Specular microscopy
Clinical specular microscopy is a practical tool not only for the cornea subspecialist in evaluating donor corneas and corneal dystrophies but also for all the ophthalmic surgeon in identifying subtle as well as macroscopic changes in the cornea prior to surgery.
Optical principle

Light striking a surface can be reflected, transmitted, or absorbed. Generally, some combination of the three effects occurs. Of primary importance in clinical specular microscopy is the light that is reflected specularly (‘mirrorlike’), where the angle of reflection is equal to the angle of incidence. It is this light that is captured by the specular microscope and forms the image of interest.
noncontact specular microscopy

- The patient blink to wet the cornea and then to hold still just prior to image capture improves image sharpness
- Noncontact specular microscopes use **internal fixation points** to provide a more standardized approach to consistently imaging the central endothelium, midperiphery, and periphery
- With noncontact instruments **imaging a thickened cornea** will sometimes fail in the automated capture mode and may require the operator to use a **manual mode**
contact specular microscope

- Anesthetic should be used
- The beam of light is directed through the pupil to ensure the placement of the cone on the most central portion of the cornea. Systematic scanning superiorly, inferiorly, nasally, and temporally will ensure a thorough evaluation of the endothelium
- Light reflexes from the iris can obscure the endothelial mosaic and are best eliminated by dilating the pupil
Methodologies in analysis software

- fixed-frame
- variable-frame
- center methods
  automated or semiautomated or manual
Specular Microscope SP-3000P

- Three modes for image capture
  - **AUTO**: With auto alignment and auto firing, this mode requires virtually no training and is quick and convenient for any user.
  - **SEMI-AUTO**: Manual alignment allows the user more control on the location of the reading. Auto firing simplifies the capture. Useful in patients with fixation difficulty or with irregular corneas.
  - **MANUAL**: Offers total control with manual alignment and manual firing. Basic method, useful in corneas with weak reflection or other anomalies.
accuracy of quantitative analysis depends on:

- Image quality: Image quality must be sufficient to enable the technician to identify the cell borders, boundaries, and centers.
- The technician's understanding.
- Technique in performing the specific analysis method.
- Technicians performing specular microscopy should be trained to recognize normal and abnormal.
The most common errors seen in the clinical setting related to specular microscopy are related to improper application of the method of analysis. Examples of common errors are double counting or missing cells when performing the center method and failing to recognize when automated analysis has traced cell borders inaccurately.
• Technician should image the region three times at the same sitting and record the average of the three images’ analyses
• Use the same image analysis method from baseline and throughout the follow-up period
• Most specular microscopic studies determine solely the endothelial cell density of the central endothelium. This may be misleading and does not necessarily reflect the impact of a surgical procedure primarily damages the peripheral cornea. Changes in the central corneal endothelium in both density and morphology may take some time (months, years)
• The paracentral and peripheral endothelium, in particular superiorly, has a higher cell density than the central endothelium
Quantitative and Qualitative Specular Microscopy

Epithelium

- Corneal epithelial cells do not normally present a flat surface suitable for specular microscopy. If a contact lens is pressed the epithelial cells can be flattened and can reflect light in a mirror-like fashion

- The normal corneal epithelium contains polygonal cells of varying brightness. Most cells can be placed into one of three groups: dark, medium, and light. Hexagonal, pentagonal, and triangular cells may be present, but rounded, enlarged, or elongated cells are considered abnormal
Elongated or enlarged corneal epithelial cells

- wound healing processes
- penetrating keratoplasty and epikeratophakia
- daily or extended soft contact lens wear
- dry eyes
- neurotrophic keratitis
- aphakia
- diabetes
- in keratoconus patients, especially near the cone's apex
Endothelium

- The normal specular micrograph of a young person should show a regular endothelial mosaic of hexagonal cells of approximately the same size. Cell boundaries should be well defined.
- With age, endothelial cells become larger and the cellular pattern becomes distinctly pleomorphic, even though the cell pattern can be pleomorphic, the cell size is not always increased.
• endothelial cell density (**ECD**) (measured as cells/mm$^2$)

• mean cell area (measured as µm$^2$/cell)
  
  coefficient of variation (**CV**) (standard deviation of cell areas/mean cell area)

• pleomorphism (usually measured as a percentage of 6, <6 or >6-sided cells)
Polymegethism Endothelial Morphology

CV=45  CD=3268
CV=76  CD=2967
CV=58  CD=3121
**corneal guttae**, are excrescences of Descemet's membrane. They can be seen with the *specular microscope* much earlier than with the *slit lamp*. They begin as small, dark structures, but, in time, grow, often becoming larger than individual endothelial cells.

