INTRODUCTION

When tears are collected from the eye and desiccated on a glass slide, the salt and protein components in the solution precipitate out in a fern-like formation (Figure 1) [1]. The spine of the fern is formed by a thick central branch with finer spindle-like branches emerging out, covering the area in which the tears have dried [2]. This crystallised network is referred to as the ‘tear fern’ pattern. With the aid of scanning electron microscopy and X-ray diffraction, the molecular constituents of the tear fern pattern have been identified as sodium chloride and potassium chloride crystals [3]. Hence changes in the tear fern pattern are thought to represent changes to tear film composition and stability [3, 4]. A lower density of tear ferning has been associated with greater discomfort and higher tear film osmolarity [5]. The association of tear ferning with other clinical variables such as lipid layer thickness (LLT) and tear film break-up time is not well understood [6, 7]. Whether treatment impacts on this tear fern pattern is also not known.

Tear ferning is currently graded subjectively using a 5-point grading system originally proposed by Rolando et al [5]. and later refined by Masmali et al [5]. This grading involves both standard reference photographs and numeric categorization [5] and has been shown to have good inter-observer repeatability [8]. Being a subjective grading scale however, the Masmali grading is still subject to bias and clinical discrepancies. A quantitative and objective method for tear fern grading would help to overcome these challenges.

Saad et al. have presented an objective, computer-based, analysis of tear ferning that utilises a vector representation for all the five grades of subjective grading system [9]. This method uses computer software that is based on the identification of orientation and spatial organization [9]. This software is then used to analyse a microscopic photograph of the tear fern for production of an objective, quantitative measurement that represents one of the 5-point grades [9]. The clinical utility of this objective grading system is currently unknown.

The current study hypothesised that the tear fern pattern will be associated with clinical tear film parameters and this will change following the instillation of ocular lubricants. The aim of this study therefore was to determine the relationship between tear ferning and the clinical parameters of LLT, tear film break-up time and comfort and how this pattern changes following the instillation of ocular lubricants. A novel computerized objective analysis technique was developed to analyse the tear ferning grades in a 0-4 scale, where '0' denotes highly dense ferning and '4' denotes no observable ferning. As a preliminary step this study set out to establish the day-to-day variability of the tear fern pattern when graded both subjectively and objectively using computational analysis.

The objective grading is based on previous work that has shown that changes in the local direction of derivatives in image luminance (i.e., shading gradients) can be used to automatically identify texture contours [10]. Texture contours are the boundaries between light and dark pixel regions in the image, which correspond to ferning contours seen in the images. The strength of pixel derivatives might therefore provide a useful objective model of perceived contour strength (i.e., ferning strength). One study has shown that such objective computations can account for perceived texture strength [11]. This study therefore assessed the feasibility of this objective measure of ferning strength by comparing it with subjective estimates of ferning to determine whether there was any consistency between these measures. Any evidence of its feasibility would support the view that the strength of local derivatives in shading gradients may serve as a psychophysically-valid objective measure of tear ferning.

METHODS

Participants

This study received ethics approval from the University of New South Wales human research ethics committee (HC15207) and adhered to the tenets of the Declaration of Helsinki (2013).

Informed consent was obtained from all 18 healthy young adults (3 male and 15 female) aged 18 to 35 years (mean age 22.9 ± 4.4 years). Sample size calculation was based on a statistically significant difference of one tear fern-grade at a 95% confidence interval and with 80% power. Tear ferning was analysed based on 5 point subjective grading suggested by Masmali et al [12], and one tear fern-grade difference in Masmali grading scale was regarded as significant difference. The following exclusion criteria were applied: a history of contact lens wear in the previous 6 months, ocular surgery in the previous 12 months, systemic conditions impacting the corneal surface, oral or topical medications, ocular lubricant use within the last month, and epilepsy.

