Specular Microscopy Image Quality Scale For Endothelial Cell Density Determination In Donor Cornea Tissue

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ABSTRACT

Objective: To develop an image quality rating (IQR) scale for specular microscopy images of donor corneal endothelium and to evaluate the accuracy and reproducibility of IQR scales based on inter- and intra-grader variation in endothelial cell density (ECD) values.

Methods: Using the Konan center method, we performed specular analysis on 536 specular images of donor corneal endothelium. All images were assessed independently for image quality using the IQR grading scale and ECD analysis was performed by trained specular microscopy graders.

Results: Of the 536 images, 462 (86%) were gradable (excellent, 65 [12%]; good, 230 [43%] fair, 81 [15%]; and poor, 86 [16%]. The remaining 74 (14%) were non-gradable by the image quality scale. Inter- and intra-grader reproducibility was good (Kappa, 0.77 and 0.88, respectively). When stratified by IQR, the image quality scale offered comparable high inter- and intra-grader repeatability (ICC≥0.98) for ECD determination at higher image quality ratings (excellent/good) and an acceptable confidence level (ICC>0.8) at lower image quality ratings (fair/poor). The agreement in ECD measurement (error<5%) is higher in excellent (96%) and good (>83%) images, but lower in fair (>66%) and poor (>55%) images, providing further validation of the quality scale.

Conclusions: For trained graders, image quality is the main factor affecting ECD reproducibility. This image quality scale shows good reproducibility for all gradable images. Even when the IQR score is poor, an acceptable ECD still can be provided.

Key Words: image quality rating, corneal endothelium, donor, specular microscope

Corneal transplant surgeons judge the quality of prospective donor tissue by endothelial cell density (ECD) and evaluate postoperative tissue cell loss by comparison to the baseline donor ECD. Qualitative and quantitative assessment of endothelial cell quality by eye banks is an important factor in both corneal donor selection and postoperative evaluation of graft health.

Previous studies showed that image quality affects the accuracy and reproducibility of ECD measurement [1,2]. A standardized image quality rating (IQR) scale provides a confidence measure for the ECD value [3]. For example, excellent images are easy to grade, reflecting high confidence, while poor images are difficult to grade, reflecting low confidence. When an image is non-gradable, no ECD value can be provided. Using an IQR scale can help the eye bank assess both the quality of the donor tissue and the reliability of the ECD value.

Many eye banks use the HAI specular microscope (HAI Laboratories, Inc. Lexington, MA, United States) for donor corneal endothelium imaging. Meanwhile, clinical trials typically employ Konan CellChek software (Konan Medical USA Inc. Irvine, CA, United States) for ECD analysis. The Konan center method [3] can accurately assess ECD based on a certain number of contiguous cells. HAI specular images can be imported into Konan CellChek software where the center method can be used to accurately assess ECD in donor corneas [3,4]. Eye bank-generated specular images from donor corneas can then be analyzed

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by outside reading centers that employ validated grading methods. To provide the best estimate of ECD, we developed an IQR scale. Herein, we evaluate the accuracy and reproducibility of our IQR scale, based on inter- and intra-grader variation in ECD values.

METHODS

Endothelial Images

A total of 536 unaltered images (640 × 480 pixels) were captured by a single eye bank (SightLife, Irvine, California) on a specular microscope for donor corneas (HAI EB-300xyz Eye Bank Specular Microscopy) and transferred electronically to the Doheny Image Reading Center (DIRC). All images were rescaled to 445× 593 pixels before they were imported into the Konan CellChek software (Ver. 4.0.1). The region with the largest possible contiguous area [5] of donor corneal endothelial cells was selected from the original image before conversion. After conversion, the Konan image represented 51.4% of the size of the original HAI image.

Image Quality Grading Scale

Our rating scale for gradable images included four levels: excellent, good, fair, and poor. Images that could not be graded were deemed non-gradable (Figure 1). The levels were defined as follows:

Excellent: The entire image can be graded with confidence. Most of the image (96% – 100%) can be included in the gradable area (dotted contiguous cells). Within the gradable area, all cell borders are clearly defined, and there is little to no difficulty in identifying cells.

