Comparison of semi-automated center-dot and fully automated endothelial cell analyses from specular microscopy images

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Abstract

Purpose: To evaluate 2 specular microscopy analysis methods across different endothelial cell densities (ECDs).

Methods: Endothelial images of 1 eye from each of 45 patients were taken by using 3 different specular microscopes (3 replicates each). To determine the consistency of the center-dot method, we compared SP-6000 and SP-2000P images. CME-530 and SP-6000 images were compared to assess the consistency of the fully automated method. The SP-6000 images from the 2 methods were compared. Intraclass correlation coefficients (ICCs) for the 3 measurements were calculated, and parametric multiple comparisons tests and Bland–Altman analysis were performed.

Results: The ECD mean value was 2425 ± 883 (range: 516–3707) cells/mm². ICC values were >0.9 for all 3 microscopes for ECD, but the coefficients of variation (CVs) were 0.3–0.6. For ECD measurements, Bland–Altman analysis revealed that the mean difference was 42 cells/mm² between the SP-2000P and SP-6000 for the center-dot method; 57 cells/mm² between the SP-6000 measurements from both methods; and −5 cells/mm² between the SP-6000 and CME-530 for the fully automated method (95% limits of agreement: −201 to 284 cell/mm², −410 to 522 cells/mm², and −327 to 318 cells/mm², respectively). For CV measurements, the mean differences were −3%, −12%, and 13% (95% limits of agreement: −18% to 11%, −26% to 2%, and −5% to 32%, respectively).

Conclusions: Despite using 3 replicate measurements, the precision of the center-dot method with the
SP-2000P and SP-6000 software was only ±10% for ECD data and was even worse for the fully automated method.

Key words: specular microscopy, low ECD, fully-automated method without any cell border correction, semi-automated center-dot method
**Introduction**

Corneal endothelial cells maintain corneal transparency by using a pumping mechanism to remove fluid from the cornea [1, 2]. Various factors, such as aging, drugs, surgery, and inflammation, reduce corneal endothelial cell density (ECD) [3-5], which leads to a loss of corneal transparency and ultimately to the need for corneal transplantation. ECD is not easily regenerated, so protecting corneal endothelial cells is critical for maintaining healthy vision over a lifetime. ECD is, therefore, an important parameter for evaluating the condition of the corneal endothelium, especially preoperatively, when accurate knowledge of the ECD is essential. Currently, assessing ECD accurately remains a challenge.

Various types of corneal endothelium measuring devices have been developed, but results have been inconsistent [6]. The most popular device is the noncontact specular microscope, which obtains images of the corneal endothelium by using tangential illumination of the corneal surface. From these images, endothelial cells can be assessed and analyzed quantitatively and qualitatively.

The first analysis method developed for noncontact specular microscopy was the semi-automated center-dot method. In this method, the examiner identifies the centers of corneal endothelial cells and estimates the boundaries of the cells from these center points, which is then used to count the cells and calculate the ECD. To obtain accurate measurements by using this method, the US Food and Drug Administration has recommended that 6 images should be acquired prior to operations and that 3 images should be acquired at postoperative visits (without actually specifying if all 3 images need to be
analyzed) [7]. Other reports have recommended that a minimum of 75 cells be counted [8], which means that acquiring accurate measurements with the semi-automated center-dot method is labor intensive and time consuming.

To enable easier and less time-consuming measurements with noncontact specular microscopes, several companies have developed a new method that is fully automated and does not use any cell border correction. In this method, the device detects captured endothelial cells and determines the cell area by identifying the boundary of each endothelial cell. The key for precise measurements is accurate determination of the boundary.

Some previous studies have reported agreement between the semi-automated center-dot method and the fully automated method without any cell border correction and with any cell border correction. However, all of their subjects had normal ECDs [9-14]. Additionally, one study compared between the fully automated method without any cell border corrections and the automated method with cell border corrections (the ECDs ranged from 417–3263 cells/mm²) [12]. The aim of our study was to evaluate and compare the consistency between the semi-automated center-dot method and fully automated method without any cell border correction and the consistency of results between devices used within each method with subjects representing wider range of ECDs, especially with low ECDs.