Study design

Each participant attended 3 visits (designated A, B and C) with a minimum separation of 24 hours. The order of all the visits was randomised for each participant using a free online program for randomisation (https://www.random.org/lists/). Visits A and B were identical in methodological procedure and served to establish baseline measurements of all tear film parameters. These baseline measurements consisted of LLT in both eyes using the LipiView® Ocular Surface Interferometer (TearScience® Inc, Morrisville, NC) followed by non-invasive keratograph break-up time (NIKBUT) measurement using the Oculus® Keratograph 5M (Oculus®, Arlington, WA, USA) and finally, collection of basal tears. At Visit C, 50µL of unpreserved Refresh® lubricant eye drops (Allergan, Inc, Dublin, Ireland) were instilled into each eye using a sterile pipette tip attached to a clinical pipette. A standardized delay of one minute was initiated after the instillation of the drop in the second eye prior to the commencement of tear film measurements. After one minute, the remainder of Visit C was conducted identically to Visit A and B, in which LLT and NIKBUT were measured and basal tears were collected again. The order of data collection (LLT, NIKBUT and tear collection) were consistent across all visits. Additionally, right eye measurements were always taken before the left eye. The examiner undertaking each measurement was kept consistent to avoid inter-examiner variability.

Clinical methods

Visual acuity for the participants was measured using Text Chart Xpert 3Di (Thomson Software Solutions, UK) at visits prior to data collection.

Ocular Surface Disease Index questionnaire

Participants completed the Ocular Surface Disease Index (OSDI) questionnaire to grade their dry eye status.

Lipid layer thickness

The LipiView® Ocular Surface Interferometer (TearScience® Inc, Morrisville, NC) was used to measure LLT. The instrument was aligned with the lower third of the cornea, about 1 mm above the inferior tear meniscus [13] and manually focused using the i controls of the instruments. The same investigator operated the LipiView® throughout the study. The following measures were recorded for each study participant: the average tear film thickness from all frame averages, the maximum and minimum thicknesses. The LipiView® has an upper cut-off of 100 interferometric colour units (ICU). Any values of 100 were excluded from the study as it was not possible to obtain the true reading [14].

Oculus® non-invasive keratograph break-up time

The Oculus® Keratograph 5M (Oculus®, Arlington, WA, USA) was used to measure (NIKBUT). The first point of break (NIKBUT-1) was recorded as well as the average break-up time for all points of break-up measured (NIKBUT-average). The same investigator operated the Oculus® throughout the study. Any values above 24.73 seconds were excluded from the analysis as this is the upper cut-off of the instrument and not considered to be the true value [14].

Tear collection

Basal tears [15] were collected using a 10 µL glass microcapillary tube (Blaubrand®, Germany) at the end of every visit. The microcapillary tube was placed in the lower tear meniscus of either eye for four minutes so that at least 4 µL was collected. Tear collection flow-rate was monitored by measuring the volume collection as a factor of the time taken to collect the tears. Tear collection ceased if reflex tearing was detected. After the sample was collected, a plunger was placed over the open, empty end of the tube to allow for its contents to be expelled into a 5 mL Eppendorf tube (Eppendorf, USA). The vial was placed inside a 4°C refrigerator for a maximum of 2 hours during participant visits until they were centrifuged (Eppendorf, USA) at 4000 rpm for 20 minutes at -4°C and placed in a -80°C freezer (Labtec Pty Ltd, Australia) until analysis.

Tear ferning analysis

Tear sample treatment

The vials were removed from the freezer and allowed to defrost for 10 minutes at a constant room temperature (24°C). The samples were then plated onto a glass slide using a clinical micro-pipette, 2µL at a time. A minimum of three 2µL drops were extracted from each sample, spaced evenly over the slide and allowed to air-dry at room temperature (24°C) for 5 minutes prior to image capture. Humidity was kept constant for tear sample desiccation by evaporating tear samples at room temperature (20-26°C) at an incubator [16].

Tear ferning image capture

An inverted microscope (IX73 Olympus, USA) was used in conjunction with cellSens© image capture software (Olympus©, USA) to photograph the samples at 10x objective (magnification) for subjective grading using the Masmali TF grading scale [5]. Care was taken in the imaging process to ensure the region of interest within each tear sample occupied approximately 95% of the image space.