Good: Most of the image can be graded with confidence. At least 75% of the image can be included in the gradable area. If the gradable area is < 96%, all cell borders are clearly defined, and there is little to no difficulty in identifying cells within the gradable area. With a larger gradable area (96% – 100%), a slightly higher level of difficulty is acceptable.

Fair: At least half of the image can be graded with confidence. Between 50% and 95% of the entire image can be included in the gradable area. If the gradable area is < 75%, all or most cell borders are clearly defined, and there is little to no difficulty in identifying cells within the gradable area. With a larger gradable area (75% – 95%), a slightly higher level of difficulty is acceptable.

Poor: At least one quarter of the image can be graded with confidence. Between 25% and 75% of the entire image can be included in the gradable area. If the gradable area is < 50%, all or most cell borders are clearly defined, and there is little to no difficulty in identifying cells within the gradable area. With a larger gradable area (50% – 74%), a slightly higher level of difficulty is acceptable.

Non-gradable: Less than 25% of the image can be graded with confidence. Up to 49% of the entire image can potentially be included in the gradable area. If the gradable area is < 25%, or image with a larger gradable area (25% – 49%) but lacking clarity, the image is not gradable.

IQR Assessment Analysis

The following instruction for grading of IQR accompanies the above stated definitions for each image quality rating.

1. Determine what percent of the image is the gradable area. The gradable area is defined as the area of dotted

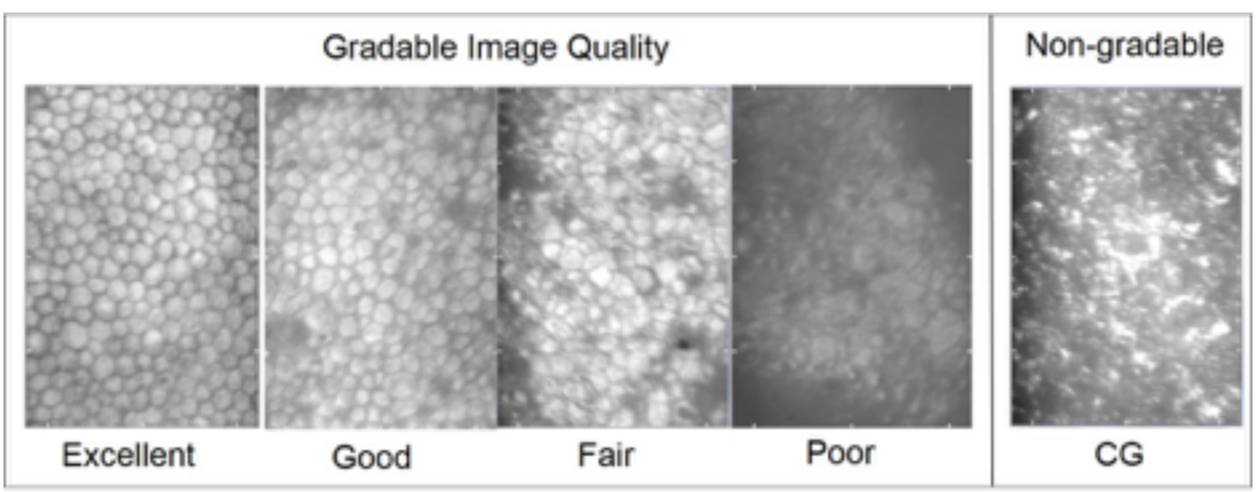

***Figure 1.** DIRC's Donor Cornea Image Quality Rating (IQR) Scale*

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(contiguous) cells. The percent gradable area is defined as the percent of the dotted area with respect to the total frame.

2. Assess the level of questionable areas within the gradable area. "Questionable areas" include anything that would increase the difficulty level in identifying cells.
3. Assign the corresponding IQR based on the flowchart distribution (Figure 2).

RESULTS

Endothelial Image Quality Classification

Of the 536 specular images of donor corneal endothelium evaluated with the DIRC image grading scale, 65 (12%) were judged to be of excellent quality, 230 (43%) of good quality, 81(15%) of fair quality and 86 (16%) of poor quality after adjudication. 14% (74) of images were judged non-gradable and therefore were excluded from ECD analysis. There was good agreement, with a Kappa value of 0.77 (95% confidence interval 0.73-0.80) on the donor specular images between the two graders with respect to image quality. The intra-grading agreement for the IQR was also good, with a Kappa value of 0.88 (95% confidence interval 0.85-0.90, Table 1).

