Title:

Evaluation of tear meniscus height using different clinical methods

Authors:

Britta Niedernolte[1], Lisa Trunk[1], James S. Wolffsohn[2], Heiko Pult[2,3,4], Stefan Bandlitz[1,3]

Author's institutions:

[1]Höhere Fachschule für Augenoptik Köln, Cologne School of Optometry, Cologne, Germany

[2]Ophthalmic Research Group, Life and Health Sciences, Aston University, Birmingham, United Kingdom

[3]Optometry and Vision Research, Weinheim, Germany

[4]School of Biomedical & Life Sciences, Cardiff University, Cardiff, United Kingdom

Running title:

Tear meniscus height measurement

Key words:

Tear meniscus height, tear film volume, dry eye diagnosis, lid thickness, eye lashes, reticule, optical coherence tomography

Clinical relevance:

The height of the tear meniscus (TMH) is a generally accepted method to evaluate tear film volume, especially in dry eye diagnoses and management.

Background:

The purpose of this study was to evaluate the ability of different methods to measure tear meniscus height accurately and repeatably.

Methods:

Lower TMH of 20 volunteers (26.8±5.6years) was measured by two observers (OI and OII) using optical coherence tomography (OCT), slit-lamp microscope image analysis, and with a reticule at low (8x) and high (32x) magnification. TMH was also evaluated by both observers by comparing TMH to the lid margin`s thickness (lid-ratio; grade 0: TMH 1/2 lid margin thickness; grade 1: 1/3; grade 2: 1/4; grade 3: 1/5; grade 4: 1/6) and to number of eyelashes fitting in the tear meniscus. Differences between observers were analysed by paired-t-test. Differences between OCT-TMH and other methods was analysed by ANOVA, and inter-observer repeatability by intra-class-correlation-coefficient (ICC). The ability to predict OCT-TMH was calculated by receiver operative characteristic curve (ROC).

Results:

There was no significant difference between OI and OII in all methods except of the eyelash-count-method (p=0.008). For OI, TMH measured using a reticule at 8x (0.20±0.05 mm) was significantly lower than OCT-TMH (0.24±0.07 mm) (p=0.032) but not at 32x (0.22±0.01 mm; p=0.435). TMH evaluated by the image software of the slit-lamp (0.20±0.05 mm) was significantly lower than OCT-TMH (p=0.022). Lid-ratio-method and eyelash-count-method resulted in grades of 2.35±1.22 and 2.85±0.81 respectively. ROC analyses showed that only the 8x and the 32x magnification method could discriminate between normal and abnormal OCT-TMH. OCT had the best repeatability (ICC=0.88;p<0.001) followed by reticule using 32x magnification (ICC=0.70;p=0.004).

Conclusion:

The most reliable method to measure TMH was OCT followed by slit-lamp using a reticule. TMH cannot be reliably evaluated by comparing it against lid margin thickness or number of eyelashes.

The tear fluid on the ocular surface is present in the exposed area between the lids, in the conjunctival sac of the upper and lower lids and in the tear menisci along the lid margins. The tear menisci hold approximately 75–90% of the overall tear fluid volume and serve as reservoirs, supplying tears to the precorneal tear film.[1-3] Tear volume assessment is an important clinical metric as it allows subclassification of dry eye disease into predominantly evaporative or aqueous deficient.[4]

Measurements of tear meniscus height (TMH), tear meniscus radius (TMR) or tear meniscus cross-sectional area (TMA) are generally accepted methods to evaluate tear film volume, especially in dry eye diagnosis and management.[4,5] Tear meniscus parameters can be influenced by time after a blink, diurnal variations, measurement location along the lids, presence of conjunctival folds, climate, and illumination technique.[4,6,7] A TMH of 0.20 mm or below is assumed to be an indicator for aqueous deficiency.[4]

Several methods to measure TMH have been published such as using a reticule at the slit-lamp, image capture, reflective meniscometry, interferometry and optical coherence tomography.[2,5,8-14] TMH obtained using those methods varied between 0.12 and 0.46 mm in healthy eyes. [15] TMH measured by OCT has shown good repeatability (ICC= 0.900 - 0.981), and high sensitivity (67.0 - 80.5%) and specificity (81- 89.3%) for the diagnosis of dry eye disease.[16-18] Interferometry allows measurement of TMH about as reliably as does OCT (ICC= 0.870 – 0.920).[10] Image capture techniques have similar repeatability to OCT, but are more repeatable than using a reticule with a slit-lamp.[9,19] Lower magnification and limited image resolution create difficulty in defining the upper edge of the meniscus.[9] To enhance visibility of the meniscus at the slit-lamp, and therefore the repeatability of TMH measurements, the use of a Tearscope has been recommended.[20] Alternatively, in measuring the TMR with reflective meniscometry there is no need to detect the upper limit of the meniscus.[21] Reflective meniscometry was suggested to be a useful surrogate for OCT measurements of TMR.[12]

While some of these methods require separate, costly instrumentation and are generally used for research purposes (e.g. interferometry and optical coherence tomography), other simpler tools that utilise the slit-lamp can easily be integrated into a routine eye examination (e.g. reticule, image capture or reflective meniscometry). Interestingly, many clinicians use alternative methods at the slit-lamp that have not been validated yet, such as the comparison of the TMH with the lower eye lid thickness or to eye lash thickness; TMH is assumed to be at least as high as the thickness of 2 eye lashes or higher than 1/3 lid thickness.[22,23]

Consequently, the aim of this prospective study was to evaluate the agreement and repeatability of different objective and subjective methods to measure tear meniscus height.

