphous corneal dystrophy	– Hyper-reflective sheet-like area which has spikes with medium intensity from the right side of it appearing in the posterior stroma[37].
Corneal amyloidosis	– Cotton candy-like reflection and fibrillar amyloid material that appears in the anterior stroma. This material has very uniform grey values[14, 45].
Fungal keratitis	– Fusarium solani reveals highly reflective hyphae of length (200-300) µm and of width (3-5) µm with branches at 90°angles in the anterior stroma and Round inflammatory cells are present [1, 46]. – Aspergillus hyphae have the same characteristics of Fusarium solani hyphae with branches at 45° angles[1, 46]. – Candida pseudiphilaments reveals high reflective elongated particles measure 10–40 µm in length and 5–10 µm located in the anterior stroma[1, 46].

Table 4: The visual signatures of the reviewed Descemet's membrane and Endothelial diseases.

Descemet's membrane and Endothelial diseases	
The disease	**The visual signatures**
Fuchs' endothelial dystrophy (Cornea guttata)	– Many roundish low intensity areas of different sizes with central light spots between the hyper-reflective endothelial cells which appear clearly[8, 14, 38, 39].

Iridocorneal endothelial syndrome (ICE syndrome)	− Endothelium appears as epithelium-like transformation with bright nuclei that appear as high intensity elliptic structures surrounded by unclear and irregular cell borders that have very low intensity[8, 40].
Posterior polymorphous corneal dystrophy (PPCD)	− Well-delineated roundish shape or elliptical endothelial lesions with low intensities near to black and appear in curvilinear pattern[8, 41].
Brown-McLean syndrome	− A highly reflective pigmentation intersperses endothelium cells which consists of an accumulation of bright round bodies with clear borders[42].

Appendix B

Table 5: Shape-based features extracted from the visual signatures of the reviewed corneal diseases.

Shape	Disease
Rounded (circle, ellipse, circular line)	**Epithelial and sub-epithelial:** • Amiodarone-induced keratopathy • Epithelial basement membrane dystrophy • Meesmann's dystrophy • Acanthamoeba infective keratitis • Viral keratitis: herpes simplex virus **Stromal:** • Fleck dystrophy **Descemet's membrane and Endothelial:** • Fuchs' endothelial dystrophy • Posterior polymorphous corneal dystrophy • Brown-McLean syndrome
Linear	**Epithelial and sub-epithelial:** • Advancing wave-like epitheliopathy

(stringy, elongated, needle-shape, tubular, striae-like, nerve, fibre)	• Epithelial basement membrane dystrophy • Salzmann's nodular degeneration • Thygeson epithelial keratitis • Recurrent Erosion Syndrome **Bowman layer:** • Reis-Bückler's dystrophy **Stromal:** • Lattice dystrophy • Schnyder crystalline corneal dystrophy • Macular corneal dystrophy • Central Cloudy Dystrophy of François • Fungal keratitis
Punctiform (granular)	**Epithelial and sub-epithelial:** • Thygeson epithelial keratitis **Bowman layer:** • Reis-Bückler's dystrophy **Stromal:** • Fleck dystrophy • Granular dystrophy • Schnyder crystalline corneal dystrophy • Macular corneal dystrophy • Central Cloudy Dystrophy of François • Pre-Descemet's membrane corneal dystrophy
Cotton (deposits with irregular shape, sheet-like, haze, pigmentation)	**Epithelial and sub-epithelial:** • Thygeson epithelial keratitis • Bacterial keratitis **Bowman layer:** • Thiel-Behnke dystrophy **Stromal:** • Granular dystrophy • Avellino corneal dystrophy • Posterior amorphous corneal dystrophy • Corneal amyloidosis
Irregular polygonal	**Epithelial and sub-epithelial:** • Salzmann's nodular degeneration • Gelatinous drop-like dystrophy **Descemet's membrane and Endothelial:** • Iridocorneal endothelial syndrome

