Some optical principles of the clinical specular microscope. WILIAM M. BOURNE AND JAY M. Enoch.

The clinical specular microscope is used to examine and photograph human endothelial cells in vivo. An optical analysis is presented here in simple form to aid the ophthalmologist or visual scientist in understanding how this instrument can be used in a quantitative manner to measure the size of the magnified endothelial cells and the thickness of the overlying cornea. An error analysis is discussed in order to demonstrate some aspects of the precision of the determination.

The clinical specular microscope allows the examination and photography of human endothelial cells in vivo. This instrument utilizes a flat-surfaced dipping cone lens in front of the objective lens of the microscope to applanate and partially stabilize the cornea. This paper discusses, in simple terms, the optical principles of such an arrangement and three practical applications concerning corneal thickness measurement, magnification variability, and photographic measurement.

Fig. 1 is a schematic diagram of the objective lens with its attached dipping cone applanating the cornea. The dipping cone may be moved 2 mm. in an anterior-posterior manner for different object distances, i.e., different corneal thicknesses. This constitutes the only means of focusing the instrument, since all other distances in the system, which ultimately focuses the image on a camera viewing screen, are fixed. The dipping cone forms an image of the corneal endothelium at the object point of the objective. After applanating the cornea, one focuses by adjusting the dipping cone separation from the objective to provide sharp imagery for the photographic record.

Corneal thickness. When focusing the device, an estimate of corneal thickness (as applanated) may be obtained by using the basic lens equation. Let U = incident reduced vergence in diopters, P = power of the lens surface in diopters, and V = resultant reduced vergence in diopters.

\[ U + P = V. \]

For the first lens surface,

\[ \frac{n_1}{u_1} + P_s = \frac{n_2}{v_1}. \]

Using the corneal endothelium as object, the thickness of the applanated cornea is \( u_c \) (in meters); this is also the object distance \( u_1 \). The corneal index of refraction of the applanated cornea, \( n_c \) is also \( n_1 \), and \( P_s = 0 \) diopters (the applanating surface is flat). \( n_2 \) is the index of the glass applanating lens and \( v_1 \) is the image distance in index \( n_3 \).

By definition,

\[ \frac{n_1}{u_1} = \frac{n_c}{u_c}, \]

and since \( P_s = 0 \)

\[ \frac{n_2}{u_c} = \frac{n_3}{v_1}. \]

For sign convention, let corneal thickness (\( u_c \)) and \( u_1 \) be negative. \( P_s \) is the second or posterior curved surface of the dipping cone which has thickness \( t \). From the diagram, \( u_2 = v_1 + t \) and \( v_1 = u_1 - t \).

Thus,

\[ \frac{n_2}{u_c} = \frac{n_1}{v_1} = \frac{n_1}{u_1 - t}. \]

Solving for \( u_c \),

\[ u_c = \frac{n_1 u_1}{n_2} + t. \]

Let \( n_s \) the space between the dipping cone and the microscope objective lens be air, hence, \( n_s = 1 \). The image point, \( v_1 \), must be located at the object point of the following microscope objective for clear focus. Any change in dipping cone position relative to the objective, by necessity, must reflect a change in corneal index

*This relationship assumes that the dipping cone is comprised of a single lens.

†Both \( v_1 \) and \( t \) are negative when determining \( u_c \).
or thickness (that is, its optical path length). The object distance to the microscope objective is $u_3$, and $u_2 = v_2 + d$. Thus, $v_1 = u_1 - d$ with $u_1 = \text{constant}$, $d$ is the variable positional factor (the separation of the second surface of the dipping cone from the first surface of the objective) of the moveable applanating dipping cone needed to focus the objective. For sign convention, $u_3$ must also be negative. $d$ in practice is dependent upon the optical constants of the dipping cone and the objective, and the index and thickness of the applanated cornea. The latter point is easily demonstrated. Again using the familiar lens equation,

$$\frac{n_1}{u_1} + P_1 = \frac{n_2}{v_2},$$

and substituting as above for $u_2$, $n_2$, and $v_2$,

$$\frac{n_3}{u_3} = \frac{1}{u_3 - d}.$$

Solving for $u_3$,

$$u_3 = \frac{n_3}{\frac{1}{u_3 - d} + P_3}.$$

$u_3$, $P_3$, $t$, and $n_2$ are constant, and $n_3$ can be assumed constant in the case of the normal cornea ($n_3 = 1.376$ from Gullstrand).\(^4\) $$u_3 = 1.376 \left(\frac{1}{1 - d} - \frac{k_3}{k_3 - d - k_3}\right)$$

(Final form, $k = \text{constants}$)

Thus, determination of $d$ provides a simple measure of corneal thickness using an assumed corneal index, and the optical constants of the dipping cone.

