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Quantitative Endothelial Biomicroscopy

Jack T. Holladay, M.D.
John E. Bishop, M.D.
Thomas C. Prager, Ph.D.

SUMMARY

Twenty patients were evaluated by quantitative endothelial biomicroscopy and the resulting endothelial cell densities compared with those from a specular microscope. Estimated cell densities by this technique, when compared with the specular microscope, demonstrated a Pearson correlation coefficient of +0.977, an average error of −7% and an absolute error of 12%. There were no errors greater than 26% except for one extremely low cell density of 318 cells/mm^2 for which the estimate was 476 cells/mm^2.

This rapid, inexpensive technique requires counting the number of endothelial cells seen across the horizontal diameter of the 0.2-mm projected spot beam of a standard biomicroscope. From this count and the known spot size the endothelial cell density may be accurately calculated. A simplified four-step technique for performing specular microscopy using only the standard biomicroscope and a method for accurately measuring the size of the projected slit lamp spot beam are explained.

The importance of the corneal endothelium in maintaining the deturgescence and optical clarity of the cornea has been well documented in recent years.[1] New developments with the specular microscope have resulted in high quality photographs or video displays which allow accurate determination of the cell density.[2]

Clinically, the endothelial cell density has been used preoperatively to identify those patients with low cell densities who are at higher risk for corneal decompensation with anterior segment surgery. Endothelial cell loss is known to occur with cataract extraction, glaucoma procedures, secondary intraocular lens implantation and secondary discissions of the posterior capsule.[3-8] Measuring endothelial cell densities has also been helpful in determining progressive endothelial cell loss with various styles of intraocular lenses so that they can be removed before corneal decompensation occurs.[9-14]

The major impediment to routine, widespread use of the specular microscope has been its prohibitive cost.[15] We have developed an inexpensive, rapid, and convenient method of estimating the endothelial cell density which uses the standard slit lamp and is accura absolute error of that obtained with a spec

SUBJECTS AND METHODS

Twenty-one subjects, nine males and were selected for inclusion in the study. P age from 21 to 97 years. Of the eight left right eyes studied, none had a history of pr trauma. Nine of the 21 eyes were normal, three had Fuchs' endothelial dystrop pterygium, one had chronic open angle gl had a cataract and open angle glaucoma

Two observers (JTH and JEB) indepen the number of endothelial cells seen alo diameter of the 0.2-mm circle projected from the Haag-Streit slit lamp. The same s to evaluate all patients. Care was taken t margin of the projected circle was in cle the eyepiece was critically focused mono the endothelial cells.

The exact projected spot size of th measured photographically to be 0.24 r diameter. This dimension was then divi find the millimeter conversion factor of lamp. This factor represents the numbe 1 mm. The number of endothelial cells c horizontal diameter of the spot was then r

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From the University of Texas Medical School, Hermann Eye Center, Department of Ophthalmology, Houston.

Requests for reprints should be addressed to Jack T. Holladay, M.D., Hermann Eye Center, P.O. Box 20420, Houston, Texas 77030.

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TABLE 1

COMPARISON OF ENDOTHELIAL CELL DENSITIES AS DETERMINED BY QUANTITATIVE BIOMICROSCOPY AND PHOTOGRAPHIC SPECULAR MICROSCOPY

	Cell Density (cells/mm^2)						
Patient No.	Est. #1	Est. #2	Mean Est.	Specular Photograph	Algebraic Difference	Algebraic Percent Error	Absolute Percent Error
1	2713	2296	2505	2830	−325	−11.5	11.5
2	2296	2500	2398	2430	−32	−1.3	1.3
3	2101	2101	2101	2183	−82	−3.8	3.8
4	2934	2500	2717	2980	−263	−8.8	8.8
5	1736	2101	1919	2203	−284	−12.9	12.9
6	2101	1736	1919	2087	−168	−8.0	8.0
7	2101	1914	2008	2137	−129	−6.0	6.0
8	1195	1129	1162	1570	−408	−26.0	26.0
9	1914	2500	2207	2416	−209	−8.7	8.7
10	999	1067	1033	1089	−56	−5.1	5.1
11	3232	2500	2866	2979	−113	−3.8	3.8
12	2101	2296	2199	2380	−181	−7.6	7.6
13	476	476	476	318	+158	+49.7	49.7
14	3164	2500	2832	3207	−375	−11.7	11.7
15	2500	2500	2500	2383	+117	+4.9	4.9
16	2713	2713	2713	3282	−569	−17.3	17.3
17	1191	1111	1151	1363	−212	−15.6	15.6
18	984	1175	1143	1321	−178	−13.5	13.5
19	1302	984	1080	1205	−125	−10.4	10.4
20	1819	1649	1734	2060	−326	−15.8	15.8
Mean	1979	1887	1933	2121	−188	−6.7%	12.1%

to find the number of endothelial cells along 1 mm. This number was then squared to find the cell density in cells/mm.^{2}

The amount of cellular pleomorphism and guttata were also graded on a clinical scale from 0 to 4+. In those patients with guttata, the calculated cell density was reduced by 10% times the clinical scale grading (i.e., 1+ = 10% reduction, 2+ = 20% reduction,..., 4+ = 40% reduction).