Figures Legend

Figure	Caption
Fig. 1	Superficial epithelial cells. (a) Polygonal cell pattern, nucleus with bright illuminated cytoplasm, and perinuclear dark halo[1]. (b) Light cell boundaries and bright visible nuclei (arrow)[16].
Fig. 2	Wing cells. (a) Upper wing cells form a regular mosaic with sharp and reflecting cellular borders. (b) Lower wing cells form a regular mosaic with sharp and reflecting cellular borders which are smaller in size than upper cells[1].
Fig. 3	Basal epithelial cells: a regular mosaic of dark cell bodies with light borders without nuclei reflectivity[8].
Fig. 4	Sub-basal nerve plexus. (a) Bright, well-defined linear long nerve fibre bundles[8]. (b) The fibres are organised in a vortex pattern in the lower quadrant of the paracentral cornea[1].
Fig. 5	Bowman's membrane: randomly arranged collagen fibrils located in between the basal cells and the stroma[1].

Fig. 6	Stroma. (a) Hyper-reflective cell nuclei, typically forming clusters, with poorly visualised cell. (b) Corneal nerves in the mid stroma dividing into branches[8]. (c) Keratocyte density is high in the anterior stroma. (d) Keratocyte density is also high in the posterior stroma[1].
Fig. 7	Endothelium: Regular hexagonal hyper-reflective pattern of honeycomb regular mosaic surrounded by hypo-reflective borders and no found of any nuclei reflection[15].
Fig. 8	Amiodarone induced keratopathy. (a) Basal cell layer clearly showing hyper-reflective cell inclusions (LSCM: HRT II-RCM). (b) Increase in the number of micro-dots between the keratocyte nuclei in anterior stroma with the period of amiodarone ingestion[18] (LSCM: HRT II-RCM).
Fig. 9	Advancing wave-like epitheliopathy. (a) Atypical elongated cells and hyper-reflective nuclei at the level of the abnormal epithelium (TSCM). (b) Confluent hyper-reflective images at the sup-epithelial level[19] (TSCM).
Fig. 10	Epithelial basement membrane dystrophy. (a) Highly reflective irregular material (arrows) intermixed with the keratocytes of the anterior stroma and posterior epithelial interface (SSCM: Confoscan 2). (b) Abnormal distended cytoplasm with very reflective nuclei (arrow) in the basal epithelium (SSCM: Confoscan 2). (c) Elliptic cysts (arrows) with poorly defined of borders, and there is irregular reflective material within them (asterisk) (SSCM: Confoscan 2). (d) Long highly reflective and linear structures (arrows) that have diffuse posterior borders and well-delineated anterior borders[20] (SSCM: Confoscan 2).
Fig. 11	Salzmann's nodular degeneration. (a) Large cells with prominent nuclei and bright margins are present in the superficial layer of the central corneal epithelium (SSCM: Confoscan 4). (b) An abnormally thick sub-basal corneal nerve (arrow) is surrounded by highly reflective cells (SSCM: Confoscan 4). (c) The branches of the corneal nerves in the mid-stroma are tortuous and thick with both highly reflective segments along the bundles (arrow) and tracts with granular aspect (arrowhead)[22] (SSCM: Confoscan 4).
Fig. 12	Gelatinous drop-like dystrophy. (a) The epithelial cells are irregular in shape and often elongated (LSCM: HRT II-RCM). (b) At the level of the Bowman's membrane, a very small number of sub-basal nerves (LSCM: HRT II-RCM). (c) Large accumulations of brightly reflective amyloid materials within the anterior stroma[23] (LSCM: HRT II-RCM).
Fig. 13	Thygeson epithelial keratitis. (a) At the basal epithelial cell layer, aggregates of highly reflective deposits that had a cotton-like appearance (LSCM: HRT II-RCM). (b) Invasion of numerous material with highly reflective dendritic structure, and epithelial cells were swelling and cell gaps were widened[24] (LSCM: HRT II-RCM).
Fig. 14	Meesmann's dystrophy. (a) Round shape and well delineated lesions that surrounded by reflective points in the cytoplasm, which probably correspond to cell nuclei (arrows), some normal cell nuclei appear as reflective round images (arrowheads)[20] (SSCM: Confoscan 2). (b) Cyst-like changes at the level of the basal epithelium[8] (LSCM: HRT II-RCM).
Fig. 15	Recurrent erosion syndrome: A mucus band appears as a bright line over the epithelial mosaic[20] (SSCM: Confoscan 2).
Fig. 16	Acanthamoeba infective keratitis. (a) Hyper-reflective single-walled, round-shaped or ovoid cysts (arrows) within the corneal epithelium and stroma[1] (LSCM). (b) Bright double-walled, round-shaped or oval particles (arrows) are observed in the surrounding epithelium[15] (TSCM).
Fig. 17	Bacterial keratitis. (a) Typical hyper-reflective defects without recognizable structure[1] (LSCM: HRT II-RCM). (b) Distinct needle-like deposits or amorphous deposits at different epithelial depths (LSCM: HRT II-RCM). (c) Increased density of hyper-reflective dendritic-like cells within the epithelium[8] (LSCM: HRT II-RCM). (d) Thin and short filamentous structures demonstrating right-

