

Retinal Nerve Fiber Layer Thickness Measurements in Rats with Spectral Domain–Optical Coherence Tomography

We read with interest the recently published article by Hein et al.¹ and congratulate the authors on their detailed evaluation of spectral domain optical coherence tomography (SD-OCT) in monitoring retinal nerve fiber layer (RNFL) degeneration in rats with autoimmune optic neuritis and their good description of the correlation of their observations with that of the histopathology obtained *ex vivo*. We have a few queries, in hope that the responses will help us to interpret the data and understand the research better.

First of all, the meaning of precision should be clear and definite. According to the British Standards Institution,^{2,3} precision (which is part of accuracy) is the closeness of agreement between independent test results obtained under stipulated conditions. The factors involved include (1) the operator, (2) the equipment used, (3) the equipment calibration, (4) the environment, and (5) the elapsed time between measurements. Precision has two conditions: repeatability and reproducibility. Under repeatability conditions, factors such as (1) to (5) are considered to be constant and do not contribute to the variability of the measurement result. Under reproducible conditions, the factors can vary. Repeatability and reproducibility are the two extremes of precision. Therefore, the standard terms *intraobserver repeatability* and *interobserver reproducibility* would have been more appropriate than the original terms *intra-* and *interreader reproducibility*.

Furthermore, the authors used mean SD to evaluate repeatability and two-way ANOVA to evaluate reproducibility. These statistical methods do not provide us with sufficient information. Most studies of repeatability and reproducibility report

additional values, such as the coefficient of variation, test-retest variability (within-subject SD \times 2.77), and intraclass correlation.^{4,5} Knowing these data would enable the reader to better understand the repeatability and reproducibility results of this investigation.

It also unclear from which data the total SD (5.53 μ m) is derived. Maybe a table showing the mean values and SD of OCT measurements at each time point would help to clarify this issue.

Last, the authors stated that “RNFL thickness measured by OCT correlated significantly with the thickness obtained from retinal sections at different investigation time points” and that it “also corresponded with histomorphometric measurements”. However, Pearson’s product moment correlation coefficient can evaluate only a possible correlation, which is no guarantee of good agreement between two different techniques measuring the same parameter.⁶ A more appropriate method would be Bland-Altman plots with 95% limits of agreement. To investigate this issue, we extracted the RNFL thickness data in Figure 4 in Hein et al. with digitizing software (GetData Graph Digitizer ver. 2.24; <http://www.getdata-graph-digitizer.com/index.php/>). The original extracted data were entered into a spreadsheet (Excel; Microsoft, Redmond, WA) and transferred to a statistical analysis program (SPSS software, ver. 13.0; SPSS Inc., Chicago, IL). We got almost the same Pearson correlation ($r = 0.726$), r^2 (0.541), and scatterplot (Fig. 1) as the authors’ results. Then, agreement between the two methods was investigated by Bland-Altman plot analysis.⁶ Figure 2 shows that, in 95% of cases, the difference in measurements between these two methods ranges from -3.4 to 7.9 μ m. This means that SD-OCT RNFL thickness measurements could be as much as 3.4 μ m below to 7.9 μ m above the histologic values. This discrepancy is small and would not be