Guttae can also be seen in the far periphery of young individuals. In this case, they are called *Hassall-Henle warts*. They usually shaped more like a dome than a mushroom, and the surrounding endothelium does not appear as abnormal.

The incidence of *corneal guttae* increases significantly with age. They appear as mushroom-shaped excrescences of Descemet's membrane.
Various dark structures can also be seen in specular micrographs. One such structure appears to be intracellular, is small, and has sharp borders. This structure is thought to represent endothelial cilia. Another intracellular dark structure is much larger and less distinct, suggesting that it is located within the cell. These are thought to represent intracellular vacuoles or blebs.
Some bright structures span several endothelial cells and have sharp borders, indicating they are likely very near the endothelial–stromal interface. These structures are believed to represent pigment deposits on the endothelial surface.

Intracellular bright structures can sometimes be seen to be associated with stressed cells such as those seen after corneal transplantation. The size of the bright structure often corresponds to the size of the endothelial cell (the larger the cell, the larger the bright structure). This structure is felt to represent the cell nucleus.
Fig. 19.8 Miscellaneous endothelial structures. Arrows indicate features mentioned. A, Isolated smooth excrescences (corneal guttae). B, Multiple coalesced excrescences. Only the bright reflection from the apex of each excrescence is clearly seen. C and D, Intracellular bright structures possibly representing cell nuclei. E, Pigmented endothelial deposits. F, Dark structures possibly representing endothelial cilia. G, Intracellular dark structures possibly representing intracellular vacuoles. H, Intercellular dark structures, believed to be invading inflammatory cells.
specular microscopy indications

When an endothelial abnormality is noted in slit lamp:

- corneal guttae
- keratic precipitates
- pigmented and inflammatory cells
- endothelial surface or Descemet's membrane irregularities
- increased corneal thickness
- A history of possible endothelial abnormality, i.e. family history of corneal dystrophy, trauma, acute narrow-angle or chronic open-angle glaucoma, uveitis, keratitis, graft rejection, previous ocular surgery, secondary intraocular lens implantation, or corneal transplantation
Clinical Applications for Specular Microscopy

- Corneal edema occurs in between 300 and 700 cells per mm$^2$
- Assuming a cell loss in the range of 0–30% for any given intraocular surgical event
- A patient should have at least 1000 to 1200 cells per mm$^2$ to safely undergo most anterior segment surgery
- There should be no significant difference in the ECD between eyes. To be meaningful, this difference should be greater than 280 cells per mm
A cornea with a coefficient of variation greater than 0.40 or the presence of less than 50% hexagonal cells should be considered abnormal and at increased risk for postoperative edema.

As in ECD measurements, there is a range of variation of polymegathism and pleomorphism in all age groups, and age alone cannot be used to predict the endothelial morphologic appearance.
When evaluating postoperative corneas, it is particularly important to utilize multiple images in the central, midperiphery, and even periphery, because a regional disparity in ECD and morphology in these postoperative corneas has been reported.
Aging

- ECD decreases (or mean cell area increases) throughout life.
- Cell loss is most rapid from birth to the first few years of life. Part of this decrease in ECD may be due to the normal enlargement of the globe during early childhood.
- ECD is rather stable from age 20 through approximately age 50 years.
- After the age of 60 years, ECD decreases significantly in most people. On average, age-related cell loss is approximately 0.5% per year.
- Polymegathism and pleomorphism is variable in correlate with age.
Fuchs’ endothelial corneal dystrophy

Specular microscopy of FECD demonstrates that an individual excrescence begins as a very small structure, much smaller than an individual endothelial cell. Adjacent endothelial cells appear normal.

In time, the excrescence grows and begins to distort overlying and adjacent endothelial cells, making the borders of these cells indistinct. The guttae themselves appear as dark spots, sometimes with bright central reflections.

In the case of FECD, they are usually more numerous centrally.
Fig. 19.14 Specular photomicrographs and drawings of various stages of corneal guttata in Fuchs’ dystrophy (×100). A and B, Stage 1: early form of corneal guttata. Ex crescences are smaller than individual endothelial cells. Dark areas are the sides of the ex crescence. Bright spots are each a reflex from the apex of the ex crescence. C and D, Stage 2: corneal guttata approximate the average endothelial cell in size. E and F, Stage 3: corneal guttata are considerably larger than the average endothelial cell. Adjacent endothelial cells are abnormal in appearance. G and H, Stage 4: individual corneal guttata have coalesced and contain more than one apical bright spot. Boundaries of adjacent endothelial cells are absent or difficult to identify. I and J, Stage 5: coalesced ex crescences and nearly complete disorganization of adjacent endothelial mosaic. Numerous bright structures presumably are collagenous material deposited at the level of Descemet’s membrane or on the posterior corneal surface.
Specular evaluation, can aid in deciding whether penetrating or endothelial keratoplasty is necessary at the time of intraocular surgery:

- If there are a significant number of peripheral endothelial cells and the guttae are primarily located centrally, there is a decreased likelihood that corneal transplantation will be necessary.