	angled branching within the epithelium (white arrow), surrounding by round to oval bright inflammatory cells (black arrow)[29] (SSCM: Confoscan 3).
Fig. 18	Viral keratitis: herpes simplex virus. (a) Increase in cell size and hyperreflectivity while there is a decrease in cell density[30] (SSCM: Confoscan 4). (b) Pseudoguttata possess a line of high reflection on the border of the elevated dark area (single arrows), and intercellular gaps appear as small black dots at the vertices of endothelial cells (double arrows)[31] (SSCM: Confoscan 3).
Fig. 19	Reis-Bückler's dystrophy. (a) Deposition of homogeneous, reflective material (arrows) interspersed with the basal epithelial cells (SSCM: Confoscan 4). (b) Highly reflective material (asterisk) at the level of Bowman's layer (SSCM: Confoscan 4). (c) Fine diffuse deposits (asterisk) interspersed between keratocyte nuclei (arrows) in the anterior stroma[27] (SSCM: Confoscan 4). (d) Deposition of highly reflective irregular and granular materials without dark shadows in the basal epithelial layer (LSCM). (e) High reflective small granular materials replaced Bowman's layer[28] (LSCM).
Fig. 20	Thiel-Behnke dystrophy. (a) Deposits with homogenous reflectivity, rounded edges and dark shadows in the basal epithelium (LSCM). (b) The homogeneous reflective materials completely replaced Bowman's layer[28] (LSCM).
Fig. 21	Lattice dystrophy. (a) Unspecified undulating and thinner structures, apparently interacting with the keratocytes in the mid-stroma (TSCM: model 165A). (b) Highly reflective deposits under the basal epithelial cells (TSCM: model 165A). (c) Bowman's layer is irregular and thicker, seen as differences in gray-scale[30] (TSCM: model 165A). (d) Poorly demarcated, hyper-reflective, linear, and branching structures of varying intensity for amyloid deposition[8] (LSCM: HRT II-RCM).
Fig. 22	Lattice dystrophy (Cont'd). (e) Reflective punctiform structures (arrows) in the basal epithelial cell layer (SSCM: Confoscan 4). (f) Diffuse, reflective deposits (asterisk) interspersed between keratocyte nuclei (arrows) in the anterior stroma (SSCM: Confoscan 4). (g) Filaments with well-defined edges (arrowheads) interspersed with normal keratocytes (arrows) in the anterior and middle stroma[27] (SSCM: Confoscan 4).
Fig. 23	Fleck dystrophy: Hyper-reflective and largely intracellular dots distributed throughout stroma, and a cluster of these hyper-reflective dots enclosed in cyst-like structures[14] (SSCM).
Fig. 24	Granular dystrophy. (a) Hyper-reflective breadcrumb-like deposits in the basal epithelial cell layer (arrows) (SSCM: Confoscan 4). (b) Reflective diffuse deposits (arrows) in the anterior stroma near the Bowman layer region, and a nerve fibre without myelin appears as a bright line (arrowhead) (SSCM: Confoscan 4). (c) Keratocyte nuclei (arrows) interspersed with punctiform reflective deposits (arrowheads) in the deep stroma[27] (SSCM: Confoscan 4).
Fig. 25	Avellino corneal dystrophy. (a) Focal deposits of highly reflective material with irregular edges in basal epithelium (LSCM: HRT II-RCM). (b) Clusters of highly reflective granular materials with irregular edges at the level of the superficial and middle stroma[34] (LSCM: HRT II-RCM).
Fig. 26	Schnyder crystalline corneal dystrophy. (a) Highly reflective material has accumulated inside and around anterior keratocytes (arrows) (TSCM: model 165A). (b) Brightly reflective deposition is associated with the sub-epithelial nerves (arrows)[31] (TSCM: model 165A). (c) Crystalline deposits with needle shaped appear in the anterior stroma (LSCM). (d) Small number of sub-epithelial nerves is detectable with high background intensity at the level of Bowman's layer[32] (LSCM). (e) Abnormal nerve branches with an irregularly curved appearance in the anterior stroma[33] (SSCM: Confoscan 2).
Fig. 27	Macular corneal dystrophy. (a) Highly reflective deposits without distinct borders in the superficial stroma (LSCM: HRT II-RCM). (b) The middle stroma shows homogeneous reflective materials with dark striae-like images[34] (LSCM: HRT II-RCM).
Fig. 28	Central Cloudy Dystrophy of François. (a) Small highly reflective granules and deposits in the superficial stromal layer (SSCM: Confoscan 2). (b) In the deep stroma, multiple dark acellular striae among extracellular matrices with increased intensities[35] (SSCM: Confoscan 2).