Materials and Methods
Study Design and Ethics Statement

This was a cross-sectional observational study approved by the Institutional Review Board of Saneikai Tsukazaki Hospital and conducted according to the tenets of the Declaration of Helsinki. Written informed consent was obtained from each subject before participation in this study.

Specular Microscopes

3 non-contact specular microscopes were used in this study: a Topcon SP-2000P (Topcon, Tokyo, Japan), a Konan Noncon ROBO SP-6000 (Konan Medical Inc., Hyogo, Japan), and a Nidek Specular Microscope CME-530 (Nidek Co, Ltd., Aichi, Japan). These 3 devices use different image analysis software to analyze endothelial cell morphology. Before screening the patients’ ECDs for recruitment, we retrospectively investigated their medical records in our hospital and checked the results of each of the microscopes.

Subjects

The subjects were recruited from among patients in our hospital between September and November 2014. Medical records were screened retrospectively to recruit 3 groups of patients according to their ECD: >3000 cells/mm², between 2000 and 3000 cells/mm², and <2000 cells/mm². These subjects were then studied prospectively. Ultimately, we recruited 45 eyes of 45 patients (28 females and 17 males;
mean age: 43.2 ± 24.8 years; age range: 5–89). Table 1 presents background data for the subjects. The
ECD mean value was 2425 ± 883 (mean ± standard deviation; range: 516–3707 cells/mm$^2$).

Fifteen of the subjects (mean age: 76.3 ± 5.8 years; age range: 67–89) had an ECD of <2000 as a main
result of previous surgery: no surgery (3 patients); cataract surgery (5 patients), Descemet’s stripping
automated endothelial keratoplasty (DSAEK, 1 patient), cataract surgery and DSAEK (1 patient);
cataract surgery and penetrating keratoplasty (2 patients), cataract surgery and glaucoma surgery (2
patients), and vitrectomy (1 patient). The mean postoperative period was 32.9 ± 21.8 months (range:
8–80 months).

Measurement of ECD

The subjects were instructed to maintain their head upright on the specular microscope’s chin rest with
their eyes to the front. Only 1 eye was assessed. Three measurements were taken with each of the
microscopes, and the mean of the 3 measurements was used for analysis. The measurements were
performed by 3 examiners who were familiar with specular microscopy. For subjects with an ECD of
<2000 cells/mm$^2$, the minimum cell count was set to 30 because counting >100 cells in these cases was
difficult.

Semi-automated Center-dot Method (SP-2000P and SP-6000)
For each subject, we used the SP-2000P and SP-6000 to obtain ≥3 images of the central cornea with the auto-control and auto-capture modes. From these endothelial images, 3 showing clear edges were selected by the examiner. The examiner plotted the centers of >30 corneal endothelial cells for the center method, and the built-in endothelial cell morphology analysis was performed consecutively in each image. The 3 analyses were all performed by the same examiner.

Fully-automated Method Without Any Cell Border Correction (SP-6000 and CME-530)

We used the SP-6000 and CME-530 to obtain ≥3 images of the central cornea, which were captured by using the auto-control and auto-capture modes. From the endothelial images captured, 3 showing clear edges were selected. To determine the endothelial cells automatically, the instruments detected the boundaries of ≥30 cells. The analysis was performed by the same examiner for each image captured consecutively. We did not adjust the boundaries between the endothelial cells in the images.

Figure 1 shows sample images from a 76-year-old male analyzed by using the semi-automated center-dot method and fully-automated method without any cell border correction.

Analysis

ECD was used to determine the agreement between devices or analysis methods. For the sub-analysis, we also evaluated the average endothelial cell area (AVG) and the coefficient of variation (CV, a
measure of the variation in endothelial form).