Subjective grading of tear ferning

Two masked observers were assigned to grade 10x magnified tear ferning images in a random order. The subjective grading was based on tear ferning grading detailed by Masmali et al. [5]. Briefly, the 5-point grading scale is a modified version of the tear ferning grading scale classified from 0 to 4, in 0.1 increments to improve grade refinement. Grade 0 has the densest ferning pattern with no spaces or gaps between the ferns and branches; the density of the ferns and branches declines in Grade 1 with the appearance of small spaces. In grade 2, the density of ferns and branches are thicker with more spaces in between, whereas no fern is present in grade 3 with only appearance of sporadic crystals. The ferning pattern is completely absent in grade 4.

Objective grading of tear ferning

Objective grading of tear ferning was estimated by computationally analysing fern density for each tear-film image using a simple measure of local contrast of image contours. Tear fern images for all 3 visits were analysed. Previous work has shown that orientation fields – the polar direction of local derivatives in image luminance – are a good computational model of perceived texture. Treating the appearance of ferning as a form a texture perception, the orientation fields of the tear-ferning images were computed to determine the local anisotropic energy across finite regions of image space. This psychophysically-based measure was intended to model perceptual grading of fern density for the same images by experienced clinical observers (Figure 2).

At first the tear ferning images were converted to greyscale for objective measurement. A 512×512 pixel region was then cropped from the centre of each tear droplet acquired at 10× magnification. This size was chosen because it sampled the central third of each droplet on average. Then the image was blurred and subtracted the blurred version from the original to eliminate vignetting. This blurring was performed using a Gaussian kernel of size 12 pixels half width. This de-vignetted image served as the input image for analysis. This process of de-vignetting is known as flat-fielding [17].

For the objective analysis of tear ferning strength, the strength of the shading gradient at each pixel location in the image was computed by differentiating values horizontally and vertically. These derivatives were then squared to added together, and then computed the square root of the result to obtain a measure of what is commonly referred to as local energy. Changes in local energy provide an indication of texture contour strength [11, 17], which in a situation corresponds to ferning contour strength.

Following from the procedures described by Kim et al. [18], the derivative of the image was obtained using a gabor filter generated by multiplying a cosine function by a Gaussian envelope (7x7 pixel kernel space). The filter was rotated over a 0° to 180° range by steps of 15°. The orientation that generated greatest local energy corresponds to the orientation of the fern contour boundary. Contour energy (E) was then computed as the normalized response range between the preferred and non-preferred filter orientations as per Equation 1, where Max and Min are the maximum and minimum responses of the filter over the 180° range, and SD is the standard deviation of responses across all orientations.

$$E = \frac{Max - Min}{SD}$$

Figure D, E and F show the image locations with positive contour energy marked in red (contour image). Note how these contours retain the visual appearance of ferning in the input flat-field images. Fern density was estimated by first computing a binary version of the contour image, where contours were 1 and non-contours were 0. This is followed by the determination of average image contrast over small local 5x5 pixel windows samples across all regions within the binary contour image. The average local image contrast was then computed and used as the raw automated objective grading of fern density.

A simple transform by multiplying each objective grading by the mean of all subjective gradings was applied to calibrate the objective measure's scale against subjective grading. The resulting values ranged between 0 (densest ferning) and 4.0 (no ferning). Should any values fall outside this range (i.e., be greater than 4.0), the model will coerce these values to 4.0 in value. This method provided an objective estimate of fern density that was comparable in scale to the subjective grading scale.

