Reviewer's report

Title: Assessment of central corneal thickness and corneal endothelial morphology using ultrasound pachymetry, non-contact specular microscopy and Confoscan 4 confocal microscope.

Version: 2 Date: 23 May 2013

Reviewer: Sven Jonuscheit

Reviewer's report:

- Major Compulsory Revisions

The author must respond to these before a decision on publication can be reached. For example, additional necessary experiments or controls, statistical mistakes, errors in interpretation.

1. The purpose and focus of the manuscript require clarification. For this study three instruments (ultrasound pachymetry (USP), SP2000 and CS4) were used to assess corneal characteristics, namely, CCT, ECD and CV. Only two of these instruments provide endothelial images that allow for analysis of ECD and CV. Two observers carried out the assessments, but it appears that USP and SP2000 were only used by one observer whereas the CS4 was used by both. It is not clear whether the focus of the manuscript is on a) the repeatability and/or the reproducibility of CCT, ECD and CV; b) a comparison of two instruments to determine ECD and CV (as US does not provide the endothelial images); c) the agreement of CCT measurements by CS 4 and SP2000 with USP as the reference technique.

2. The methods-section requires more detail. For example, a definition of repeatability and reproducibility would be helpful. How were ECD and CV determined? Was the software of the SP2000 and CS4 used to determine ECD and CV? If so, which option was chosen (e.g. the automated)? Or were images downloaded and analysed by an external analysis software programme?

3. Endothelial cell counts can be highly variable and automated, semi-automated and manual analysis can all be carried out on endothelial images obtained with either a specular (SP2000) or confocal (CS4) microscope. A number of previous studies and reviews have addressed this, e.g. McCarey et al., Cornea 2008;27:1–16. In the present manuscript, a mention was made in the text referring to the area of interest, but information on the exact type of endothelial cell analysis is missing and would be very useful for the reader.

4. The results-section should be re-structured and follow the revised focus of the manuscript. One possibility could be to present CCT data first (3 instruments), followed by ECD and CV (2 instruments). Alternatively, the authors could either concentrate on the comparison of CCT as measured with the three instruments or on ECD and CV and the comparison of images from the specular and the
confocal microscope plus the respective endothelial analysis technique. This relates to comment 1 (focus of the manuscript).

5. The conclusions should be entitled ‘Discussion’. In the second paragraph of the conclusions the authors state that “… there was no statistically significant difference in intra-observer repeatability for observer 1 of ECD, CV and CCT with SP2000 and USP…” This sentence and the following are not correct because the ultrasound pachymeter does not provide any endothelial data, so ECD and CV can therefore not be obtained by using this instrument. This links back to comment number 1 and supports the suggestion that the manuscript needs a clearer focus.

6. The mean differences between measurements are mentioned the conclusions/discussion, but should be part of the results-section. Mean differences and limits of agreement should be given for all variables and all instruments and not only for the inter-observer data of one instrument (CS4, Table 3).

- Minor Essential Revisions
The author can be trusted to make these. For example, missing labels on figures, the wrong use of a term, spelling mistakes.

7. The references are listed inconsistently.

8. The symbols used in Fig1-3 vary, it is not clear what they represent. A better illustration is Fig 5, which is presented in a much clearer way.

9. Fig 6 is confusing and should be deleted. If the new focus of the manuscript were on CCT alone, then the presentation of three separate BA-plots (USP vs SP2000; USP vs CS4; and SP2000 vs CS4) would be beneficial.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interests