Photographs were taken with the Pocklington specular microscope, and the endothelial cells were counted using the standard technique as specified by the manufacturer. Specular photographs of sufficient clarity to permit accurate determination of cell density could not be obtained in one eye. On the remaining 20 eyes a Pearson correlation coefficient was calculated and used to compare the estimated and specular microscopic cell densities.

RESULTS

Table 1 lists the endothelial cell densities for all 20 subjects as estimated by each observer and that obtained with the specular microscope. The absolute and percentage algebraic difference between the mean cell density estimates and that obtained with the specular microscope are listed for each subject. The average algebraic difference between the two methods of assessing cell density was −188 cells/mm.^{2} The mean absolute error of the estimates was 12.1%, and the mean algebraic error was −6.7%.

These data are further illustrated in Figure 1. For each

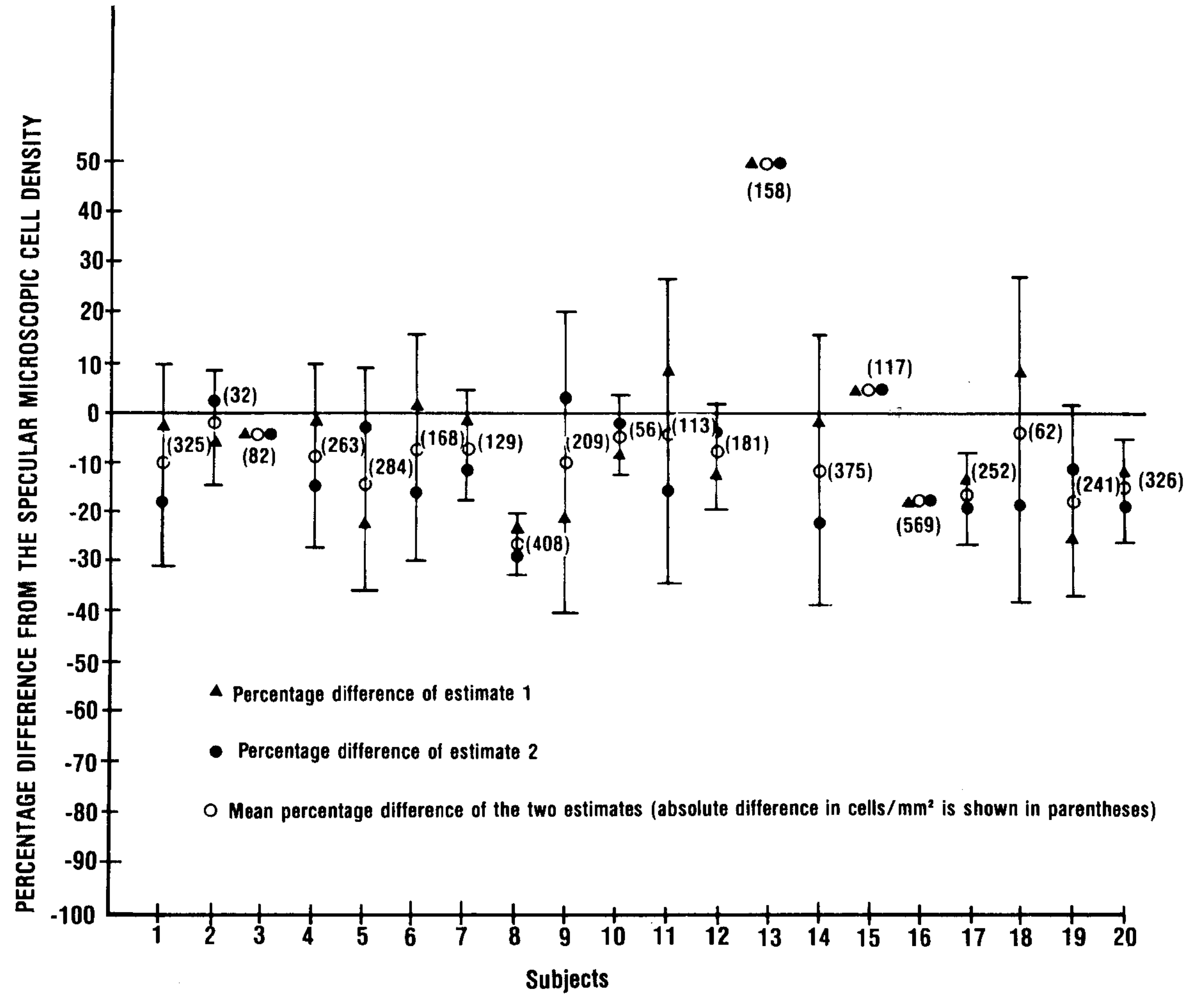

FIGURE 1: (Holladay, Bishop and Prager). The mean percent error (O) of the endothelial cell density as determined by quantitative endothelial biomicroscopy is compared with photographic specular microscopy. The absolute cell density difference is shown in parentheses. The percentage difference for estimate 1 (▲) and estimate 2 (●) is also depicted. Brackets indicate 99% confidence levels.

subject the average percentage difference from the specular microscopic cell density is plotted as well as the absolute cell density difference, in parentheses. The 99% confidence interval of the mean and the percent deviation for each observer are also depicted for each of the 20 subjects. In Figure 2 a best fit linear regression line was determined between the mean estimated cell density and the cell density determined with the specular microscope. A Pearson product-moment correlation of $R = +0.977$ was shown to be highly significant ($p < .0005$).

DISCUSSION

Several studies have shown the value of determining the endothelial cell density as a predictor of the cornea's ability to withstand anterior segment surgery.[1] There is no established cell density below which the cornea will decompensate with surgery since there are many other factors that contribute to the optical clarity of the cornea. Some of these other factors include the functional capability of each cell, the total number of cells distributed over the posterior cornea, cellular pleomorphism, the intraocular pressure, and the corneal thickness.[16–18] Although these other factors are important, the endothelial cell density is a major factor and is also one of the easiest to measure.[1]

Using endothelial photographs, early researchers were able to determine the importance of the endothelial cell density *in vivo*. Newer instruments are still quite expensive and primarily reserved for research purposes. These newer