METHODS

Participants

Twenty participants with a mean age of 26.8 ± 5.6 (SD) years (10 females) were recruited from the students of the Höhere Fachschule für Augenoptik Köln (Cologne School of Optometry), Cologne, Germany. To analyse accuracy of the techniques the height of the tear meniscus was evaluated by optical coherence tomography (OCT, iVue, Optovue, Inc., Fremont, USA) and compared to the height of the tear meniscus, evaluated using different techniques, as described below. Furthermore, inter-observer repeatability was analysed. The TMH of the participants was measured by the two observers (OI and OII), with all four techniques, in a randomised order 15 to 60min apart. The observers were masked against each other. Measurements were obtained between 9 and 12 o’clock, and 3–4 seconds after a normal blink. The study was conducted in a room with controlled temperature (20°C to 23°C) and humidity (44% to 53%).

Participants were excluded if they had a current or previous condition known to affect the ocular surface or tear film, if taking medication known to affect the ocular surface and/or tear film, and/or if they had worn any type of contact lens on the measurement day. All participants gave written informed consent before taking part in the study. The procedures obtained the approval of the Cardiff School of Optometry and Vision Sciences Human Ethics Committee and were conducted in accordance with the requirements of the Declaration of Helsinki. Given a repeated measure standard deviation of ~0.15mm for slit lamp imaging,[24] the sample size allowed a difference of 0.05 mm to be detected with 80% power (G*Power, Franz Faul, University Kiel, Germany).

Procedures

Optical Coherence Tomography:

The height of the central, lower TM of the participants was measured using optical coherence tomography (OCT, iVue, Optovue, Inc., Fremont, USA) (Figure 1). TMH on the OCT images was measured using the inbuilt calipers of the manufacturer provided software. The mean of 3 consecutive measurements was noted.

