assessment in eye banks and that by specular microscopy in the recipient is important to quantify the postoperative cell loss dynamics. In Europe, where organ culture is common, postoperative endothelial cell density (ECD) is assessed either by manual counting by observation through a microscope reticle or by using computer-assisted analysers. We studied the agreement between ECD and morphometry determined by the non-contact small-field (0.08 mm²) specular microscope Topcon SP-2000P (Topcon, Tokyo, Japan) and that determined by a wide-field (1.2 mm²) flow, A-line eye bank analyser Sambacornea (Sambatechnologies, Meylan, France) using light microscopy images.

Methods
Fifty one patients (comprising keratoconus 52%, lattice dystrophy 27% and others 21%) with somewhat clear central cornea were subjected to preoperative specular microscopy. The excised cornea was immediately (always within 30 min) stained with Alizarin Red and the central endothelium assessed by Sambacornea after appropriate calibration. For either analysis, an automated cell border detection was followed by manual touch-up of incorrectly drawn cells. Assessment of ECD, coefficient of variation (CV) of cell area and hexagonality was performed on the maximum number of cells possible. Agreement was determined using the Bland–Altman method.

Results
Specular counts were performed on a mean (SD, range) of 102 (58, 4–223) cells compared with 2340 (1571), 163–6600) cells for Sambacornea (p<0.001). Specular microscopy underestimated the ECD by a mean of 221 cells/mm² (95% confidence interval (CI) 141 to 301) corresponding to an 11% (95% CI 6 to 15) underestimation independently of the ECD itself (fig 1). No correlation was found between the number of cells used for ECD determination with specular microscopy and the difference observed between the two methods (r = −0.053 and p = 0.713). In the subgroup (n = 35) of specular counts performed on >75 cells (‘minimum standard’ for reliability), the mean underestimation was 202 cells/mm² (95% CI 94 to 310) corresponding to 6% (95% CI 1 to 11) (fig 2). The percentage of hexagonal cells and coefficient of variation were comparable: 55 (21, 0 to 100) and 32 (9, 10 to 56) for specular and 55 (11, 29 to 76) and 34 (13, 23 to 78) for Sambacornea (p = 0.596 and 0.588, respectively).

Comment
Few studies have compared specular and light microscopic endothelial assessments until now. A direct correlation has been shown between ECD from histological cross-sections and in vitro specular microscopy on eye bank corneas. In the only morphometric comparison performed using wide-field contact specular microscopy, the authors did not find any significant difference between specular and post-staining cellular morphology, and in particular no evidence of tissue retraction. Our protocol was designed to remove any possibility of tissue retraction as counting immediately followed excision. Calibration problems have been shown in Topcon SP-2000P, producing underestimation in ECD values up to 9%, however, with any effect on morphometry. Image quality has also been shown to influence morphometric parameters. Using Sambacornea, counting of Alizarin-stained well-demarcated cells on a field 15 times larger than specular microscopy is expected to be more consistent with reality. Our morphometric findings remained comparable. The ECD underestimation by specular microscopy could help to explain the part of the step between the preoperative measurement in the eye bank and the first ECD measured postoperatively.

References

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