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

2 microscopy images

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24

25 **Abstract**

26 **Purpose:** To evaluate 2 specular microscopy analysis methods across different endothelial cell densities

27 (ECDs).

28 **Methods:** Endothelial images of 1 eye from each of 45 patients were taken by using 3 different specular

29 microscopes (3 replicates each). To determine the consistency of the center-dot method, we compared

30 SP-6000 and SP-2000P images. CME-530 and SP-6000 images were compared to assess the consistency

31 of the fully automated method. The SP-6000 images from the 2 methods were compared. Intraclass

32 correlation coefficients (ICCs) for the 3 measurements were calculated, and parametric multiple

33 comparisons tests and Bland−Altman analysis were performed.

34 **Results:** The ECD mean value was 2425 ± 883 (range: 516–3707) cells/mm². ICC values were >0.9 for

35 all 3 microscopes for ECD, but the coefficients of variation (CVs) were 0.3–0.6. For ECD measurements,

36 Bland−Altman analysis revealed that the mean difference was 42 cells/mm² between the SP-2000P and

37 SP-6000 for the center-dot method; 57 cells/mm² between the SP-6000 measurements from both

38 methods; and −5 cells/mm² between the SP-6000 and CME-530 for the fully automated method (95%

39 limits of agreement: −201 to 284 cell/mm², −410 to 522 cells/mm², and −327 to 318 cells/mm²,

40 respectively). For CV measurements, the mean differences were –3%, –12%, and 13% (95% limits of

41 agreement: –18% to 11%, −26% to 2%, and −5% to 32%, respectively).

42 **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

50

51 **Introduction**

52 Corneal endothelial cells maintain corneal transparency by using a pumping mechanism to remove fluid

53 from the cornea [1, 2]. Various factors, such as aging, drugs, surgery, and inflammation, reduce corneal

54 endothelial cell density (ECD) [3-5], which leads to a loss of corneal transparency and ultimately to the

55 need for corneal transplantation. ECD is not easily regenerated, so protecting corneal endothelial cells is

56 critical for maintaining healthy vision over a lifetime. ECD is, therefore, an important parameter for

57 evaluating the condition of the corneal endothelium, especially preoperatively, when accurate knowledge

58 of the ECD is essential. Currently, assessing ECD accurately remains a challenge.

59 Various types of corneal endothelium measuring devices have been developed, but results have been

60 inconsistent [6]. The most popular device is the noncontact specular microscope, which obtains images

61 of the corneal endothelium by using tangential illumination of the corneal surface. From these images,

62 endothelial cells can be assessed and analyzed quantitatively and qualitatively.

63 The first analysis method developed for noncontact specular microscopy was the semi-automated

64 center-dot method. In this method, the examiner identifies the centers of corneal endothelial cells and

65 estimates the boundaries of the cells from these center points, which is then used to count the cells and

66 calculate the ECD. To obtain accurate measurements by using this method, the US Food and Drug

67 Administration has recommended that 6 images should be acquired prior to operations and that 3 images

68 should be acquired at postoperative visits (without actually specifying if all 3 images need to be

69 analyzed) [7]. Other reports have recommended that a minimum of 75 cells be counted [8], which means

70 that acquiring accurate measurements with the semi-automated center-dot method is labor intensive and

71 time consuming.

72 To enable easier and less time-consuming measurements with noncontact specular microscopes, several

73 companies have developed a new method that is fully automated and does not use any cell border

74 correction. In this method, the device detects captured endothelial cells and determines the cell area by

75 identifying the boundary of each endothelial cell. The key for precise measurements is accurate

76 determination of the boundary.

77 Some previous studies have reported agreement between the semi-automated center-dot method and the

78 fully automated method without any cell border correction and with any cell border correction. However,

79 all of their subjects had normal ECDs [9-14]. Additionally, one study compared between the fully

80 automated method without any cell border corrections and the automated method with cell border

81 corrections (the ECDs ranged from 417–3263 cells/mm^2) [12]. The aim of our study was to evaluate and

82 compare the consistency between the semi-automated center-dot method and fully automated method

83 without any cell border correction and the consistency of results between devices used within each

84 method with subjects representing wider range of ECDs, especially with low ECDs.