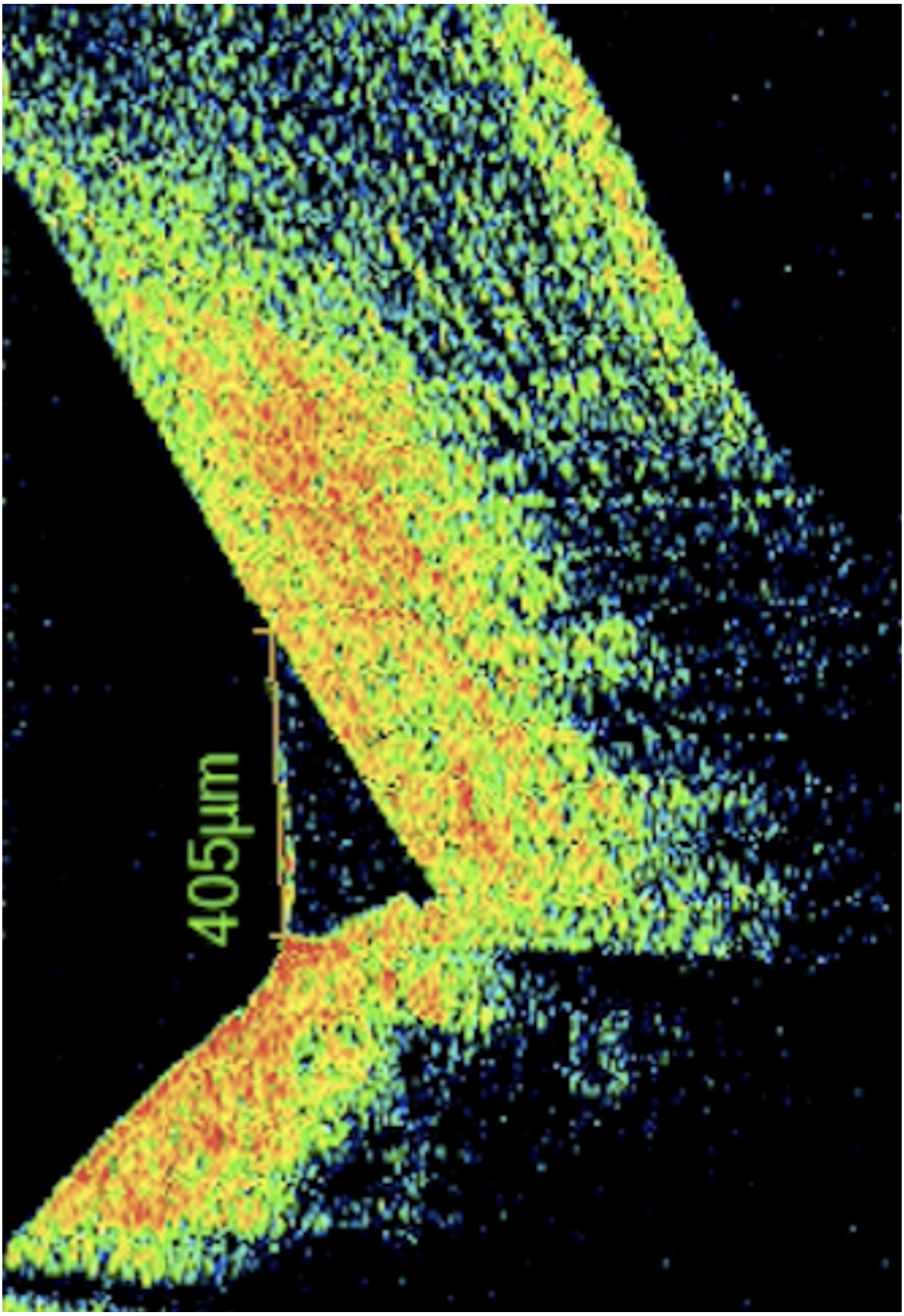

Figure 1. Tear meniscus height measured by OCT (iVue, Optovue, Inc., Fremont, USA)

Slit-Lamp Microscope – Reticule Method:

The central TMH of the lower lid was observed using a reticule at the slit-lamp (Zeiss SL 120, Zeiss, Oberkochen, Germany) with low (8x) and high (32x) magnification (Figure 2). To enhance visibility of the meniscus at the slit-lamp without affecting the TMH, a Tearscope Plus (Keeler Ltd, Windsor, GB) was used. The mean of 3 consecutive measurements was noted. However, each reticule is calibrated to a certain magnification. Therefore, if a higher magnification is used, the readings of the reticule need to be divided by the quotient of higher magnification / calibrated magnification. In this study, the reticule was made to be used with 8x magnification and when using it with a 32x magnification, the measured TMH was divided by 4.