To determine the consistency of the semi-automated center-dot method, we used the more common SP-6000 as a benchmark to compare with the results obtained from the SP-2000P. For the inter-method comparison, the semi-automated center-dot method and fully-automated method without any cell border correction were compared by using images obtained from the SP-6000. For the analysis of the consistency of the fully-automated method without any cell border correction, images from the CME-530 and SP-6000 were compared.

**Statistical Analysis**

Statistical analysis was performed by using JMP version 10.0.0 software (SAS Institute Inc., Cary, NC, USA) and Statcel 3 (OMS Publishing Ltd., Tokyo, Japan). Data are expressed as the mean ± standard deviation (SD). *P* values < 0.05 were considered as indicating statistical significance.

The repeatability of 3 consecutive measurements for each specular microscope was evaluated by calculating intraclass correlation coefficients, ICCs (1,1) (i.e., intrarater reliability, one-way random effects model). An ICC value of 0 would indicate the level of agreement produced by chance alone, whereas a value of 1 would indicate perfect, positive agreement.

Interdevice differences were initially evaluated by using analysis of variance (ANOVA) to detect any significant divergences in the 3 specular microscopes as a group and then by Tukey–Kramer post-hoc
analysis to check for significant differences between each device.

In the Bland–Altman analysis, the distribution of the measurements was expressed as the mean difference and SD between 2 devices; in addition, the 95% limits of agreement (LOA), which were defined as the mean difference ± 1.96 SD, were determined to assess agreement between the devices [15, 16].

Results

The ICC values showing the consistency of results between the devices and between analysis methods, each obtained from 3 measurements, are shown in Table 2. The calculated ICC values for the measurements of ECD and AVG from repeated assessments ranged from 0.92 to 0.99. The calculated ICC values in the measurements of CV, from repeated assessments, ranged from 0.34 to 0.69.

One-way ANOVA showed no significant differences among the 3 devices combined with the 2 analysis methods for the ECD and AVG values ($p = 0.95$ and 0.96, respectively). However, there was a statistically significant difference among the CV values ($p < 0.01$). Post-hoc analysis using the Tukey–Kramer test showed no significant difference between the two devices (SP-2000P and SP-6000) for the semi-automated center-dot method; however, there were significant differences for the SP-6000 between the two analysis methods ($p < 0.01$), as well as between the SP-6000 and CME-530 for the fully-automated method without any cell border correction ($p < 0.01$, Table 3).
Table 4).}

181

**Endothelial Cell Density**

182 Figures 2A–C show Bland–Altman plots for the values of ECD obtained from the 3 devices and 2

183 analysis methods.

184 A: The mean difference was 42 cells/mm², the 95% LOA was narrow (486 cells/mm²), and rs was low

185 (0.067).

186 B: The semi-automated center-dot method tended to give smaller measurement values than those of the

187 fully-automated method without any cell border correction for ECD of <2034 cells/mm². The mean

188 difference was 56 cells/mm², but the 95% LOA was wide (932 cells/mm²), and rs was high (0.7).

189 C: The mean difference was only ~5 cells/mm², the 95% LOA was relatively narrow (646 cells/mm²), and

190 rs was low (0.091).

191

**Average Endothelial Cell Area**

193 Figures 3A–C show the Bland–Altman plots for the values of AVG obtained from the 2 devices and 2

194 analysis methods.
A: The SP-2000P semi-automated center-dot method gave smaller measurements than those of the
SP-6000 semi-automated center-dot method when the AVG increased from the approximate line based on
the scatter plot of the results. The mean difference was only $-11 \mu m^2$, the 95% LOA was narrow ($128
\mu m^2$), and $rs$ was low ($-0.11$).

B: The mean difference was only $4 \mu m^2$, the 95% LOA was narrow ($302 \mu m^2$), and $rs$ was low ($0.39$).

These results indicate good agreement between the 2 methods in measuring the AVG when it was $\leq 400
\mu m^2$; however, for larger AVG values, the variance was greater, which suggested that the agreement was
poor especially for low ECD.