85

86 **Materials and Methods**

87 **Study Design and Ethics Statement**

88 This was a cross-sectional observational study approved by the Institutional Review Board of Saneikai

89 Tsukazaki Hospital and conducted according to the tenets of the Declaration of Helsinki. Written

90 informed consent was obtained from each subject before participation in this study.

91

92 **Specular Microscopes**

93 3 non-contact specular microscopes were used in this study: a Topcon SP-2000P (Topcon, Tokyo, Japan),

94 a Konan Noncon ROBO SP-6000 (Konan Medical Inc., Hyogo, Japan), and a Nidek Specular

95 Microscope CME-530 (Nidek Co, Ltd., Aichi, Japan). These 3 devices use different image analysis

96 software to analyze endothelial cell morphology. Before screening the patients' ECDs for recruitment,

97 we retrospectively investigated their medical records in our hospital and checked the results of each of

98 the microscopes.

99

100 **Subjects**

101 The subjects were recruited from among patients in our hospital between September and November

102 2014. Medical records were screened retrospectively to recruit 3 groups of patients according to their

103 ECD: >3000 cells/mm^2, between 2000 and 3000 cells/mm^2, and <2000 cells/mm^2. These subjects were

104 then studied prospectively. Ultimately, we recruited 45 eyes of 45 patients (28 females and 17 males;

105 mean age: 43.2 ± 24.8 years; age range: 5–89). Table 1 presents background data for the subjects. The

106 ECD mean value was 2425 ± 883 (mean ± standard deviation; range: 516–3707 cells/mm^2).

107 Fifteen of the subjects (mean age: 76.3 ± 5.8 years; age range: 67–89) had an ECD of <2000 as a main

108 result of previous surgery: no surgery (3 patients); cataract surgery (5 patients), Descemet's stripping

109 automated endothelial keratoplasty (DSAEK, 1 patient), cataract surgery and DSAEK (1 patient);

110 cataract surgery and penetrating keratoplasty (2 patients), cataract surgery and glaucoma surgery (2

111 patients), and vitrectomy (1 patient). The mean postoperative period was 32.9 ± 21.8 months (range:

112 8–80 months).

113

114 **Measurement of ECD**

115 The subjects were instructed to maintain their head upright on the specular microscope's chin rest with

116 their eyes to the front. Only 1 eye was assessed. Three measurements were taken with each of the

117 microscopes, and the mean of the 3 measurements was used for analysis. The measurements were

118 performed by 3 examiners who were familiar with specular microscopy. For subjects with an ECD of

119 <2000 cells/mm^2, the minimum cell count was set to 30 because counting >100 cells in these cases was

120 difficult.

121

122 **Semi-automated Center-dot Method (SP-2000P and SP-6000)**

123 For each subject, we used the SP-2000P and SP-6000 to obtain ≥3 images of the central cornea with the

124 auto-control and auto-capture modes. From these endothelial images, 3 showing clear edges were

125 selected by the examiner. The examiner plotted the centers of >30 corneal endothelial cells for the center

126 method, and the built-in endothelial cell morphology analysis was performed consecutively in each

127 image. The 3 analyses were all performed by the same examiner.

128

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

130 We used the SP-6000 and CME-530 to obtain ≥3 images of the central cornea, which were captured by

131 using the auto-control and auto-capture modes. From the endothelial images captured, 3 showing clear

132 edges were selected. To determine the endothelial cells automatically, the instruments detected the

133 boundaries of ≥30 cells. The analysis was performed by the same examiner for each image captured

134 consecutively. We did not adjust the boundaries between the endothelial cells in the images.

135 Figure 1 shows sample images from a 76-year-old male analyzed by using the semi-automated

136 center-dot method and fully-automated method without any cell border correction.