- If there is complete confluence of guttae even in the peripheral cornea associated with increased corneal thickness of over 0.68 microns (µm), one might consider a combined procedure.

- Some surgeons do not consider keratoplasty combined with another intraocular procedure unless evidence of significant stromal edema is already present preoperatively.
Iridocorneal endothelial syndrome

- Rounding off of cell angles
- There is a loss of cellular definition and hexagonal shape. Many pentagonal cells are evident
- An increased granularity of the intracellular details
- Small, centric dark areas in individual cells which can enlarge and become completely blacked-out areas within the cell
- As the disease progresses, the endothelial monolayer may no longer be recognizable as a mosaic of cells. A ‘reversal appearance’ may develop, with black central areas and white borders
Posterior polymorphous corneal dystrophy

- When examined with the specular microscope, the vesicle has a thick, dark border yielding a doughnut-like appearance. This structure appears to lie anterior to undistorted endothelial cells, which are generally more recognizable toward the center of the lesion.

- The endothelial cells adjacent to the structure are distorted and commonly smaller than normal.
Keratoconus

- The most striking abnormality in keratoconus, is elongation of endothelial cell
- Localized areas of endothelial cells that are 7 to 10 times larger than normal near the site of rupture. Away from the site of rupture, however, the endothelial cells are of a more normal size and morphologic appearance
- Presence of dark bodies completely contained within an otherwise normal-appearing cell. These dark bodies occur less frequently in larger endothelial cells and are consistent in appearance with blebs or vacuoles seen with the electron microscope
Glaucoma

- Persistently elevated intraocular pressure likely results in gradual loss of endothelial cells and progressive loss of endothelial function; however, revealed no morphologic changes despite elevated pressures.

- These experiments suggest that endothelial cell loss is not a direct result of increased pressure, but rather some other disturbance such as prolonged low oxygen concentration in the aqueous humor. If the pressure is medically controlled, cell loss is reduced.

- Studies have shown varying cell loss connected with various glaucoma procedures, in particular trabeculotomy and tube shunts.
Intraocular inflammation

During an acute episode of anterior uveitis, mononuclear inflammatory cells penetrate apical junctional complexes and infiltrate both between endothelial cells and between endothelial cells and Descemet's membrane. Endothelial cells, however, are not generally harmed by this process, but in the most severe case can become dislodged and float free in the aqueous humor.
Endothelium of a patient with acute anterior uveitis. Note the numerous dark structures and the dislodged endothelial cells in the center of the image.
Cataract extraction with intraocular lens implantation

- Endothelial cell loss following uncomplicated phacoemulsification and posterior chamber intraocular lens implantation using viscoelastic and modern, small-incision techniques is quite low, ranging from no detectable cell loss to 20%.

- No statistical difference between endothelial cell loss resulting from phacoemulsification versus extracapsular cataract extraction.
- suture fixation of a posterior chamber intraocular lens or placement of an intraocular lens in the sulcus appears to be no more traumatic and results in no more endothelial cell loss than a posterior chamber intraocular lens placed in the capsular bag.

- The implantation of an anterior chamber lens, however, has been shown to result in not only higher endothelial cell loss due to the procedure itself but also continued endothelial loss which is greater than that of posterior chamber intraocular lenses.
Refractive surgery

- neither LASIK nor PRK results in a decreased endothelial density
- Laser ablation of the stroma within 200 µm of the corneal endothelium, however, will result in endothelial structural changes and the formation of the amorphous substance deposited onto Descemet's membrane
many investigators have described an increase in central endothelial cell density after PRK and LASIK. This is felt to be a result of endothelial migration initiated by the discontinuation of contact lens wear and not a result of refractive surgery
• mitomycin-C during LASEK have also found no significant changes in the corneal endothelium

• intrastromal corneal ring, Significant endothelial cell loss was not present at the 6, and 12-month visits, but a decrease in ECD was found at the 24-month visit, which may or may not be related to surgery
long-term studies on the FDA-approved Artisan/Verisyse phakic intraocular lens (IOL) have found acceptable mean cell loss rates of 1.8% per year after insertion to correct high myopia. Hexagonality and coefficient of variation during a 4-year follow-up study were comparable to preoperative numbers.