C: The mean difference was only $33 \mu m^2$, the 95% LOA was narrow ($423 \mu m^2$), and $rs$ was low ($0.23$).

These results show that agreement was good between the devices when using the fully automated method
without any cell border correction for $AVG \leq 400 \mu m^2$; however, higher AVG values showed greater
variance, which suggested that the agreement was especially poor for low ECD.

**Coefficient of Variation**

Figures 4A–C shows Bland–Altman plots for the values of CV obtained from the 3 devices and 2 analysis
methods.

A: The mean difference was only $-3.4\%$, the 95% LOA was narrow ($29.6\%$), and $rs$ was low ($0.13$). The
results indicate good agreement between the 2 devices when using the center-dot method to measure CV.
B: The SP-6000 semi-automated center-dot method gave smaller measurements than those of the SP-6000 fully-automated method without any cell border correction when the CV increased from the approximate line based on the scatter plot of the results. The mean difference was only $-12.0\%$, the 95% LOA was narrow (28.7%), and rs was low ($-0.28$). Overall, the SP-6000 fully-automated method without any cell border correction gave higher measurements for CV than those of the SP-6000 semi-automated center-dot method.

C: The SP-6000 gave larger measurements than those of the CME-530 when CV increased from the approximate line based on the scatter plot of the results. The mean difference was only 13.4%, the 95% LOA was wide (36.8%), and rs was low (0.26). Overall, the CME-530 gave smaller measurements for CV than those of the SP-6000 when using the fully-automated method without any cell border correction.

Discussion

It has also been reported that the semi-automated center-dot method is time-consuming but more appropriate than the fully automated method without any cell border correction that produces inaccurate measurements [10, 17]. However, in daily clinical practice where time is limited, the fully-automated method without any cell border correction has attracted clinicians’ attention as a useful method for evaluating the state of endothelial cells more efficiently. It is, therefore, important to know the level of agreement between the 2 methods. Because previous studies only included patients with ECD in the
normal range, it was essential to compare the 2 methods in patients with low ECD.

Even though the present study included patients with ECD of \(<2000 \text{cell/mm}^2\), the assessment of ECD measurement repeatability showed ICCs of \(\geq 0.9\) for all pairings of devices and methods. Furthermore, Bland–Altman analysis revealed stronger agreement between the 2 microscopes used in the semi-automated center-dot method (95\% LOA of 486 cells/mm\(^2\)) than that between the semi-automated center-dot method and the fully automated method without any cell border correction (95\% LOA of 932 cells/mm\(^2\)) and between the 2 microscopes used in the fully automated method without any cell border correction (95\% LOA of 646 cells/mm\(^2\)). The data in Figure 2A show that the outcome measures for ECD were within 1 grade point for density estimates, but this was not the case for comparisons between the semi-automated center-dot method and the fully automated method without any cell border correction (Fig. 2B), and comparisons between the 2 fully automated methods without any cell border correction (Fig. 2C) were on the borderline of acceptability. The data in Figure 3A show that the outcome measures for AVG were \(\leq 1\) grade point, but this was not the case for comparisons between the semi-automated center-dot method and the fully automated method without any cell border correction (Fig. 3B) and comparisons between the 2 fully automated methods without any cell border correction (Fig. 3C). The data in Figures 4A–C show that the outcome measures for CV were within 1 grade point.

Figure 5 shows the 3 images of an 82-year-old man with extremely low ECD. The images were analyzed by using both software systems and the fully automated method without any cell border correction. The
values obtained by the fully automated method without any cell border correction were thought to be influenced by the device’s individual software programs. When the SP-6000 fully automated method without any cell border correction is used, the software identifies the cells by attempting to detect as many cell partitions as possible. This system often misidentifies large cells as small cells, especially in subjects with low ECD. This commonly observed cell-detection error caused high CV measurements (39.7 ± 8.5%) and overestimation of ECD (1380 ± 612 cells/mm²) in 15 patients with ECD of <2000 cells/mm². In contrast, the CME503 fully automated method without any cell border correction only measures cells that can be found easily. This commonly observed cell-detection error caused low CV measurements (33.7 ± 9.3%) and overestimation of ECD (1383 ± 453 cells/mm²) in 15 patients with ECD of <2000 cells/mm². The fully automated method without any cell border correction used with both the SP6000 and CME530 showed high variance in image quality, so multiple replicate measurements should be used [7], especially for patients with low ECD.