137

138 **Analysis**

139 ECD was used to determine the agreement between devices or analysis methods. For the sub-analysis,

140 we also evaluated the average endothelial cell area (AVG) and the coefficient of variation (CV, a

141 measure of the variation in endothelial form).

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

148

149 **Statistical Analysis**

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

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

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

159 analysis to check for significant differences between each device.

160 In the Bland–Altman analysis, the distribution of the measurements was expressed as the mean

161 difference and SD between 2 devices; in addition, the 95% limits of agreement (LOA), which were

162 defined as the mean difference ± 1.96 SD, were determined to assess agreement between the devices [15,

163 16].

164

165 **Results**

166 The ICC values showing the consistency of results between the devices and between analysis methods,

167 each obtained from 3 measurements, are shown in Table 2. The calculated ICC values for the

168 measurements of ECD and AVG from repeated assessments ranged from 0.92 to 0.99. The calculated

169 ICC values in the measurements of CV, from repeated assessments, ranged from 0.34 to 0.69.

170 One-way ANOVA showed no significant differences among the 3 devices combined with the 2 analysis

171 methods for the ECD and AVG values ($p = 0.95$ and 0.96, respectively). However, there was a

172 statistically significant difference among the CV values ($p < 0.01$). Post-hoc analysis using the

173 Tukey–Kramer test showed no significant difference between the two devices (SP-2000P and SP-6000)

174 for the semi-automated center-dot method; however, there were significant differences for the SP-6000

175 between the two analysis methods ($p < 0.01$), as well as between the SP-6000 and CME-530 for the

176 fully-automated method without any cell border correction ($p < 0.01$, Table 3).

177

178 **Bland–Altman analysis**

179 Agreement among the devices and methods in the values obtained for ECD, AVG, and CV was analyzed

180 by using Bland–Altman plots (Table 4).

181

182 **Endothelial Cell Density**

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

184 analysis methods.

185 A: The mean difference was 42 cells/mm^2, the 95% LOA was narrow (486 cells/mm^2), and rs was low

186 (0.067).

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

188 fully-automated method without any cell border correction for ECD of <2034 cells/mm^2. The mean

189 difference was 56 cells/mm^2, but the 95% LOA was wide (932 cells/mm^2), and rs was high (0.7).

190 C: The mean difference was only –5 cells/mm^2, the 95% LOA was relatively narrow (646 cells/mm^2), and

191 rs was low (0.091).

192

193 **Average Endothelial Cell Area**

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

195 analysis methods.

196 A: The SP-2000P semi-automated center-dot method gave smaller measurements than those of the

197 SP-6000 semi-automated center-dot method when the AVG increased from the approximate line based on

198 the scatter plot of the results. The mean difference was only−11 μm^2, the 95% LOA was narrow (128

199 μm^2), and rs was low (−0.11).

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

201 These results indicate good agreement between the 2 methods in measuring the AVG when it was ≤400

202 μm^2; however, for larger AVG values, the variance was greater, which suggested that the agreement was

203 poor especially for low ECD**.**

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

205 These results show that agreement was good between the devices when using the fully automated method

206 without any cell border correction for AVG ≤400 μm^2; however, higher AVG values showed greater

207 variance, which suggested that the agreement was especially poor for low ECD.

208

209 **Coefficient of Variation**

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

211 methods.

212 A: The mean difference was only−3.4%, the 95% LOA was narrow (29.6%), and rs was low (0.13). The

213 results indicate good agreement between the 2 devices when using the center-dot method to measure CV.

214 B: The SP-6000 semi-automated center-dot method gave smaller measurements than those of the SP-6000

215 fully-automated method without any cell border correction when the CV increased from the approximate

216 line based on the scatter plot of the results. The mean difference was only −12.0%, the 95% LOA was

217 narrow (28.7%), and rs was low (−0.28). Overall, the SP-6000 fully-automated method without any cell

218 border correction gave higher measurements for CV than those of the SP-6000 semi-automated center-dot

219 method.