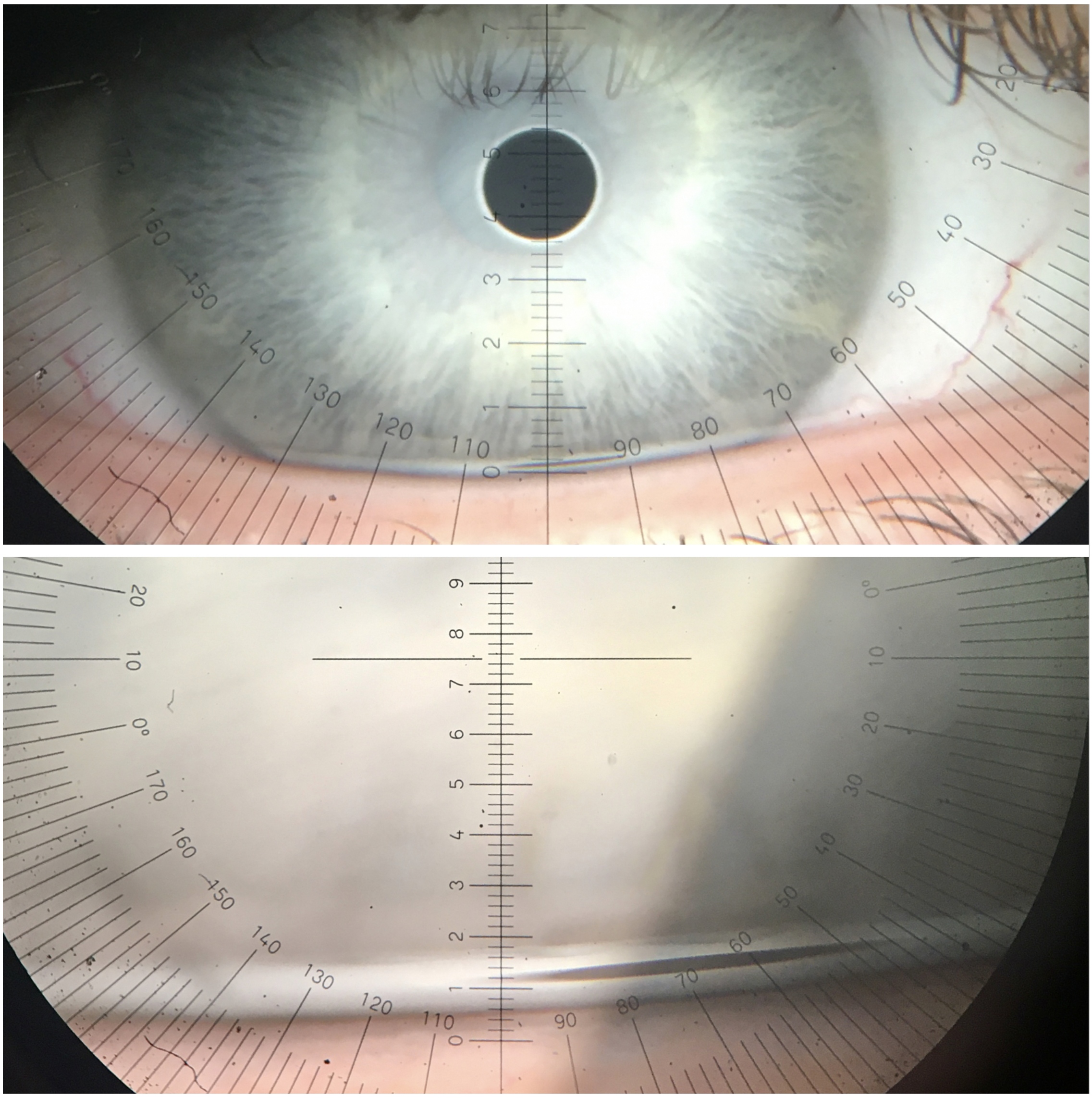

Figure 2. Tear meniscus height measured with reticule at the slit-lamp, top (8x), bottom (32x).

Slit-Lamp Microscope – Image Analyses:

The TM was photographed at 32x magnification using the in-built video camera (Check & Review Cam, Wagner and Guder Medical GmbH, Bad Sulza, Germany) of the slit lamp microscope (Figure 3). To enhance visibility of the meniscus at the slit-lamp without affecting the TMH, a Tearscope Plus (Keeler Ltd, Windsor, GB) was used. The height of the photographed TM was measured using ImageJ 1.53 (http://rsbweb.nih.gov/ij) image analyses software. The mean of 3 consecutive measurements was noted.

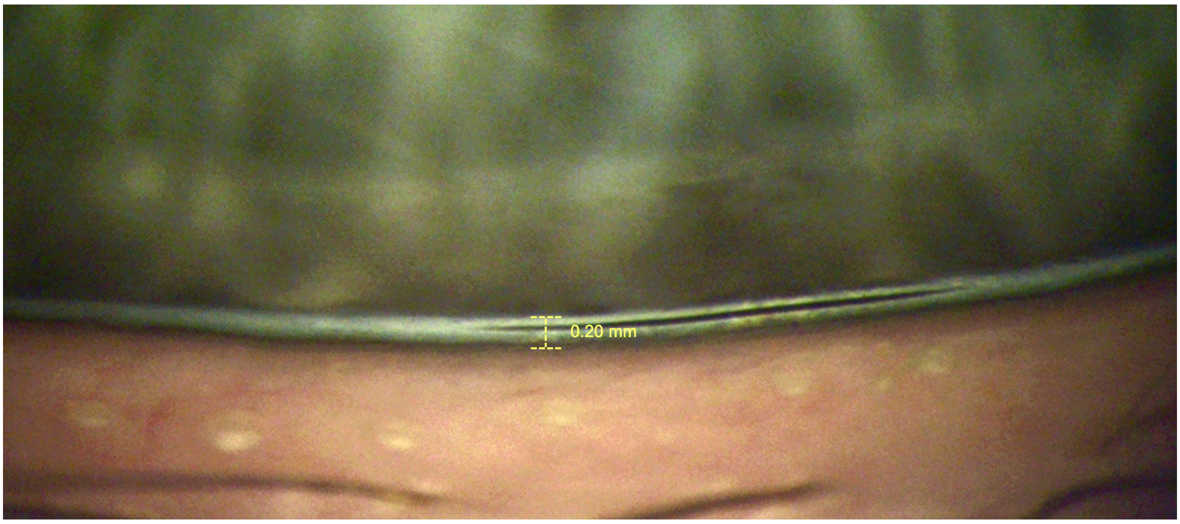

Figure 3. Tear meniscus height measured with image analysis at the slit-lamp.

Slit-Lamp Microscope – Comparison Method:

In the "comparison method" TMH was compared to eye lid or eye lash thickness (Figure 4). To enhance visibility of the meniscus at the slit-lamp without affecting the TMH, a Tearscope Plus (Keeler, Windsor, GB) was used. The tear meniscus was quantified by comparing the height of the tear meniscus to the lid margin`s thickness (lid ratio; grade 0: TMH 1/2 lid margin thickness; grade 1: 1/3; grade 2: 1/4; grade 3: 1/5; grade 4: 1/6) and to number of the lashes fitting in the tear meniscus (eye lashes count).