If the cornea is edematous and increases its thickness from approximately 0.5 mm. to 0.7 mm., the index of the edematous addition is 1.334 (water—assume no entry of albumin, etc., in this case). This approaches a "worst case analysis." To estimate corneal index of refraction (swollen), $n'_c$, we add a proportionate corneal addition composed of water,

$$n'_c = \frac{0.5}{0.7} \left(1.376\right) + \frac{0.2}{0.7} \left(1.334\right) = 1.364.$$ 

We then take the ratio of estimated corneal index (swollen) to corneal index (normal),

$$\frac{n'_c}{n_c} = \frac{1.364}{1.376} = 0.991.$$ 

This simple exercise demonstrates that the error in estimation of corneal index of refraction would only be about 1 per cent in this nearly "worst case" edematous cornea if one used a constant corneal index (normal). This would lead to a comparable 1 per cent error in the determination of corneal thickness ($u_3$). The justification for this approach comes from interference microscopy where it is stated that the index of a biological medium is given by the following formula:

$$n_{\text{medium}} = n_{\text{water}} + kC$$

where $k$ is a constant and C is concentration of solids. One assumes that the addition of water simply lowers the concentration of solids (proteins, lipids, and lipoproteins)\(^4\) in a given medium.

Hence, a reasonably accurate means exists for measuring corneal thickness at the observation point while one is in the act of adjusting the dipping cone for best microscope focus of the corneal endothelium. The measure can be made
sensitive by fine calibration of the dipping cone adjustment mechanism.

**Magnification variability.** Another question to consider is how much the magnification of the final endothelial image on film varies for corneas of different thicknesses. Using the basic lens equation for magnification,

\[ M = \frac{U}{V} \]

and

\[ M_{\text{total}} = M_{\text{FDC}} \times M_{\text{BDC}} \times M_{\text{Obj}} \times M_{\text{EP}} \]

where FDC = front surface of dipping cone, BDC = back surface of dipping cone, Obj = objective lens, and EP = eyepiece. In the terms we have been using for this instrument,

\[ M_{\text{total}} = \frac{U_i}{V_i} = 1. \]

In addition, in this instrument \( U_i/V_i \) and all subsequent terms are held constant. Hence, the only variable term is \( U_i/V_i \). Thus,

\[ M_{\text{total}} = M_{\text{FDC}} = \frac{U_i}{V_i} = 1. \]

As derived above,

\[ U_i = \frac{n_i}{u_i} = \frac{n_2}{n_e + t} = \frac{1}{u_e + \frac{t}{n_e}} \]

and by substitution,

\[ M_{\text{total}} = 1 + P_i \left( \frac{u_e}{n_e} + \frac{t}{n_i} \right) \cdot \]

We may use the “worst case” analysis illustrated previously to compare the image magnifications obtained through a normal cornea 0.5 mm. thick and a swollen cornea 0.7 mm. thick. In our instrument, \( P_i = +40 \) diopters, \( t = 12.5 \) mm., and \( n_i = 1.56883 \).

For the normal cornea,

\[ M'_{\text{total}} = 1.4998 \]

for the abnormal cornea,

\[ M'_{\text{total}} = \frac{1}{1 + 40 \left( \frac{-0.0007}{1.376} + \frac{-0.0125}{1.56883} \right)} = 1.5134 \]

Taking the ratio of the two magnifications (all other factors do not vary and hence cancel),

\[ \frac{M'_{\text{normal}}}{M'_{\text{abnormal}}} = \frac{1.5134}{1.4998} = 1.0091 \]

or a 0.91 per cent change in magnification.

One may conclude that the variation in magnification is less than 1 per cent over a broad range of corneal thicknesses through which the endothelium may be seen.

**Photographic measurement.** A third practical optical consideration with the clinical specular microscope is a knowledge of the exact magnification of the image obtained on film, so that cell size, etc., may be measured. Analysis is simplified because essentially only flat surfaces are involved when applanating the cornea through use of the dipping cone. If the dipping cone is kept at a constant distance from the objective, then the optical system cannot be varied, and all the terms in the optical expression of the instrument become constants. Therefore, magnification is constant. One merely needs to set the dipping cone at the proper position for focusing the endothelium of a representative normal cornea (\( U_i = U_c \)) and then use the instrument to photograph a flat calibrated surface through any liquid medium. To be in focus, rays from the surface used for calibration must have a vergence of \( U_i \). Therefore, the focused surface will be at a distance of \( U_i \) from the dipping cone, depending upon the index of refraction \( n_i \) of the medium through which the photograph is taken as defined by the equation

\[ U_i = \frac{n_i}{u_i} \cdot \]

The magnification of the image of the flat calibrated surface on film will then be the same as that of the endothelium of the normal applanated cornea, and appropriate size comparisons between photographs can be made. As noted in the previous discussion, any error in measured magnification in corneas of abnormal thickness will be approximately 1 per cent or less.