220 C: The SP-6000 gave larger measurements than those of the CME-530 when CV increased from the

221 approximate line based on the scatter plot of the results. The mean difference was only 13.4%, the 95%

222 LOA was wide (36.8%), and rs was low (0.26). Overall, the CME-530 gave smaller measurements for CV

223 than those of the SP-6000 when using the fully-automated method without any cell border correction.

224

225 **Discussion**

226 It has also been reported that the semi-automated center-dot method is time-consuming but more

227 appropriate than the fully automated method without any cell border correction that produces inaccurate

228 measurements [10, 17]. However, in daily clinical practice where time is limited, the fully -automated

229 method without any cell border correction has attracted clinicians' attention as a useful method for

230 evaluating the state of endothelial cells more efficiently. It is, therefore, important to know the level of

231 agreement between the 2 methods. Because previous studies only included patients with ECD in the

232 normal range, it was essential to compare the 2 methods in patients with low ECD.

233 Even though the present study included patients with ECD of <2000 cell/mm^2, the assessment of ECD

234 measurement repeatability showed ICCs of ≥ 0.9 for all pairings of devices and methods. Furthermore,

235 Bland–Altman analysis revealed stronger agreement between the 2 microscopes used in the

236 semi-automated center-dot method (95% LOA of 486 cells/mm^2) than that between the semi-automated

237 center-dot method and the fully automated method without any cell border correction (95% LOA of 932

238 cells/mm^2) and between the 2 microscopes used in the fully automated method without any cell border

239 correction (95% LOA of 646 cells/mm^2). The data in Figure 2A show that the outcome measures for ECD

240 were within 1 grade point for density estimates, but this was not the case for comparisons between the

241 semi-automated center-dot method and the fully automated method without any cell border correction

242 (Fig. 2B), and comparisons between the 2 fully automated methods without any cell border correction

243 (Fig. 2C) were on the borderline of acceptability. The data in Figure 3A show that the outcome measures

244 for AVG were ≤ 1 grade point, but this was not the case for comparisons between the semi-automated

245 center-dot method and the fully automated method without any cell border correction (Fig. 3B) and

246 comparisons between the 2 fully automated methods without any cell border correction (Fig. 3C). The

247 data in Figures 4A–C show that the outcome measures for CV were within 1 grade point.

248 Figure 5 shows the 3 images of an 82-year-old man with extremely low ECD. The images were analyzed

249 by using both software systems and the fully automated method without any cell border correction. The

250 values obtained by the fully automated method without any cell border correction were thought to be
251 influenced by the device's individual software programs. When the SP-6000 fully automated method
252 without any cell border correction is used, the software identifies the cells by attempting to detect as
253 many cell partitions as possible. This system often misidentifies large cells as small cells, especially in
254 subjects with low ECD. This commonly observed cell-detection error caused high CV measurements
255 (39.7 ± 8.5%) and overestimation of ECD (1380 ± 612 cells/mm^2) in 15 patients with ECD of <2000
256 cells/mm^2. In contrast, the CME503 fully automated method without any cell border correction only
257 measures cells that can be found easily. This commonly observed cell-detection error caused low CV
258 measurements (33.7 ± 9.3%) and overestimation of ECD (1383 ± 453 cells/mm^2) in 15 patients with ECD
259 of <2000 cells/mm^2. The fully automated method without any cell border correction used with both the
260 SP6000 and CME530 showed high variance in image quality, so multiple replicate measurements should
261 be used [7], especially for patients with low ECD.
262 Figure 6 shows the differences among the 3 images of the same patient shown in Figure 5 that were
263 analyzed by both software systems using the semi-automated center-dot method. In the semi-automated
264 center-dot method, the examiners identified and counted cells that were easily recognized; this resulted in
265 a lower CV and ECD for this method (CV: SP-2000P, 29.1 ± 9.8%; SP-6000, 31.6 ± 5.6%; ECD:
266 SP-2000P, 1240 ± 481 cells/mm^2; SP-6000, 1228 ± 472 cells/mm^2) in 15 patients with ECD of <2000
267 cells/mm^2. These differences in methodology caused variations in the analytical results even for images