Candidates who narrowly meet the minimal ECD of 2000 cells/mm² are suggested to have greater anterior chamber depth due to greater cell loss.
Penetrating keratoplasty

- 5 to 10 years after successful PKP for a variety of indications including keratoconus and endothelial dysfunction disorders, endothelial cell loss progresses at a rate 7 times faster than normal. However, between 10 and 15 years, this rate substantially slows and approaches the rate of loss of endothelial cells in normal aging.

- **donor age**: younger age group (12–65 years old) experienced a substantial but slightly lower 69% median percentage cell loss, compared to the older group (66–75 years old) of 75% cell loss.
Phakic patients lose significantly more endothelial cells during PKP than do aphakic patients. This finding was attributed to the deeper anterior chamber and the absence of endothelial trauma from the lens–iris diaphragm in the aphakic eye.
endothelial permeability, rather than the variation in cell size (polymegathism) or cell shape (pleomorphism) of endothelial cells, is the major factor that determines the thickness of a clear corneal graft.

Besides quantitative determination of changes in cell density and morphometric parameters, qualitative specular microscopy can detect early evidence of graft rejection not detectable by clinical examination of value in following certain high-risk patients.

During graft rejection episodes, one sees intercellular bright bodies, black inflammatory cells, and generally recognizable keratic precipitates on the endothelial surface.
Endothelial keratoplasty

Most authors have reported significantly greater cell loss in the first 6 months after EK compared to PKP. Interestingly, although there is greater loss at 1 year when compared to PKP, the rate of cell loss begins to level off around 6 months, unlike PKP.
• No significant differences in cell loss have been shown between donor posterior lenticule prepared by the surgeon versus the eye bank.

• In regards to wound size, a smaller-sized incision for EK surgery results in greater acute endothelial area damage than larger size (5 mm) incisions.
Donor corneas

- The minimum donor ECD is established for keratoplasty, for most eye banks is at 2000 cells/mm²
- Ancillary Study has reported that baseline donor ECD does not predict graft failure 5 years after PKP; however, the 6-month ECD was predictive of subsequent failure
Intraocular irrigating solutions

- Endothelial structure and function are best maintained when solutions resemble aqueous humor in composition
- Irrigating solutions not satisfying the basal metabolic requirements of the corneal endothelium. In vitro perfusion experiments demonstrate that these endothelial changes and the resultant corneal swelling are caused by intraocular irrigating solutions that are deficient in certain essential components.
Vitreocorneal contact

Some endothelial cells are markedly enlarged and grossly abnormal in shape. Others contain abnormal bright or dark structures within their cell boundaries. Abnormal cell intersections and side length distribution are encountered, and the central guttate excrescences of FECD are often seen.

Despite the seeming irreversibility of the endothelial changes, clinical reversal of corneal edema may occur on occasion in cases of moderately prolonged vitreous contact.
Evidence of prior endothelial damage was revealed only by clinical specular microscopy. The endothelial cells were enlarged and the central endothelial cell density was only 47% of that of the opposite, normal eye. Although it is suspected that in such instances the residual endothelial cells might be more susceptible to subsequent trauma than normal cells, no additional cell loss was documented following cataract extraction by phacoemulsification in this particular case.
- The impact of small, nonpenetrating foreign bodies on the cornea may give rise to posterior annular keratopathy, clinically apparent gray rings on the corneal endothelium. Specular microscopic studies demonstrate that posterior annular keratopathy occurring after blunt corneal trauma in humans represents a contusion injury and consists of disrupted and swollen endothelial cells.

- The degree of endothelial cell loss appears to be related to the severity of the injury.
Contact lens wear

- Both acute and chronic endothelial changes are seen with the specular microscope following contact lens wear.
- Within minutes of application of a contact lens, small dark endothelial blebs occur that disappear quickly if the lens is removed. These endothelial blebs reach a maximum size in 20–30 minutes from the time the contact lens is placed on the cornea and then gradually decrease in size, may represent the effects of hypoxia or lactate accumulation.
- Long-term wear of either hard or soft contact lenses results in an increased polymegathism that is not reversed upon cessation of lens wear, although some recovery towards normal might occur. The degree of polymegathism increases as the period of time the lenses are worn increases. The degree of polymegathism also depends upon the type of lens worn.
Diabetes

- In diabetes the cell density significantly decreases with age. However, no difference in corneal thickness or endothelial permeability to fluorescein has been described in two studies.
- Diabetic corneas also exhibit increased polymegathism and pleomorphism and a decreased percentage of hexagonality.
- Topical administration of aldose reductase inhibitor can reverse these morphologic changes, suggesting that aldose reductase may be involved in the etiology of corneal endothelial variations in diabetic patients.