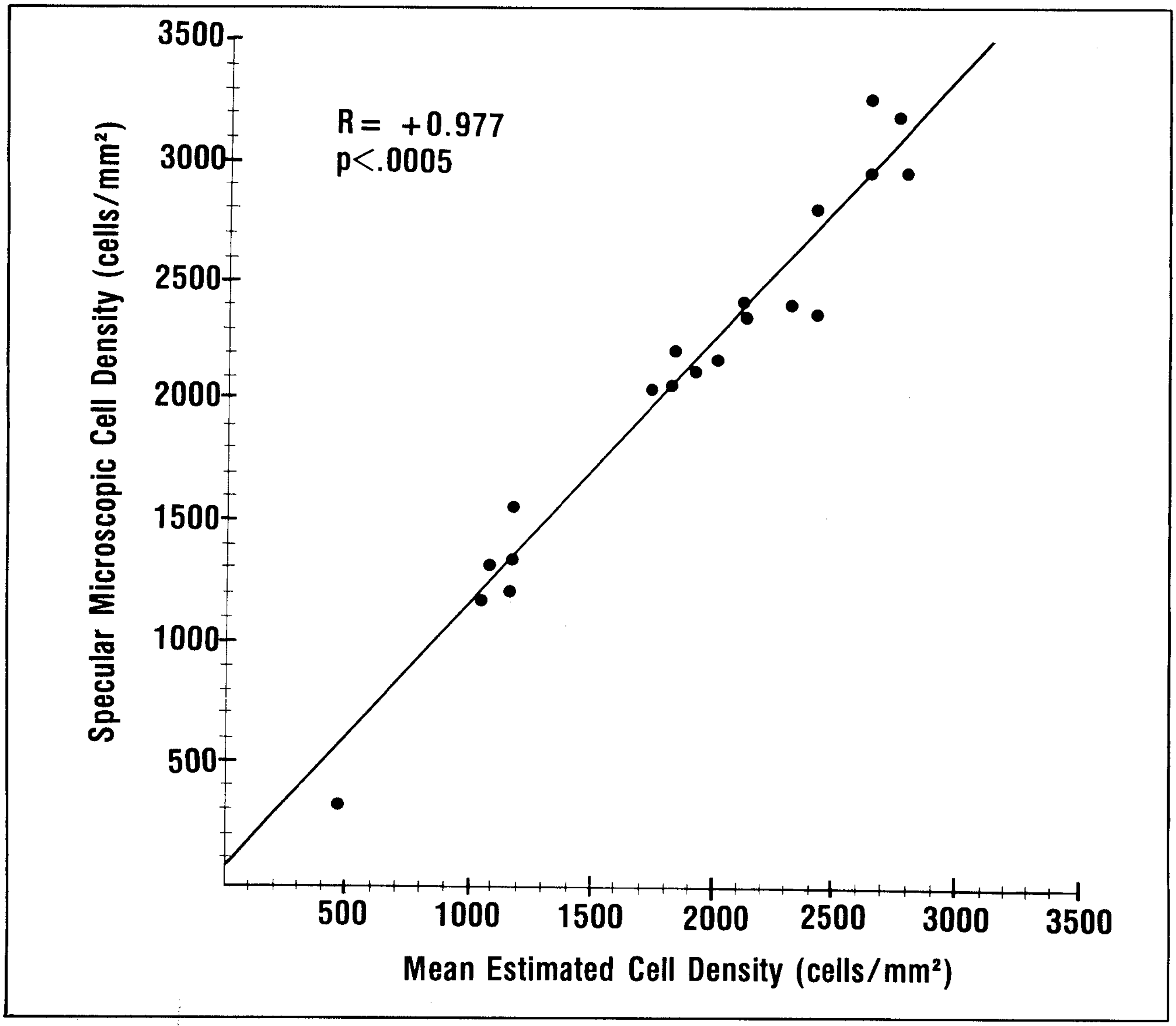

FIGURE 2: (Holladay, Bishop and Prager). The correlation of endothelial cell densities estimated by quantitative endothelial biomicroscopy and photographic specular microscopy is shown. A linear regression line is also plotted.

generations of endothelial cell cameras allow instant feedback by using a video monitor display. Cell densities can be obtained easily and rapidly by having a standardized grid on the monitor screen. These instruments are superb technical devices, but, unfortunately their cost is prohibitive to many ophthalmologists.[15]

Since anterior segment surgery, such as cataract extraction, primary and secondary intraocular lens implantation, and glaucoma procedures is commonly performed by most ophthalmologists, there is a need for a simple, convenient, rapid, and inexpensive technique to determine the endothelial cell density. In an effort to satisfy these requirements for the clinician, two estimation techniques have been developed previously.

The first method, a 25X eyepiece with reticle, allows the observer to view the endothelium and compare it with an adjacent reticle that is graduated in four steps from 500 to 4000 cells/mm.[2] This technique has been reported to have a mean algebraic error of 10% to 21% with a range from 25% to 105%.[15,19,20] The cost is very reasonable, and the only disadvantage is having to remove the special 25X eyepiece with reticle from the slit lamp for routine biomicroscopy.

The second technique has been called "mosaic matching." This method requires the observer to memorize the pattern of endothelial cells within the small spot on the slit beam and then match this pattern with that shown on a printed comparative card (Personal communication, John R. Karickhoff, M.D., May, 1982). No published data is available on the accuracy of this subjective technique. It also may be difficult for most observers to remember the image of the endothelial cells as seen through the biomicroscope until they look at the comparative card.