268 captured from the same patients. For AVG, the repeatability was good for any pairing of device and

269 analytical method (all ICCs > 0.9). However, the ability to correctly detect the cell areas became weak in

270 both the fully automated method without any cell border correction and semi-automated center-dot

271 method in patients with low ECD for whom cell partitions were not clearly displayed. For CV, in addition

272 to the variation caused by differences in the analytical methods between devices, when even a small

273 number of abnormal cells exist in the cell area, the CV tends to be higher, as reported in previous studies.

274 Therefore, it is still difficult to appropriately evaluate CV [9, 18]. For the patients with low ECD in our

275 study, variations in detecting cell areas tended to occur, which resulted in low ICC values.

276 Our study had 2 limitations. First, it has been suggested that examiners should correct cell-detection

277 errors when using the fully automated method without any cell border correction to minimize variation

278 and increase correlation [11, 12, 19]. In this study, we did not make such adjustments so that we could

279 better understand the actual performance of these devices when using the fully automated method without

280 any cell border correction to analyze images with low ECD. The second limitation was that we included

281 cases with only approximately 30 cells that could be counted in the data. However, even counting 30 cells

282 was often difficult in the subjects with low ECD, so further research is needed to develop a counting

283 method suitable for use with low ECD.

284

285 **Conclusion:** Despite using 3 repeated measures, use of the semi-automated center-dot method with the

286 SP-2000P and SP-6000 software only yielded ECD results with a precision of ± 10% and even lower

287 precision for the results obtained by using the fully automated method without any cell border correction

288 on the SP-6000 and CME-530. Additionally, specular microscopy analysis had greater errors in patients

289 with low ECD.

290

291

292

Table 1. Subject demographics

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

293

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

	ICC (1,1)	95% CI
SP2000P center-dot		
ECD	0.989	0.981–0.993
AVG	0.991	0.985–0.995
CV	0.691	0.553–0.803
SP6000 center-dot		
ECD	0.986	0.977–0.992
AVG	0.989	0.982–0.994
CV	0.341	0.157–0.529
SP6000 automated		
ECD	0.974	0.869–0.985
AVG	0.917	0.869–0.951
CV	0.552	0.384–0.701
CME530 automated		
ECD	0.992	0.987–0.995
AVG	0.986	0.977–0.992
CV	0.672	0.529–0.789

ICC (1, 1): intraclass correlation coefficients, one-way random effects model

95% CI: 95% confidence interval

294

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

	SP2000P center-dot	SP6000 center-dot	SP6000 automated	CME-530 automated
ECD（mean ± SD）(cells/mm²)	2483 ± 973（520–3679）	2441 ± 953（516–3707）	2385 ± 824（579-3424）	2390 ± 793（589–3303）
AVG (mean ± SD) (μm²)	531 ± 376（212–1925）	542 ± 383（270–1938）	537 ± 373（292–1743）	505 ± 297（296–1701）
CV (mean ± SD) (%)	27.8 ± 6.8（18–54）	31.2 ± 5.6（22–50)†	43.3 ± 7.2（29–59)†*	29.8 ± 6.4（19–51）*

295 †significant different in CV was found between SP-6000 center method and SP-6000 boundary method

296 by the Tukey–Kramer test.

297 *significant different in CV was found between SP-6000 boundary method and CME-530 boundary

298 method by the Tukey–Kramer test.