Fig. 29	Pre-Descemet's membrane corneal dystrophy. (a) Anterior stroma shows normal keratocyte nuclei and a few irregular highly reflective particles (SSCM: Confoscan 2). (b) Posterior stroma shows hyper-reflective, non-homogenous structures with numerous tiny inclusions (arrows)[36] (SSCM: Confoscan 2).
Fig. 30	Posterior amorphous corneal dystrophy. Hyper-reflective sheet-like opacities with spikes extending from the posterior stroma[37] (SSCM: Confoscan 3).
Fig. 31	Corneal amyloidosis: Sub-epithelial and anterior stromal gray white, extracellular, hyper-reflective, and cotton candy-like deposits[14].
Fig. 32	Fungal keratitis. (a) Highly reflective hyphae (Fusarium solani) with branches at 90°angles in the anterior stroma (white arrows), and round inflammatory cells (black arrows)[46] (LSCM: HRT II-RCM). (b) High reflective elongated particles (Candida pseudiphilaments) in the anterior stroma (white arrows)[46] (LSCM: HRT II-RCM).
Fig. 33	Fuchs' endothelial dystrophy. (a) Roundish hypo-reflective areas of varying size surrounded by hyper-reflective endothelial cells[39] (TSCM: Topcon SP 2000 P). (b) Descemet's membrane displays significant fibrosis[38] (SSCM: Confoscan 2).
Fig. 34	Iridocorneal endothelial syndrome: Epithelium-like transformation with prominent bright nuclei, and with irregular and indistinct borders[8] (LSCM: HRT II-RCM).
Fig. 35	Posterior polymorphous corneal dystrophy. (a) Round lesions containing endothelial cells with surrounding haze[8] (LSCM: HRT II-RCM). (b) A prominent hyper-reflective band lesion at the level of descemet's membrane (arrow) (SSCM: Confoscan 2). (c) Endothelial vesicular lesions in curvilinear pattern with associated endothelial pleomorphism and polymegathism (SSCM: Confoscan 2). (d) Prominent corneal nerves at the level of Bowman's membrane in patient with more advanced posterior polymorphous dystrophy[41] (SSCM: Confoscan 2).
Fig. 36	Brown-McLean syndrome. (a) Masked stromal structure with no clear outline of the stromal cells (SSCM: Confoscan 2). (b) At endothelium, pigmentation appears as round, bright, hyper-reflective bodies[42] (SSCM: Confoscan 2).