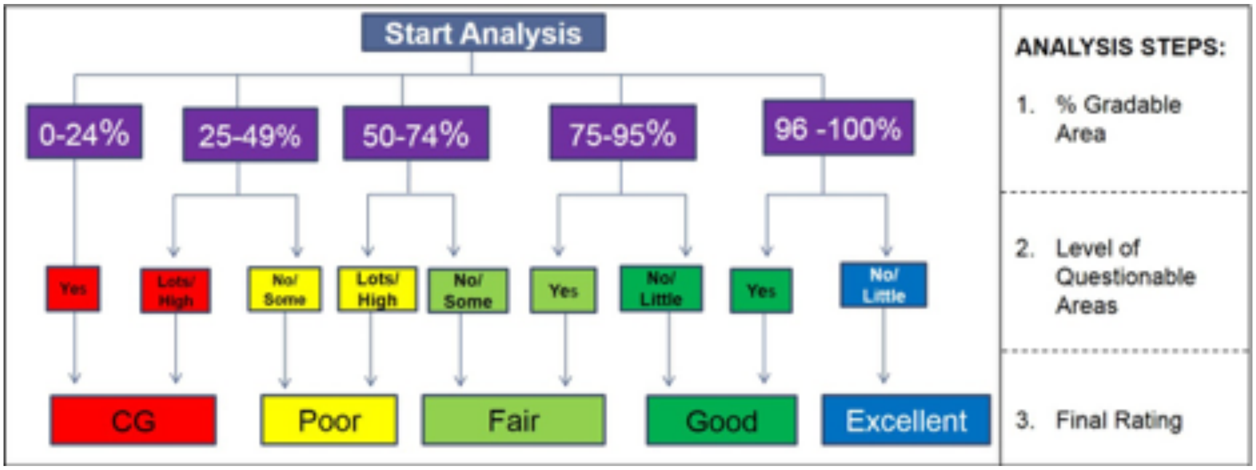

Figure 2. *DIRC's Donor Cornea Image Quality Rating (IQR) Flowchart*

Image Quality Assessment and Cell Density Determination

All images were analyzed in a masked fashion by two trained graders. When the 2 graders were not in consensus on IQR, the adjudicator's determination of image quality was declared the final image quality. Endothelial cell density was determined by the Konan center method, in which the largest area of contiguous cells in the image that could be accurately distinguished was selected by the grader. If image quality was judged to be non-gradable after the adjudication process, no ECD analysis was done.

ECD variation of inter- and intra-grader was determined based on the absolute percent error between ECD values:

$$\text{Absolute error (\%)} = \frac{\text{ECD high-ECD low}}{\text{ECD low}} \times 100$$

Statistical Analysis

Statistical analysis was performed with SPSS 19.0 (SPSS, Inc. Armonk, NY, United States). The absolute mean difference, percentage error and standard using MedCalc version 12 (MedCalc software bvba; Ostend, Belgium). Deviation of ECD values between grading's were calculated. Inter-grader agreement (Kappa)[6] and intra-class correlation coefficient (ICC) [7] were also calculated to evaluate reproducibility.

Table1. *IQR determination using DIRC scale: Reproducibility of intra- and inter- grader.*

	Kappa (95% confidence interval)
Intra-grader	0.88 (0.85-0.90)
Inter-grader	0.77 (0.73-0.80)

Endothelial Cell Density Comparison

All images were stratified based on the DIRC IQR specular image quality scale and inter- and intra- grading reproducibility of ECD analysis was evaluated. For excellent quality images there was no statistical difference in ECD values between graders; ECD was 2719 ± 171 cells/mm^2 by first grader and 2717 ± 154 cells/mm^2 by second grader (P=0.916). Similarly, for good quality images, average ECD values were not statistically significantly different obtained by the first and second grader (2633 ± 489 cells/

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mm² and 2600 ± 501 cells/mm², P=0.063). However, there was a statistically significant difference in inter-grading in fair images (2582 ± 397 cells/mm² and 2534 ± 379 cells/mm², P<0.001) and poor quality images (2627 ± 410 cells/mm² and 2520 ± 423 cells/mm², P<0.001) between first and second grader (Figure 3).