299

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

	Bland–Altman Analysis						
			Difference Between				
	Correlation	Coefficient	2 Measurements			LOA (cells/mm²)	
	rs	P	Mean	SD	Lower	Upper95%	Width

			(cells/mm²)	(cells/mm²)	95%		of 95%
ECD (cells/mm²)							
SP-2000P and SP-6000 center-dot	0.067	0.65	42	124	−202	284	486
SP-6000 center-dot and SP-6000 automated	0.7	<0.001	56	238	−410	522	932
SP-6000 and CME-530 automated	0.091	0.54	−5	165	−328	318	646
AVG (μm²)							
SP-2000P and SP-6000 center-dot	−0.11	0.45	−11	33	−76	52	128
SP-6000 center-dot and SP-6000 automated	0.39	0.009	4	77	−146	155	302
SP-6000 and CME-530 automated	0.23	0.13	33	108	−179	244	423
CV (%)							
SP-2000P and SP-6000 center-dot	0.13	0.4	−3	8	−18	11	30
SP-6000 center-dot and SP-6000 automated	−0.28	0.06	−12	7	−26	2	29
SP-6000 and CME-530 automated	0.26	0.08	13	9	−5	32	37

rs: Regression on differences

LOA: 95% limits of agreement

300

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303

304 **Authors' Contributions**

305 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.,

306 conducted the study, S.M., and S.N., statistically analyzed the results of the study and all authors gave

307 their final approval of the article for submission.

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357

358 **Figure captions**

359 **Fig 1.** Images from a 76-year-old male analyzed by using the semi-automated center-dot method and fully

360 automated method without any cell border correction obtained by using 3 different devices

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

362 and 2 analysis methods

363 Comparison between SP2000P semi-automated center-dot method and SP6000 semi-automated

364 center-dot method for ECD estimates

365 The line shows a regression analysis on the net differences

366 **Fig 2B.** Comparison between SP6000 semi-automated center-dot method and SP6000 fully-automated

367 method without any cell border correction for ECD estimates

368 The line shows a regression analysis on the net differences

369 **Fig 2C.** Comparison between SP6000 fully-automated method without any cell border correction and

370 CME530 fully-automated method without any cell border correction for ECD estimates

371 The line shows a regression analysis on the net differences

372 **Fig 3A.** Bland–Altman plots for the values of average endothelial cell area (AVG) obtained from the 3

373 devices and 2 analysis methods

374 Comparison between SP2000P semi-automated center-dot method and SP6000 semi-automated center-dot

375 method for estimates of AVG

376 The line shows a regression analysis on the net differences

377 **Fig 3B.** Comparison between SP6000 semi-automated center-dot method and SP6000 fully-automated

378 method without any cell border correction for estimates of AVG

379 The line shows a regression analysis on the net differences

380 **Fig 3C.** Comparison between SP6000 fully-automated method without any cell border correction and

381 CME530 fully-automated method without any cell border correction for estimates of AVG

382 The line shows a regression analysis on the net differences

383 **Fig 4A.** Bland–Altman plots for the values of the coefficients of variation (CVs) obtained from the 3

384 devices and 2analysis methods.

385 Comparison between SP2000P semi-automated center-dot method and SP6000 semi-automated center-dot

386 method for estimates of CV in ell area

387 The line shows a regression analysis on the net differences

388 **Fig4B.** Comparison between SP6000 semi-automated center-dot method and SP6000 fully-automated

389 method without any cell border correction for estimates of the CV in ell area

390 The line shows a regression analysis on the net differences

391 **Fig4C.** Comparison between SP6000 fully-automated method without any cell border correction and

392 CME530 fully-automated method without any cell border correction for estimates of the CV in ell area

393 The line shows a regression analysis on the net differences

394 **Fig 5.** An 82-year-old-man with extremely low ECD analyzed by using both software systems and the

395 fully automated method without any cell border correction. Each of the 3 images have many variations

396 and there are many differences in the way the cells are identified.

397 **Fig 6.** The same patient with extremely low ECD in Figure 5 analyzed by using both software systems

398 and the fully automated method without any cell border correction.

399 Each of the 3 images have many variations, but there are fewer differences in the ways the cells are

400 identified in Figure 6 than in Figure 5.

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