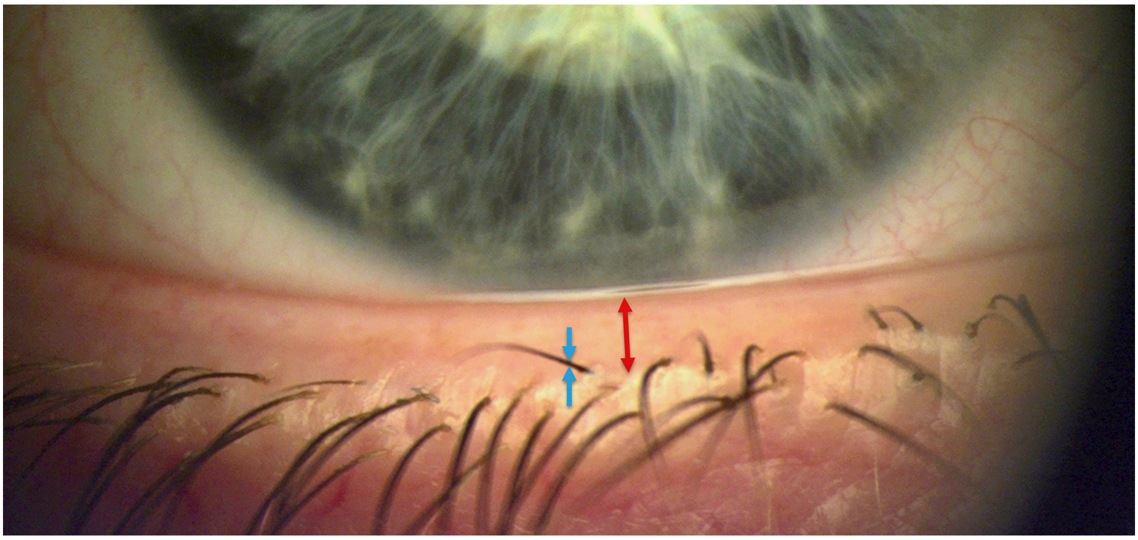

Figure 4. Tear meniscus height estimation by the comparison method. Tear meniscus height was compared to eye lid thickness (red arrow) or eye lash thickness (blue arrows).

Statistical analyses

Data were tested using the Shapiro-Wilk test and were not statistically different from a normal distribution ($p>0.05$). Differences between the measurements of the two observers were analysed by paired t-test and the 95% limit of agreement (LoA) was calculated using the Bland Altman method. The ability of the different techniques to differentiate between normal and abnormal OCT tear meniscus height was analysed by receiver operative characteristic curve (ROC). The areas under the curve (AUC) were calculated using OCT cut-off values of 0.20 mm, 0.22 mm and 0.24 mm. OCT-TMH was considered as the gold-standard; hence, differences between OCT measurements and those of the other techniques were analysed using an ANOVA followed by the Holm-Sidak test for the observer OI. The intra-class correlation coefficient (ICC) was calculated to analyse intra-rater reliability, with the following ranges: 0-0.20 slight; 0.21-0.40 fair; 0.41-0.60 moderate; 0.61-0.80 substantial; 0.81-1.00 (almost) perfect.[25] Data were analysed using BiAS, (Espilon-Verlag, Frankfurt, Germany) and SPSS Version 22.0. (IBM Corp, Armonk, USA).

RESULTS

The descriptive data of the measurements of both observers with the different methods are summarised in Table 1. Based on the paired t-test, there was no significant difference between OI and OII for all methods except the eye lashes count method.

Optical Coherence Tomography:

There was no statistically significant difference between OCT-TMH measured by OI (0.24 ±0.07 mm) and OII (0.24 ±0.09 mm) (paired t-test: p=0.911; 0.00 mm; 95% LoA -0.10 to 0.10 mm).

Slit-Lamp Microscope – Reticule Method:

For the reticule at 8x magnification, TMH measured by OI (0.20 ±0.05 mm) and OII (0.21 ±0.04 mm) was not statistically significant different (p=0.940; +0.01 mm; 95% LoA -0.10 to 0.10 mm). At 32x magnification there was also no statistically significant difference between OI (0.22 ±0.01 mm) and OII (0.20 ±0.05 mm) (p=0.198; -0.02 mm; 95% LoA -0.13 to 0.09 mm).

Slit-Lamp Microscope – Image Analyses:

The mean TMH measured by OI (0.20 ±0.05 mm) was not statistically significant different from OII (0.21 ±0.07 mm) (p=0.153; +0.01 mm; 95% LoA -0.10 to 0.14 mm).

Slit-Lamp Microscope – Comparison Method:

The lid ratio method resulted in a similar grade for OI (0.25 ±0.07) and OII (0.25 ±0.04) (p=0.300). However, for the lash count method, the mean grade for OI (2.85 ±0.08) was statistically significantly greater than for OII (2.38 ±0.06) (p=0.008).

ROC analyses:

ROC analyses showed, that only the 8x and the 32x magnification method were able to discriminate between normal and abnormal OCT-TMH (Table 2). Based on the DeLong test, the AUC for these two methods were not statistically different (p=0.892). When using a higher cut-off value, all 3 slit-lamp methods were able to detect an abnormal tear meniscus (Table 2). No significant difference in terms of predictive ability was identified by the DeLong test (p>0.801).

Comparison between the different methods:

TMH evaluated by the image software of the slit lamp was significantly lower than OCT-TMH (p=0.022, mean difference=0.04 ±0.06 mm). However, there were no statistically significant differences between the 3 methods: 8x magnification, 32x magnification and image analyses (p=0.228). TMH measured using a reticule at 8x magnification was significantly lower than OCT-TMH (p=0.032, mean difference=0.04 ±0.06), but not at 32x magnification (p=0.435, mean difference=0.02 ±0.07 mm). OCT-TMH results were grouped by (i) lid ratio grades and (ii) eye lashes count (Table 3). There were no statically significant differences between the OCT measured TMH between each of the grades of the lid ratio (p=0.542) or between each of the grades of eye lashes count (p=0.298) and there was no obvious trend of increase OCT TMH with grade value.