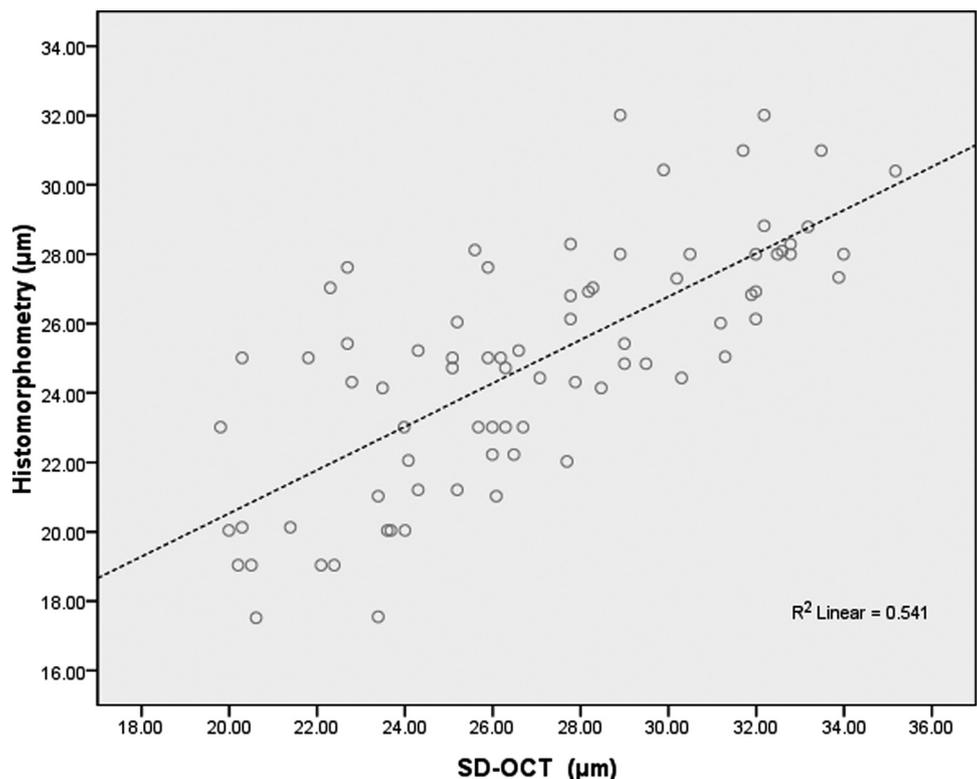


FIGURE 1. Correlation of retinal nerve fiber layer thickness measurement by spectral domain optical coherence tomography (SD-OCT) and histology.

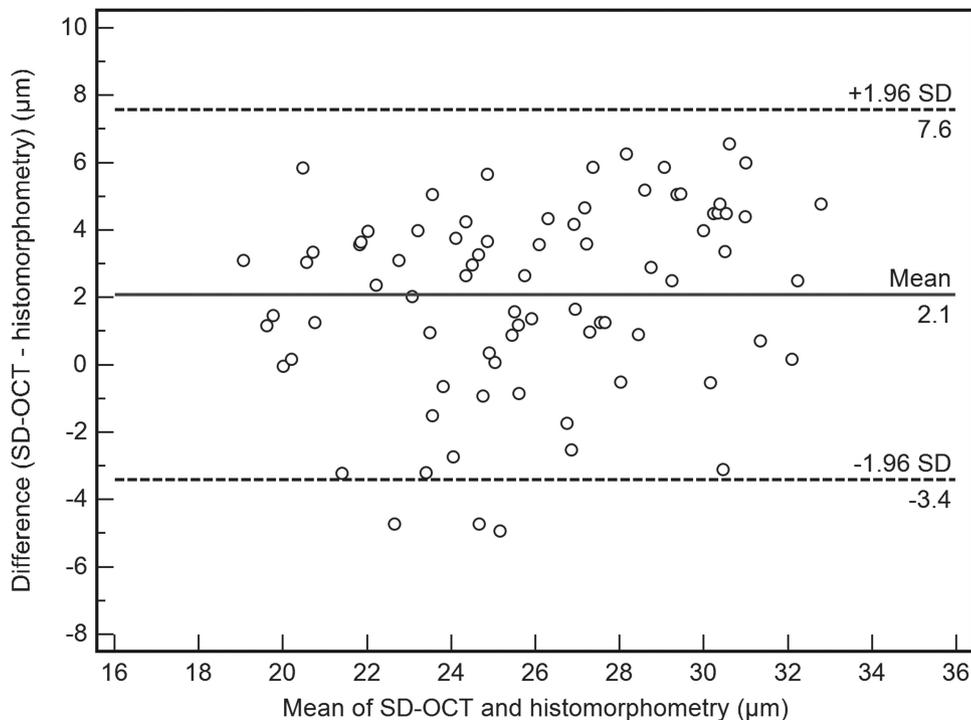


FIGURE 2. Bland-Altman plot of agreement between spectral domain optical coherence tomography (SD-OCT) against histology measurements.

clinically significant in humans (so that both methods may be used interchangeably for most clinical purposes). However, we still don't know whether it is clinically significant in rats, whose RNFL thickness is approximately four times thinner than in healthy humans.⁷

Jinbai Huang¹
Giacomo Savini²
Yifan Feng¹
Qinmei Wang¹

¹School of Optometry and Ophthalmology and Eye Hospital, Wenzhou Medical College, Wenzhou, Zhejiang, China; and the ²G. B. Bietti Eye Foundation, IRCCS (Istituto di Ricerca e Cura a Carattere Scientifico), Rome, Italy.
E-mail: wqm6@mail.eye.ac.cn

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