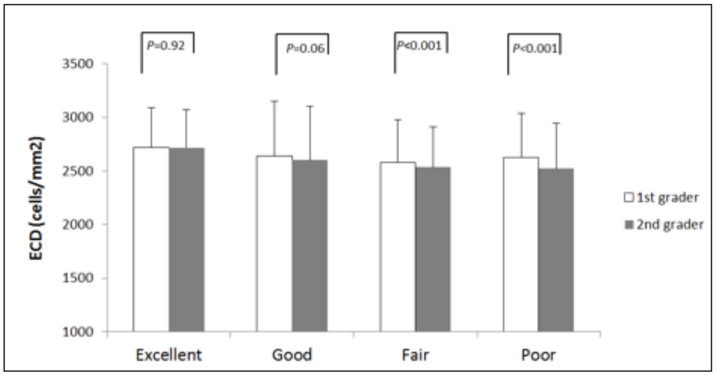

Figure 3. *ECD values of inter-grader with different image quality.*

In terms of intra-grading reproducibility, across all gradable image quality ratings, there was no statistically significant difference between the ECD values of intra-grading. The average ECD value was 2719 ± 171 cells/mm² by first grading and 2702 ± 358 cells/mm² by second grading in excellent quality images (P=0.13). Likewise, mean ECD values was 2633 ± 489 cells/mm² by first grading and 2631 ± 500 cells/mm² by second grading in good quality images (P=0.07). Even in fair quality images (2582 ± 397 cells/mm² and 2568 ± 375 cells/mm², P=0.38) and poor quality images (2627 ± 410 cells/mm² and 2597 ± 359 cells/mm², P=0.20) there was no significant difference in ECD analysis (Figure 4).

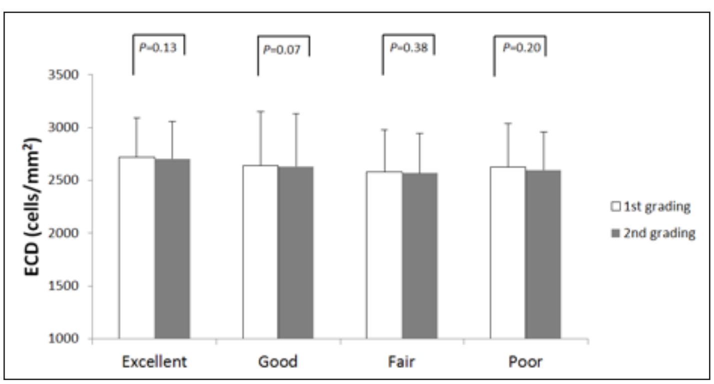

Figure 4. *ECD values of intra-grader with different image quality.*

The reproducibility of ECD measurement by the two graders was excellent (Table 3). ICC ≥ 0.98 in excellent and good quality images; even in poor images, ICC > 0.8. The inter-grader absolute error was <5% for 98.5% for excellent images, 83.5% for good images, 66.7% for fair images and 57.0% for poor images, while the intra-grader absolute error was <5% for 96.9% for excellent images, 91.3% for good images, 70.4% for fair images and 59.3% for poor images (Tables 2, 3).

Table 2. *Inter- and intra-grader agreement for ECD values with different image quality.*

Image Quality rating	Inter-grader Agreement	Intra-grader Agreement
	(error <5%)	(error <5%)
Excellent (n=65)	98.5% (64/65)	96.9% (63/65)
Good (n=230)	83.5%% (192/230)	91.3% (210/230)
Fair (n=81)	66.7% (54/81)	70.4% (57/81)
Poor (n=86)	57.0% (49/86)	59.3% (51/86

Table 3. *Reproducibility of ECD measurement with DIRC image quality scales.*

	Image Quality Rating	Intraclass Correlation (95% confidence interval)
Intra-grader	Excellent	0.99 (0.98-0.99
	Good	0.99 (0.98-0.99)
	Fair	0.95 (0.92-0.96)
	Poor	0.90 (0.84-0.93)
Inter-grader	Excellent	0.96 (0.94-0.98)
	Good	0.98 (0.97-0.99)
	Fair	0.94 (0.91-0.96)
	Poor	0.86 (0.79-0.91)

DISCUSSION

The qualitative and quantitative assessment of corneal endothelium has implications for the distribution of donor corneas by eye banks to surgeons. With all of the variables that may affect ECD determination, an IQR scale is necessary before we can provide the most reliable ECD determination.