Repeatability of the methods:

Based on ICC calculation, all types of TMH evaluation were substantially repeatable (intraclass coefficient (ICC) >0.67, p>0.05), except the lid ratio approach. The best repeatability in terms of absolute agreement was shown by OCT (ICC 0.88, p<0.001), followed by reticule using 32x magnification (ICC=0.70, p=0.004) (Table 4).

DISCUSSION

This study analysed the repeatability and usefulness of different clinical methods to measure tear meniscus height. The TMH measurements using the slit-lamp microscope with reticule (0.20 ±0.05 mm and 0.22 ±0.01 mm) as well as the OCT measurements (0.24 ±0.07 mm) are in good agreement with literature.[19,26-28] Imamura et al.[19] reported a significantly higher TMH measured with a swept-source optical coherence tomography (SS-OCT) in comparison to conventional slit lamp microscope method with a reticule. However, this was only the case when using 8x magnification, not 32x magnification. This difference might be due to the higher-resolution OCT images and higher slit-lamp magnification that enhances the ability to identify the upper and lower limits of the tear meniscus.

Subjects were grouped between low tear meniscus height (OCT-TMH ≤0.20 mm) and normal tear meniscus height (OCT-TMH >0.20 mm) and the ability of the different methods to discriminate between both was analysed by ROC. However, only slit-lamp observation using a reticule was a significant discriminator but not all the other methods. This was not surprising for the lid ratio or the eye lashes count, since lid and eye lash thickness can be highly variable in humans.[29,30]

As OCT-TMH appears to be higher in the literature than when using a slit-lamp,[19] higher cut-off values than reported in the TFSO DEWS II report were also applied. Interestingly, using amended cut-off value of 0.22 mm or even 0.24 mm showed that image analyses are also a significant discriminator of normal and abnormal OCT-TMH, too – but still not lid ratio or the eye lashes count method. The amended cut-off values were calculated by adding the differences between OCT and slit lamp observations - evaluated in this study - to the cut-off value of 0.20 mm, recommended by the TFOS DEWS II Report.[4]

Anecdotally, the tear meniscus height should be at least 1/3 of the lid thickness or 2 eye lashes thick.[22,23] The lid ratio method is defined to be the comparison between TMH and the thickness of the lid margin measured from the eye lash line to the posterior edge of the lid margin. This approach is questionable, as every patients lid thickness may be different and such this would result in different TMH. Furthermore, this study showed, that regardless which ratio was observed, almost the same effective OCT-TMH of 0.23 mm to 0.27 mm (lid ratio) or 0.23 mm to 0.26 mm (eye lash count) was measured. Furthermore, there seems to be a perspective distortion when lid thickness is measured in frontal view with the slit-lamp, as lid thickness measurements are on average 2.7x larger when measured from side view with the Pentacam.[30,31] The lid ratio cut-off for abnormal TMH is anecdotically reported to be “TMH = 1/3 of lid thickness”, that one of eye lash count “2 lashes”. The results listed in table 3 clearly demonstrated the huge possible number of false positives in both methods.

Inter-observer repeatability was analysed, and all of the methods appeared to be repeatable, except the lid ratio method. In terms of the intra class coefficient the most repeatable method was OCT, followed by use of a reticule at 32x magnification and image analyses. The alternative methods “lid ratio” was not repeatable (p=0.092) but the “eye lash count” demonstrated fair repeatability.

However, repeatability simply means, that two observers would give the same score, degree or measurement of an evaluation for the same observation. As described above, both methods “lid ratio” and “eye lash count” are not able to detect differences between TMH nor are usable to discriminate between normal and abnormal TMH. In contrast, slit-lamp observation using a reticule or image analyses software appears to be an accurate method.

For TMH measurement, Arita et al.[10] reported an intraclass coefficient (ICC) of 0.87 for an interferometry device and of 0.89 for a swept source OCT. Using the same swept source OCT, Imamura et al.[19] found an ICC of 0.93 and for the slit-lamp reticule technique an ICC of 0.761. In the present study the results were close to these numbers, with an almost perfect ICC of 0.89 for spectral domain OCT, and a substantial ICC of 0.70 for the slit-lamp reticule technique (32x magnification). The results of the slit-lamp reticule technique using an 8x magnification was moderately repeatable. Thus, a higher magnification at the slit-lamp seems to improve the reproducibility of the TMH measurements which can attributed to the higher measurement resolution of the image.[9]

CONCLUSION

OCT-TMH measures were higher than those obtained using slit-lamp biomicroscopy with a reticule or image analysis software. However, this difference may be marginal from a clinical point of view. TMH cannot be evaluated by comparing it against lid margin thickness or the number of eye lashes. The most repeatable method to measure TMH was OCT, followed by a reticule using 32x magnification of the slit lamp microscope. In clinical practice, a higher magnification of the slit lamp microscope may improve accuracy and repeatability when measuring the TMH.

REFERENCES

1 Gaffney EA, Tiffany JM, Yokoi N et al. A mass and solute balance model for tear volume and osmolarity in the normal and the dry eye. *Prog Retin Eye Res* 2010; 29: 59-78.