Figure 6 shows the differences among the 3 images of the same patient shown in Figure 5 that were analyzed by both software systems using the semi-automated center-dot method. In the semi-automated center-dot method, the examiners identified and counted cells that were easily recognized; this resulted in a lower CV and ECD for this method (CV: SP-2000P, 29.1 ± 9.8%; SP-6000, 31.6 ± 5.6%; ECD: SP-2000P, 1240 ± 481 cells/mm²; SP-6000, 1228 ± 472 cells/mm²) in 15 patients with ECD of <2000 cells/mm². These differences in methodology caused variations in the analytical results even for images.
captured from the same patients. For AVG, the repeatability was good for any pairing of device and analytical method (all ICCs > 0.9). However, the ability to correctly detect the cell areas became weak in both the fully automated method without any cell border correction and semi-automated center-dot method in patients with low ECD for whom cell partitions were not clearly displayed. For CV, in addition to the variation caused by differences in the analytical methods between devices, when even a small number of abnormal cells exist in the cell area, the CV tends to be higher, as reported in previous studies. Therefore, it is still difficult to appropriately evaluate CV [9, 18]. For the patients with low ECD in our study, variations in detecting cell areas tended to occur, which resulted in low ICC values. Our study had 2 limitations. First, it has been suggested that examiners should correct cell-detection errors when using the fully automated method without any cell border correction to minimize variation and increase correlation [11, 12, 19]. In this study, we did not make such adjustments so that we could better understand the actual performance of these devices when using the fully automated method without any cell border correction to analyze images with low ECD. The second limitation was that we included cases with only approximately 30 cells that could be counted in the data. However, even counting 30 cells was often difficult in the subjects with low ECD, so further research is needed to develop a counting method suitable for use with low ECD.

**Conclusion:** Despite using 3 repeated measures, use of the semi-automated center-dot method with the
SP-2000P and SP-6000 software only yielded ECD results with a precision of ± 10% and even lower precision for the results obtained by using the fully automated method without any cell border correction on the SP-6000 and CME-530. Additionally, specular microscopy analysis had greater errors in patients with low ECD.
Table 1. Subject demographics

<table>
<thead>
<tr>
<th></th>
<th>ECD &gt; 3000 (cells/mm²)</th>
<th>2000 &lt; ECD &lt; 3000 (cells/mm²)</th>
<th>ECD &lt; 2000 (cells/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Age (range) (y)</td>
<td>24.8 ± 9.6 (5–41)</td>
<td>28.5 ± 7.2 (22–47)</td>
<td>76.3 ± 5.8 (67–89)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>80</td>
<td>60</td>
<td>47</td>
</tr>
<tr>
<td>History of surgery (%)</td>
<td>0</td>
<td>0</td>
<td>73</td>
</tr>
<tr>
<td>Target eye: right (%)</td>
<td>46</td>
<td>80</td>
<td>53</td>
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</tbody>
</table>