Because of the disadvantages mentioned above with each of the estimation techniques and the prohibitive cost of the endothelial cell cameras, we decided to develop a technique using only the standard biomicroscope. This technique requires counting the number of endothelial cells along the horizontal diameter of the 0.2-mm spot beam as seen on the posterior cornea during specular microscopy. Since the diameter of the spot is known, the number of cells counted along the horizontal diameter can be multiplied by the number of spots necessary to fill one millimeter. This would then give the number of endothelial cells along one millimeter. If this number is then squared, the density of endothelial cells per square millimeter is thus determined. For the standard Haag-Streit slit lamp in which the spot beam is nominally 0.2 mm, it would require multiplying the horizontal cell count by 5 (the millimeter conversion factor) and then squaring this number. For example, if 10 cells were counted along the horizontal diameter of the spot, there would be 50 cells in one millimeter or 2500 cells/mm.[2]

The data in Figures 1 and 2 show the average accuracy of this technique to be approximately 12%. For spot sizes greater than 0.25 mm, such as found on the Zeiss slit lamp (0.30 mm), estimates are more difficult due to the greater number of cells which must be counted.

In teaching this technique to our residents and other ophthalmologists, it has become apparent that the most difficult part of the entire procedure is the technique of specular microscopy. We have, therefore, described in detail four simple steps to easily visualize the corneal endothelial mosaic.

