Contact and noncontact specular microscopy

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SUMMARY A contact specular microscope was compared with a noncontact endothelial camera on 20 eyes of 10 consecutive patients presenting for cataract surgery. No difference was found in the percentage of photographs acceptable for cell counting or the time for photography by one or other camera. A close correlation was found in cell density estimations by the 2 methods. Most patients prefer the noncontact mode of examination.

The potential uses of clinical specular microscopy are reflected in diverse clinical applications. These include the assessment of the endothelium in donor corneae,¹⁻³ in monitoring different modes of anterior segment surgery,⁴⁻⁷ in the assessment of surgical techniques,⁸⁻¹¹ the longitudinal effects of surgery,¹² the action of agents in clinical use,¹³⁻¹⁵ and the effects of naturally occurring diseases.¹⁶⁻¹⁸

The earlier generation of clinical specular microscopes as developed by Laing *et al.*,¹⁹ Bourne and Kaufman,²⁰ and Sherrard¹⁴ were essentially similar in design. They all had objectives which applanated the cornea, and the size of the field photographed was small. Such microscopes offer high magnification and good image resolution but have limitations. Their field may be adequate for accurate estimation of cell density in normal corneae but not after a high percentage of cell loss. Laing *et al.*²¹ have calculated that a minimum sample size of 50 to 100 cells will produce a reliable estimate of cell density in normal corneae. In patients with low cell density it may be impossible to obtain a field of more than 50 cells with these microscopes, while variability in cell size will require a larger sample of cells to minimise sampling errors.

Specular microscopy may also yield useful clinical information in the study of cellular morphology,²²⁻²⁴ but it is not yet possible even with high magnification to define intracellular events which reflect subtle physiological or biochemical changes. While polymorphism may suggest recent patchy cell loss or a more vulnerable endothelium, there is no evidence yet to confirm this supposition. Until a method is devised to obtain a better cell image, or to quantify morphological characteristics

currently available, we are still mainly concerned with cell numbers to give an indication of cellular reserve.

Since macrophotography of the corneal endothelium was first accomplished,²⁵ it has become possible to photograph the endothelium with a modified slit-lamp microscope.²⁶⁻²⁸ The noncontact technique has so far been critically compared with contact specular microscopy only by Olsen²⁹ for a small number of normal eyes.

The purpose of this paper is to report a study comparing the performance of a commercially available contact specular microscope with that of a noncontact specular microscope in terms of patient acceptability, image quality, and the accuracy of cell density estimation.

Materials and methods

The specular microscopes used in the study were the Heyer-Schulte corneal endothelial camera (Model HS CEM 3) with an Olympus OMI camera unit and the Nikon noncontact endothelial camera.

The Heyer-Schulte microscope was calibrated by photographing a standard graticule through a Duragel 85 contact lens (refractive index 1.37), 0.5 mm thick, to simulate the cornea. The magnification of the endothelial cells on the film was found to be 80.5 times.

The on-film magnification of a standard graticule in air was found to be $\times 9.96$ with the Nikon camera. In calibration of the noncontact microscope it is necessary to correct for the distortion of the endothelial image due to curvature of the cornea. The final endothelial magnification was calculated from a factor derived by Olsen²⁹ to correct for horizontal distortion.

No vertical disparity in cell density was found on counting contiguous areas of cells along a 2 mm vertical slit in a patient with high cell density, which supports Olsen's calculation that no vertical correction factor is necessary.

Ten consecutive patients presenting for cataract surgery were recruited into the study. Their ages ranged from 22 to 85 years, with a mean of 66.2 years.

Ten photographs were taken of the central corneal endothelium of each eye with the 2 cameras in turn. Noncontact specular microscopy always preceded contact microscopy to avoid any epithelial disturbance interfering with image resolution. When the contact microscope was used, photography was attempted whenever the observer was presented with a clear image of the endothelium. For noncontact specular photography the observer could alter the illumination slit and change the subject's point of fixation between photographs in an attempt to achieve the best balance between width of field and clarity.

All photography was undertaken by one of us (H.C.) who is experienced in the use of both microscopes. In comparing the 2 instruments the following features were assessed:

Time. The time taken to complete photography of both eyes with each camera was recorded by an independent observer. This included all preparative steps such as the instillation of topical anaesthetic in the case of the contact microscope.

Quality of photographic material. Ilford FP4 (ASA 125) films were used with the contact microscope and were developed in Acutol developer at 68°F (20°C) for 12 minutes. Kodak Panatomic film (32 ASA) was used with the noncontact microscope and was developed in Ilford ID 11 fine grain developer at 68°F (20°C) for 5 to 7 minutes.

Table 1 Endothelial areas used in cell density estimations

	Area	Counts per eye
Sugar <i>et al.</i> ⁸	0.0100 mm ²	2-6
Vannas <i>et al.</i> ³⁰	0.0120 mm ²	3
Olsen ³⁸	0.0179 mm ²	3
Sperling and Gundersen ³¹	0.030 mm ²	4
Bourne and Kaufman ²⁰	0.040 mm ²	3
Laing <i>et al.</i> ³¹	Variable frame analysis of 50 cells	5
Sturrock, Sherrard, and Rice ³¹	40-100 cells	7-10

All the negatives were examined to determine the percentage of acceptable photographs achieved for each patient with each camera. For the contact microscope a negative was deemed acceptable if countable cells were visible in at least 4 of the 10 grid squares (Fig. 1). This is equivalent to an endothelial area of 0.017 mm², which is comparable with that quoted by other workers (Table 1). A minimum area of countable cells equivalent to 0.05 mm² was considered acceptable for the noncontact microscope (Fig. 2).