Table 2. Average ICC values (n=3) for each device and analysis method

<table>
<thead>
<tr>
<th></th>
<th>ICC (1,1)</th>
<th>95% CI</th>
</tr>
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<tbody>
<tr>
<td><strong>SP2000P center-dot</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECD</td>
<td>0.989</td>
<td>0.981–0.993</td>
</tr>
<tr>
<td>AVG</td>
<td>0.991</td>
<td>0.985–0.995</td>
</tr>
<tr>
<td>CV</td>
<td>0.691</td>
<td>0.553–0.803</td>
</tr>
<tr>
<td><strong>SP6000 center-dot</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECD</td>
<td>0.986</td>
<td>0.977–0.992</td>
</tr>
<tr>
<td>AVG</td>
<td>0.989</td>
<td>0.982–0.994</td>
</tr>
<tr>
<td>CV</td>
<td>0.341</td>
<td>0.157–0.529</td>
</tr>
<tr>
<td><strong>SP6000 automated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECD</td>
<td>0.974</td>
<td>0.869–0.985</td>
</tr>
<tr>
<td>AVG</td>
<td>0.917</td>
<td>0.869–0.951</td>
</tr>
<tr>
<td>CV</td>
<td>0.552</td>
<td>0.384–0.701</td>
</tr>
<tr>
<td><strong>CME530 automated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECD</td>
<td>0.992</td>
<td>0.987–0.995</td>
</tr>
<tr>
<td>AVG</td>
<td>0.986</td>
<td>0.977–0.992</td>
</tr>
<tr>
<td>CV</td>
<td>0.672</td>
<td>0.529–0.789</td>
</tr>
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</table>
ICC (1, 1): intraclass correlation coefficients, one-way random effects model
95% CI: 95% confidence interval

Table 3. Mean ECD, AVG, and CV values for the 3 devices and 2 analysis methods.

<table>
<thead>
<tr>
<th></th>
<th>SP2000P center-dot</th>
<th>SP6000 center-dot</th>
<th>SP6000 automated</th>
<th>CME-530 automated</th>
</tr>
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<tbody>
<tr>
<td>ECD (mean ± SD) (cells/mm²)</td>
<td>2483 ± 973</td>
<td>2441 ± 953</td>
<td>2385 ± 824</td>
<td>2390 ± 793</td>
</tr>
<tr>
<td>AVG (mean ± SD) (µm²)</td>
<td>531 ± 376</td>
<td>542 ± 383</td>
<td>537 ± 373</td>
<td>505 ± 297</td>
</tr>
<tr>
<td>CV (mean ± SD) (%)</td>
<td>27.8 ± 6.8</td>
<td>31.2 ± 5.6</td>
<td>43.3 ± 7.2</td>
<td>29.8 ± 6.4</td>
</tr>
</tbody>
</table>

†significant different in CV was found between SP-6000 center method and SP-6000 boundary method by the Tukey–Kramer test.

*significant different in CV was found between SP-6000 boundary method and CME-530 boundary method by the Tukey–Kramer test.

Table 4. Bland–Altman Analysis for ECD, AVG, and CV values for 3 devices and 2 analysis methods

<table>
<thead>
<tr>
<th>Bland–Altman Analysis</th>
<th>Difference Between</th>
<th>Correlation Coefficient</th>
<th>2 Measurements</th>
<th>LOA (cells/mm²)</th>
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<tr>
<td>rs</td>
<td>P</td>
<td>Mean</td>
<td>SD</td>
<td>Lower Upper 95%</td>
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<table>
<thead>
<tr>
<th></th>
<th>(cells/mm²)</th>
<th>(cells/m²)</th>
<th>95%</th>
<th>of 95%</th>
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<td><strong>ECD (cells/mm²)</strong></td>
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<td></td>
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<tr>
<td>SP-2000P and SP-6000</td>
<td>0.06</td>
<td>0.65</td>
<td>42</td>
<td>124</td>
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<tr>
<td>center-dot</td>
<td>7</td>
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<tr>
<td>SP-6000 center-dot and SP-6000 automated</td>
<td>0.7</td>
<td>&lt;0.001</td>
<td>56</td>
<td>238</td>
</tr>
<tr>
<td>SP-6000 and CME-530 automated</td>
<td>0.09</td>
<td>0.54</td>
<td>−5</td>
<td>165</td>
</tr>
<tr>
<td><strong>AVG (µm²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP-2000P and SP-6000</td>
<td>−0.1</td>
<td>0.45</td>
<td>−11</td>
<td>33</td>
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<tr>
<td>center-dot</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SP-6000 center-dot and SP-6000 automated</td>
<td>0.39</td>
<td>0.009</td>
<td>4</td>
<td>77</td>
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<tr>
<td>SP-6000 and CME-530 automated</td>
<td>0.23</td>
<td>0.13</td>
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<td>108</td>
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<tr>
<td><strong>CV (%)</strong></td>
<td></td>
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<tr>
<td>SP-2000P and SP-6000</td>
<td>0.13</td>
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<td>center-dot</td>
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<tr>
<td>SP-6000 center-dot and SP-6000 automated</td>
<td>−0.2</td>
<td>0.06</td>
<td>−12</td>
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<tr>
<td>SP-6000 automated</td>
<td>8</td>
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</tr>
<tr>
<td>SP-6000 and CME-530 automated</td>
<td>0.26</td>
<td>0.08</td>
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</tbody>
</table>