The preceding simplified analyses demonstrate how the clinical specular microscope may be used to measure accurately the size of the magnified endothelial cells and the thickness of the overlying cornea.

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REFERENCES

Experimental studies with Staphylococcus aureus in M-K media. FREDERICK S. BRIGHTBILL, CATHERINE TERRONES, and SHIRLEY GOULD.

This study reviewed the potential for survival of a pathogenic bacteria when inoculated into McCarey-Kaufman modified tissue culture media 199. A clinically isolated specimen of Staphylococcus aureus was selected and rabbit eyes inoculated with 0.1 ml of the organism in a suspension of 1 to 3 x 10^5. Upon enucleation 12 hours later no clinical signs of infection were noted. Limbal-conjunctival cultures were obtained on all eyes before and after application of the antibiotic. Corneas stored in M-K media with standard penicillin-streptomycin added and cultured up to 48 hours. Our studies showed some recovery of the infecting organism from the media with topical antibiotic application but none following complete immersion of eyes in antibiotic. Direct cultures from corneal buttons taken 48 to 72 hours after antibiotic application by either method showed 1 to 3 colonies per plate in 25 per cent of eyes. In this study improperly applied antibiotic allowed some survival of S. aureus in the media.

Rapid acceptance of McCarey-Kaufman modified tissue culture media 199 used for short-term corneal cold storage has come about with little supporting evidence for proper sterilization procedures. The media supplier (Warner Lambert Research Institute, Morris Plains, N. J.) guarantees sterility of the solution and container but donor corneas with known bacterial flora may serve as potential media contaminants. Treatment of donor eyes prior to grafting with antibiotic solutions has proved effective in reducing corneal and conjunctival organisms (Table I). The purpose of this study was to determine whether M-K modifications are sufficient to suppress survival of donor eye pathogens introduced when corneal-scleral buttons are placed into the media.

Penicillin-resistant Staphylococcus aureus was isolated from a patient with acute conjunctivitis and inoculated into rabbit eyes. Globes were enucleated 12 hours later, cultured, treated with neomycin-sulfate-Polymyxin B sulfate by either of two methods, recultured, and corneal-scleral buttons placed into M-K media for culture up to 48 hours.

Material and methods. Albino rabbits weighing between 1 and 2 kilograms were used. The lower cul-de-sac of each eye was inoculated with 0.1 c.c. of S. aureus in a suspension of 1 to 3 x 10^8 cells. The isolate was a penicillin-resistant strain obtained from a human eye with acute conjunctivitis. A 24-hour culture of the organism was suspended in Tryptose phosphate broth and plate dilutions were done to determine the concentration.

At 15 hours after inoculation the eyes were examined for clinical signs of infection, the rabbits sacrificed, and the enucleated eyes placed into sterile moist chambers kept at room temperature. Within 2 hours, all eyes were cultured prior to antibiotic treatment by swabbing 360° around the limbus using a moistened cotton tip applicator.

Two methods of antibiotic application were employed. Group A consisted of 25 eyes to which 20 drops of Polyspectrin (Neomycin sulfate Polymyxin B sulfate) were placed directly onto the cornea. Fifty-four eyes in group B were irrigated with 20 c.c.'s of sterile normal saline then individually immersed in Polyspectrin for three minutes. All eyes were kept in sterile glass chambers at room temperature until cultures were obtained 30 minutes after antibiotic treatment.

Scleral-corneal buttons were then removed and placed in the McCarey-Kaufman media containing

Table I. Preferred methods of sterilizing donor eyes

<table>
<thead>
<tr>
<th>Authors</th>
<th>Method</th>
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<tbody>
<tr>
<td>Doctor and Hughes</td>
<td>10 minute immersion in</td>
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<td></td>
<td>Neosporin followed by 2</td>
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<tr>
<td></td>
<td>minute saline rinse.</td>
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<tr>
<td>Rollins and Stocker</td>
<td>5 minute rinse with 10 c.c.'s</td>
</tr>
<tr>
<td>Polack, Locatcher, Khorazo, and Gutierrez</td>
<td>2 minute drip of 0.5 c.c.'s</td>
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<td></td>
<td>Neosporin.</td>
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