Step 1 (Figure 3): In preparation, the slit lamp should be placed so that the angle between the slit beam and the oculars is between 60° and 80°. The slit lamp should be adjusted to the 0.2-mm spot beam and set at the highest available magnification (25X with the Haag-Streit or 40X with the Zeiss).

The patient is now positioned at the slit lamp for normal viewing of the cornea. When the slit beam is directed from the observer's left, three images will be seen on the cornea as shown in Figure 3. The lower two corneal reflexes will be the anterior and the posterior spot beam circles on the epithelial and endothelial cell surfaces. The bright upper corneal reflex will be the mirror image of the slit lamp filament, which will be slightly above and to the left of the previous two images. If the filament image cannot be located easily, enlarge the size of the slit beam momentarily, and the image will become brighter. Once the mirror image of the filament is located, return to the 0.2-mm spot size. It is very important to note that the only point at which the specular microscopic reflex can be seen is where the mirror reflex of the filament appears on the cornea.

To see the specular microscopic reflex, it is necessary to take the posterior endothelial circle and move it upward and to the left so that it is superimposed on the mirror image of the filament. When this occurs, a brilliant light reflex will be projected back into one of the observer's eyes, confirming that the proper endpoint has been achieved. By carefully keeping the epithelial reflex out of this mirror image, some of the glare from the unwanted epithelial specular reflex can be avoided.

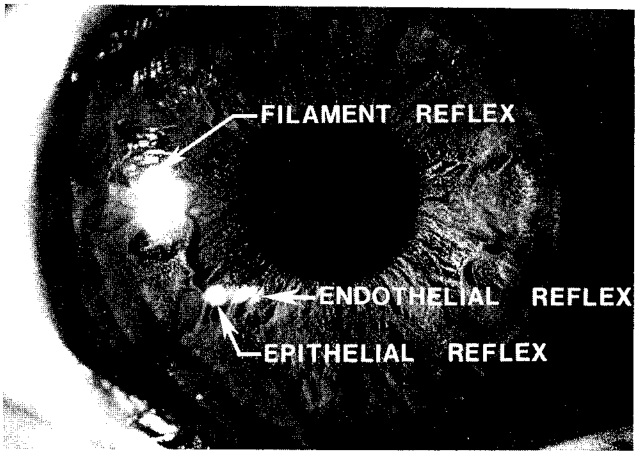

FIGURE 3: (Holladay, Bishop and Prager). The normal appearing corneal reflexes visible during biomicroscopy are shown with the slit beam projected from the observer's left. The upper reflex is the mirror image of the slit lamp filament. The lower two reflexes are the epithelial (left) and endothelial (right) images from the slit beam using the 0.2 mm spot size (X10 Haag-Streit biomicroscope).

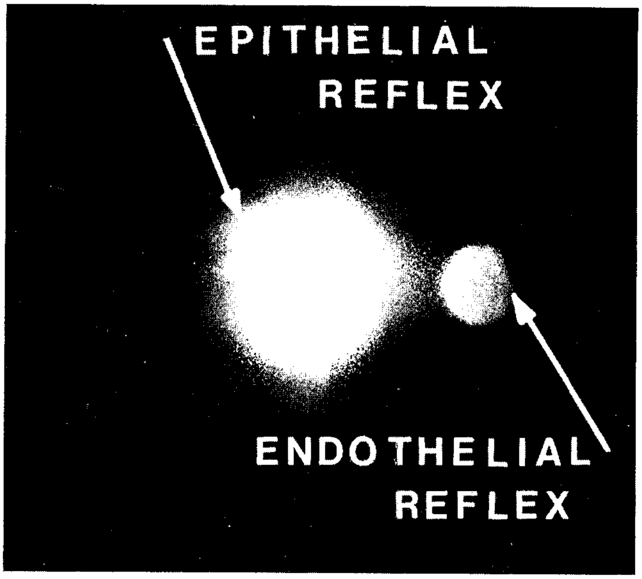

FIGURE 4: (Holladay, Bishop and Prager) The appearance of the epithelial (left) and endothelial (right) images from the slit beam using the 0.2-mm spot size when superimposed on the mirror image of the filament are illustrated. Specular microscopy can be achieved only when this relationship exists. (X10 Haag-Streit biomicroscope).

Step 2 (Figure 4): Once the endothelial spot is superimposed on the mirror image of the filament, as shown in Figure 4, it is then necessary to assure that the margin of the projected spot is in clear focus on the corneal