rs: Regression on differences
LOA: 95% limits of agreement
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Authors’ Contributions

S.M., S.N., C.K., H.T., T.C., and Y.K. were involved in designing the study, S.M., N.M., and K.Y., conducted the study, S.M., and S.N., statistically analyzed the results of the study and all authors gave their final approval of the article for submission.
References


Figure captions

**Fig 1.** Images from a 76-year-old male analyzed by using the semi-automated center-dot method and fully automated method without any cell border correction obtained by using 3 different devices

**Fig 2A.** Bland–Altman plots for the values of endothelial cell density (ECD) obtained from the 3 devices and 2 analysis methods

Comparison between SP2000P semi-automated center-dot method and SP6000 semi-automated center-dot method for ECD estimates

The line shows a regression analysis on the net differences

**Fig 2B.** Comparison between SP6000 semi-automated center-dot method and SP6000 fully-automated method without any cell border correction for ECD estimates

The line shows a regression analysis on the net differences

**Fig 2C.** Comparison between SP6000 fully-automated method without any cell border correction and CME530 fully-automated method without any cell border correction for ECD estimates

The line shows a regression analysis on the net differences

**Fig 3A.** Bland–Altman plots for the values of average endothelial cell area (AVG) obtained from the 3 devices and 2 analysis methods

Comparison between SP2000P semi-automated center-dot method and SP6000 semi-automated center-dot method for estimates of AVG
The line shows a regression analysis on the net differences

**Fig 3B.** Comparison between SP6000 semi-automated center-dot method and SP6000 fully-automated method without any cell border correction for estimates of AVG

The line shows a regression analysis on the net differences

**Fig 3C.** Comparison between SP6000 fully-automated method without any cell border correction and CME530 fully-automated method without any cell border correction for estimates of AVG

The line shows a regression analysis on the net differences

**Fig 4A.** Bland–Altman plots for the values of the coefficients of variation (CVs) obtained from the 3 devices and 2 analysis methods.

Comparison between SP2000P semi-automated center-dot method and SP6000 semi-automated center-dot method for estimates of CV in ell area

The line shows a regression analysis on the net differences

**Fig 4B.** Comparison between SP6000 semi-automated center-dot method and SP6000 fully-automated method without any cell border correction for estimates of the CV in ell area

The line shows a regression analysis on the net differences

**Fig 4C.** Comparison between SP6000 fully-automated method without any cell border correction and CME530 fully-automated method without any cell border correction for estimates of the CV in ell area

The line shows a regression analysis on the net differences
Fig 5. An 82-year-old-man with extremely low ECD analyzed by using both software systems and the fully automated method without any cell border correction. Each of the 3 images have many variations and there are many differences in the way the cells are identified.

Fig 6. The same patient with extremely low ECD in Figure 5 analyzed by using both software systems and the fully automated method without any cell border correction. Each of the 3 images have many variations, but there are fewer differences in the ways the cells are identified in Figure 6 than in Figure 5.