2 Savini G, Barboni P, Zanini M. Tear meniscus evaluation by optical coherence tomography. *Ophthalmic Surg Lasers Imaging* 2006; 37: 112-118.

3 Holly FJ. Physical chemistry of the normal and disordered tear film. *Trans Ophthalmol Soc U K* 1985; 104 (Pt 4): 374-380.

4 Wolffsohn JS, Arita R, Chalmers R et al. TFOS DEWS II Diagnostic Methodology report. *Ocul Surf* 2017; 15: 539-574.

5 Bandlitz S, Purslow C, Murphy PJ et al. Influence of Conjunctival Folds on Calculated Tear Meniscus Volume Along the Lower Eyelid. *Ocul Surf* 2016; 14: 377-384.

6 Bandlitz S, Purslow C, Murphy PJ et al. The relationship between tear meniscus regularity and conjunctival folds. *Optom Vis Sci* 2014; 91: 1037-1044.

7 Srinivasan S, Chan C, Jones L. Apparent time-dependent differences in inferior tear meniscus height in human subjects with mild dry eye symptoms. *Clin Exp Optom* 2007; 90: 345-350.

8 Johnson ME, Murphy PJ. The agreement and repeatability of tear meniscus height measurement methods. *Optom Vis Sci* 2005; 82: 1030-1037.

9 Santodomingo-Rubido J, Wolffsohn JS, Gilmartin B. Comparison between graticule and image capture assessment of lower tear film meniscus height. *Cont Lens Anterior Eye* 2006; 29: 169-173.

10 Arita R, Yabusaki K, Hirono T et al. Automated Measurement of Tear Meniscus Height with the Kowa DR-1alpha Tear Interferometer in Both Healthy Subjects and Dry Eye Patients. *Invest Ophthalmol Vis Sci* 2019; 60: 2092-2101.

11 Stegmann H, Werkmeister RM, Pfister M et al. Deep learning segmentation for optical coherence tomography measurements of the lower tear meniscus. *Biomedical optics express* 2020; 11: 1539-1554.

12 Bandlitz S, Purslow C, Murphy PJ et al. Comparison of a new portable digital meniscometer and optical coherence tomography in tear meniscus radius measurement. *Acta Ophthalmol* 2014; 92: e112-118.

13 Savini G, Goto E, Carbonelli M et al. Agreement between stratus and visante optical coherence tomography systems in tear meniscus measurements. *Cornea* 2009; 28: 148-151.

14 Chan HH, Zhao Y, Tun TA et al. Repeatability of tear meniscus evaluation using spectral-domain Cirrus(R) HD-OCT and time-domain Visante(R) OCT. *Cont Lens Anterior Eye* 2015; 38: 368-372.

15 Mainstone JC, Bruce AS, Golding TR. Tear meniscus measurement in the diagnosis of dry eye. *Curr Eye Res* 1996; 15: 653-661.

16 Czajkowski G, Kaluzny BJ, Laudencka A et al. Tear meniscus measurement by spectral optical coherence tomography. *Optom Vis Sci* 2012; 89: 336-342.

17 Canan H, Altan-Yaycioglu R, Ulas B et al. Interexaminer reproducibility of optical coherence tomography for measuring the tear film meniscus. *Curr Eye Res* 2014; 39: 1145-1150.

18 Binotti WW, Bayraktutar B, Ozmen MC et al. A Review of Imaging Biomarkers of the Ocular Surface. *Eye Contact Lens* 2020; 46 Suppl 2: S84-S105.

19 Imamura H, Tabuchi H, Nakakura S et al. Usability and reproducibility of tear meniscus values generated via swept-source optical coherence tomography and the slit lamp with a graticule method. *Int Ophthalmol* 2018; 38: 679-686.

20 Fodor E, Hagyo K, Resch M et al. Comparison of Tearscope-plus versus slit lamp measurements of inferior tear meniscus height in normal individuals. *Eur J Ophthalmol* 2010; 20: 819-824.

21 Bandlitz S, Purslow C, Murphy PJ et al. A new portable digital meniscometer. *Optom Vis Sci* 2014; 91: e1-8.

22 Tamm M. Hochleistungshydrogel oder Silikonhydrogel – Wann passe ich was an? [High performance hydrogel or silicone hydrogel - When do I fit which lens?]. In, 2009.

23 Poeltner G, Sulzer M. Spaltlampe Traenenmeniskus [Tear meniscus at the slit-lamp]. In. https://www.youtube.com/watch?v=jaMSOJQKHnQ, 2016.

24 Nichols KK, Mitchell GL, Zadnik K. The repeatability of clinical measurements of dry eye. *Cornea* 2004; 23: 272-285.

25 Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; 33: 159-174.

26 Garcia-Resua C, Santodomingo-Rubido J, Lira M et al. Clinical assessment of the lower tear meniscus height. *Ophthalmic Physiol Opt* 2009.

27 Patel S, Wallace I. Tear meniscus height, lower punctum lacrimale, and the tear lipid layer in normal aging. *Optom Vis Sci* 2006; 83: 731-739.