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The DIRC specular image quality scale uses the fixed frame and center method approach to assess donor specular image quality and ECD. The DIRC IQR scale classified image quality by the percent area of contiguous countable cells relative to the maximum Konan field of cells (240 μm × 400 μm) and level of "questionable" area that increases the difficulty of identifying cells within the gradable area. Using this scale, there is good agreement for both intra- and inter-grading with respect to image quality rating. In terms of variation in ECD values, both inter-grader and intra-grader differences were amplified with decreasing image quality. Analysis of ECD variation stratified by IQR shows that the DIRC scale offers a high intra/inter-grader reproducibility (ICC≥0.98) at higher image quality ratings (excellent/good). When the IQR is fair or poor, inter- and intra-grader agreement was decreased. For inter-grader reproducibility, there was a statistically significant difference in ECD values for fair and poor images. However, the agreement of ECD values between graders was greater than 50% and an acceptable ECD value could still be provided at a confidence level (ICC>0.8). This classification will be important in the final assessment of the reliability of the mean endothelial density in donor cornea tissue.

The Cornea Donor Study Group [1, 8] published the Specular Microscopy Reading Center Image Quality Classification (SMRC) of the corneal endothelium. Images were classified as excellent, good, fair and unanalyzable. The image quality grading scale used multiple-field variable frame analysis [9] for the image quality rating and ECD determination of HAI specular images. Benetz et al [8] used a dual-grading procedure and adjudication process to classify image quality and determine ECD. They reported that the methods used in the Specular Microscopy Ancillary Study were reliable for determining central corneal ECD in a multicenter eye bank study. However, the IQR scale used for the Specular Microscopy Ancillary Study included only three categories (excellent, good, fair) for gradable images. In addition, the large gap between the grade of fair and that of non-gradable precluded many images from ECD analysis. In contrast, the DIRC IQR scale extended the gradable scale to include an image quality grade of poor, allowing an additional 16% of corneas to be analyzed which would otherwise not be offered for transplant for lack of reportable ECD value. Meanwhile, using the DIRC IQR scale, only 14% of images are excluded from ECD analysis because of non-gradable image quality compared to 30% by the scale in the Specular Microscopy Ancillary Study.

A cornea suitable for transplantation with an ECD of 2000 cells/mm^2 [10-12] will have approximately 150 countable cells in the entire Konan field. On the other hand, a cornea with less than 100 cells in the entire Konan field is unsuitable for transplantation of the endothelium, as it will have an ECD value is lower than 1000 cells/mm^2. When determining IQR of a specular image using the DIRC scale, percentage gradable area and level of questionable areas that increase the difficulty of identifying cells within the gradable area are taken into account. These factors are evaluated independently of the absolute number of countable cells. This eliminates the discrepancy between a clear image with low number of countable cells because of low ECD as compared to a poor quality image in which most cells in the field are too unclear or obscured to be counted. In other words, within a fixed surface area (240 μm × 400 μm), the same low number of cells available for counting could explained by either low number of endothelial cells in a good quality image (low ECD but good IQR) or a poor quality image that precludes confident identification of all normal cells within the frame (good ECD but poor IQR). Because we have shown that the ECD quantification of inter- and intra- grading are consistent, both low and high ECD values can be regarded as reliable and accurate in images of gradable IQR. Thus, the surgeon can make a confident, educated decision on donor selection by eliminating the donor corneas with low ECD.

In summary, we report on a new IQR scale for specular microscopy, specifically for use in eye banking. High reproducibility in terms of both intra- and inter-grader agreement demonstrates that this scale is reliable for assessment of donor cornea specular image quality. As expected, ECD values associated with excellent and good quality images have higher consistency than those of fair and poor quality images. Nevertheless, even when IQR is poor, acceptable ECD can still be provided. Finally, less than 15% of images were of non-gradable image quality, precluding ECD analysis.

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