Counting methods. The 3 best photographs taken of each eye with each camera were used for estimating cell density. The contact microscope negatives were back-projected and cells counted within a superimposed grid by means of the counting techniques described by Sperling and Gundersen.³² For photographs taken by the noncontact camera the 3 best negatives for each eye were printed to a known magnification ($\times 148$) and cell counts undertaken within standard grids.

Patient tolerance. This was assessed by a simple questionnaire in which patients were asked whether photography with either microscope was unpleasant

Fig. 1 Specular photograph taken with Heyer-Schulte contact endothelial camera.

Fig. 2 Specular photograph taken with Nikon noncontact camera. Grid inset to indicate minimum area used for counting.

or uncomfortable, and which camera they would prefer for future photography.

Results

The time taken for photography did not vary significantly between the 2 modes of photography (Fig. 3). Photography of both eyes was completed in less than 12 minutes in almost every case.

The percentage of acceptable photographs obtained with the two microscopes was similar (Fig. 4). Less than half the photographs were found to be

acceptable in 2 out of 20 eyes when the noncontact microscope was used, while in the case of the contact microscope fewer than half the photographs were acceptable in only one eye. The mean acceptability for the contact camera was 74.4% and that for the noncontact system 75.2%.

Endothelial cell density determined for the same eye by means of photographs taken by the two cameras (Fig. 5) showed a coefficient of correlation of 0.9303 with a least-squares slope not significantly different from 1 and an intercept of +45 cells on the abscissa. The mean difference in endothelial

Fig. 3 Time to complete photographs for each patient. Noncontact vs. contact microscope.

Fig. 4 Percentage 'acceptable' photographs of each eye. Noncontact vs. contact microscope.

Fig. 5 Estimated endothelial cell density for each eye. Noncontact vs. contact microscope.

density estimations was -0.45% with a standard deviation of 4.3% .

The mean endothelial area of cells counted in the noncontact photographs was 0.069 mm^2 with a maximum of 0.15 mm^2 compared with a mean field size of 0.031 mm^2 for the contact microscope, with a maximum of 0.045 mm^2 .

The results of our questionnaire indicate patient preference for the noncontact camera (Table 2).

Discussion

While specular microscopy is becoming a routine clinical investigation, one may need different

Table 2 Results of patient questionnaire

	Contact	Noncontact	(n = 10)
Uncomfortable/unpleasant Reason	5	0	
2 Contact			
1 Light intensity			
1 Excessive lacrimation			
1 Position			
Preference for future photography	1	7	No preference—2

microscopes to fill different clinical needs. Clearly morphological characteristics of individual cells are best studied with a highly resolved and magnified image, while density studies require a large field as well. Before any microscope can be used it must be shown to be acceptable in terms of accuracy and reliability for cell density estimation. Calculation of the magnification of the noncontact microscope must allow for distortion of the endothelial image due to curvature of the cornea. We have shown that, provided this is corrected for, a high degree of correlation is achieved in estimated cell density for the contact and noncontact microscopes.

Hirst *et al.*³³ have emphasised that, while a sample size of 50 cells may be adequate for estimation of cell density in regular endothelial mosaics, it is inadequate when considering those corneae with low density and variable cell size. For representative sampling a relatively large area of endothelium must be photographed. The largest field of cells we have photographed with the Nikon noncontact endothelial camera is in excess of 0.2 mm^2 , compared with a maximum area photographed by the Heyer-Schulte specular microscope of 0.045 mm^2 . The problems of field size will not be encountered

Fig. 6 Noncontact specular photograph of peripheral cornea. Arrows to indicate reflection from iris.

with the recently introduced Pocklington microscope³⁴ and that developed by Koester,³⁵ but the Nikon noncontact camera is a good alternative and offers an adequate field of several hundred cells.

An important feature of any endothelial camera is its ability to photograph different parts of the

cornea, including the periphery. While this is difficult with the contact microscope, almost the whole cornea can be easily photographed with the noncontact camera, the limiting factor being reflection from the iris, which interferes with image resolution (Fig. 6).

Fig. 7 Montage of prints taken on different occasions to show repeatable field of cells (noncontact camera). Arrows indicate same endothelial feature.

It is extremely difficult to rephotograph the same field of cells with conventional contact specular microscopes. Relocation of the same area of the cornea with the noncontact camera is still difficult, but, if there are identifiable landmarks, repeated photography of the same field is possible (Fig. 7).

We found that the time taken for photography and the photographic standards achieved were almost identical for the 2 microscopes. This indicates that one instrument is not intrinsically more difficult to use than the other, given sufficient practice.

The patients' preference for the noncontact microscope may reflect their dislike of contact of the dipping cone with the cornea. Such contact may entail other disadvantages. It may not be desirable to applanate an eye recently operated on, or one with an infected or otherwise diseased cornea, and the introduction of a contact lens between dipping cone and cornea adds a further step which complicates training of nonclinical staff.

Our study has shown that noncontact specular microscopy with a modified slit-lamp allows accurate estimation of endothelial cell density. The advantages of the noncontact method outlined above make it a useful alternative to contact specular microscopy. It is a simple procedure which is particularly useful in clinical situations where large numbers of patients are to be photographed and where cell counting is more important than a study of morphology.

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