28 Fukuda R, Usui T, Miyai T et al. Tear meniscus evaluation by anterior segment swept-source optical coherence tomography. *Am J Ophthalmol* 2013; 155: 620-624, 624 e621-622.

29 Na JI, Kwon OS, Kim BJ et al. Ethnic characteristics of eyelashes: a comparative analysis in Asian and Caucasian females. *Br J Dermatol* 2006; 155: 1170-1176.

30 Pult H, Korb DR, Murphy PJ et al. A new model of central lid margin apposition and tear film mixing in spontaneous blinking. *Cont Lens Anterior Eye* 2015; 38: 173-180.

31 Niedernolte B, Trunk L, Wolffsohn J et al. Evaluation of tear meniscus height using different methods. *Contact Lens and Anterior Eye* 2018; 41: 87.

Corresponding author name and e-mail address

Stefan Bandlitz

Höhere Fachschule für Augenoptik Köln

(Cologne School of Optometry)

Bayenthalgürtel 6-8

D-50968 Köln, Germany

e-mail: bandlitz@hfak.de

Table 1. Descriptive data for mean and SD values of observations grouped by observer I and observer II.

	Observer I		Observer II			
	Mean	**SD**	**Mean**	**SD**	**p-value**	**95 % LoA**
TMH OCT [mm]	0.24	±0.07	0.24	±0.09	0.911	-0.10 to 0.10
TMH Reticule 8x [mm]	0.20	±0.05	0.21	±0.04	0.940	-0.10 to 0.10
TMH Reticule 32x [mm]	0.22	±0.01	0.20	±0.05	0.198	-0.13 to 0.09
TMH Image Analyses [mm]	0.20	±0.05	0.21	±0.07	0.153	-0.10 to 0.14
TMH Lid-Ratio	0.25	±0.07	0.25	±0.04	0.300	-0.14 to 0.11
TMH Lid-Ratio [mm]	0.14	±0.04	0.13	±0.03	0.272	-0.09 to 0.07
TMH Eye Lashes Count	2.85	±0.08	2.38	±0.06	0.008	-1.77 to 0.82
TMH Eye Lashes [mm]	0.19	±0.05	0.15	±0.04	0.008	-0.12 to 0.05

Table 2. Ability of the different methods to discriminate between normal and abnormal OCT-TMH at different cut-off values using receiver operative characteristic curve (ROC). Area under the curve (AUC), standard deviation (SD), 95% confidence interval (CI) and p-value (p).

Cut-off 0.20mm	AUC	SD	95% CI		p
TMH Reticule 8x [mm]	0.946	±0.055	0.839	1.000	<0.001
TMH Reticule 32x [mm]	0.905	±0.078	0.753	1.000	<0.001
TMH Image Analyses [mm]	0.691	±0.149	0.398	0.983	0.201
TMH Lid Ratio Grade	0.732	±0.139	0.464	1.000	0.090
TMH Eye Lashes Count	0.661	±0.115	0.436	0.885	0.161
Cut-off 0.22mm					
TMH Reticule 8x [mm]	0.823	±0.080	0.668	0.979	<0.001
TMH Reticule 32x [mm]	0.742	±0.117	0.514	0.971	0.038
TMH Image Analyses [mm]	0.758	±0.112	0.537	0.978	0.022
TMH Lid Ratio Grade	0.581	±0.133	0.321	0.841	0.543
TMH Eye Lashes Count	0.546	±0.128	0.295	0.796	0.721
Cut-off 0.24mm					
TMH Reticule 8x [mm]	0.833	±0.079	0.679	0.987	<0.001
TMH Reticule 32x [mm]	0.817	±0.099	0.624	1.000	0.001
TMH Image Analyses [mm]	0.802	±0.104	0.599	1.000	0.004
TMH Lid Ratio Grade	0.651	±0.130	0.396	0.906	0.245
TMH Eye Lashes Count	0.516	±0.104	0.261	0.771	0.904

Table 3. OCT measurements sorted by lid ratio or eye lashes count approach (none of the OCT measurements showed a lid ratio of 1/2 or degree 1 in terms of the eye lashes approach). There are no significant differences between the grades of the lid ratio (p=0.542) or between the grades of eye lashes count (p=0.298).

TMH – Lid Ratio	Mean TMH - OCT	SD	TMH – Eye Lashes Count	Mean TMH - OCT	SD
1/2	n/a	n/a	1	n/a	n/a
1/3	0.26	±0.06	2	0.23	±0.04
1/4	0.24	±0.07	3	0.26	±0.08
1/5	0.23	±0.04	4	0.25	±0.04
1/6	0.27	±0.08			

Table 4. Inter-observer repeatability based on the intraclass coefficient (ICC) and standard deviation (SD). Observer I (OI) and observer II (OII).

	ICC	SD	p
OCT-TMH	0.884	±0.000	<0.001
TMH Reticule 8x	0.594	±0.033	<0.001
TMH Reticule 32x	0.702	±0.004	<0.001
TMH Image Analyses	0.685	±0.007	0.077
TMH Lid Ratio	0.289	±0.902	0.092
TMH Eye Lashes	0.402	±0.764	0.031