Data Analysis

All data were analysed using SPSS software version 23.0 (SPSS, Inc., Chicago, IL) and GraphPad software (Prism version 7; La Jolla, CA). Results are presented as means ± standard deviation (SD). All LLTs are expressed in nanometers (nm) and NIKBUT in seconds. Coefficients of repeatability (1.96 x the SD of mean difference between replicates) [19] were calculated. Normality was tested using the Shapiro-Wilk test. Repeated measures analysis of variance (RMANOVA) was used to compare techniques and had two within-subject factors: *eyes* and *days*. The assumption of sphericity was verified using Mauchly's test. Where main effects were significant, Student *t*-tests with Bonferroni correction were used. Agreement between pairs of subjective and objective grading was analysed using the Bland-Altman plot [20]. In the Bland-Altman plots, the difference between the measurements with the two different methods is plotted against their mean. The linear regression of the 95% limits of agreement (mean difference±1.96 standard deviation) provides the distance between the measurements by the methods with 95% confidence. Pearson's correlation analysis was performed to test for associations between tests and comfort scores. A *P* value of 0.05 or less was considered statistically significant.

RESULTS

Repeatability

Tear collection flow-rate

Tear flow rate was 0.4 ± 0.2 μL/min at visit A, 0.5 ± 0.3 μL/min at visit B and 0.4 ± 0.3 μL/min at Visit C and there was no difference between visits ($p = 0.97$).

Tear ferning

Inter-observer repeatability

The mean difference between observers in terms of tear ferning grading was -0.3 grades. The coefficient of repeatability was 1.9 grades and the range of the limits of agreement was 1.6 to -2.2. The two observers were not significantly different ($p = 0.08$).

Day-to-day variability

The grades from the two observers were averaged to yield one result for each visit. Visits A and B were compared to establish day-to-day variability (Table1). There was no significant difference ($p = 0.36$) between the baseline visits for the subjective grading.

Day-to-day variability – objective grading

Similar to subjective grading, objective grading did not find any significant difference between the baseline visit A and B ($p= 0.64$).

Lipid layer thickness

Eye-to-eye repeatability

Eye-to-eye repeatability was assessed for LipiView® average, maximum and minimum at the two baseline visits (Table 1). There was no significant difference between the eyes at baseline visits A and B for either the LipiView® average, maximum or minimum (p = 0.58, 0.11 and 0.13, respectively).

Day-to-day variability

As there was no significant difference between right and left eyes, eyes were averaged, and visits A and B were compared (Table 2). The CoR between days was greater than that between eyes. This is not surprising given that there would be greater physiological variability expected between days than between eyes. There was no difference between days for either LipiView® average, maximum or minimum (p = 1.00, 0.90, 0.40).

Non-invasive keratograph break-up time

Eye-to-eye repeatability

Table 2 presents the differences between eyes for NIKBUT-1 and NIKBUT-average at the baseline visits. There were no significant differences between eyes at for either NIKBUT-1 or NIKBUT-average (p = 0.50, p = 0.32, respectively).

Day-to-day variability

As there was no significant difference between right and left eyes, eyes were averaged, and days were compared. The CoR between days for NIKBUT-1 and NIKBUT-average did not differ from that between eyes, indicating perhaps that NIKBUT demonstrates as much variability between eyes as it does between days. Unlike LLT assessment using the LipiView® interferometer, NIKBUT measurements require the participant to keep his/her eyes open for the duration of the measurement. This very process may destabilize the blink, increasing the variability between eyes to the level of that seen between days (i.e., physiological variability). Hence, it may not be the best measure to assess for inter-eye differences. There was no significant difference between days for NIKBUT-1 or NIKBUT-average (p = 0.50, p = 0.32 respectively).

The effect of lubricants on clinical parameters

Tear ferning

Subjective grading

As there was no significant difference between baseline days, baseline days were averaged and compared to Visit C when refresh plus was instilled. There was no significant difference between the baseline visit and following post-lubrication instillation (Table 3; F = 0.34, p = 0.57).

Objective grading

Objective grading for the combined visit A and B was 1.4 ± 0.9 and for the visit C it was 1.4 ± 0.8 (Table 3). There was no significant difference between the baseline and post-lubrication instillation visits (p = 0.82).

Lipid layer thickness

Because there was no difference between visits A and B, the LLTfor these visits was averaged as the baseline result and compared to visit C with topical lubrication to determine the effect of lubricants on LLT (Table 3). There was no significant difference between the baseline visit and Visit C for either LipiView® average (F = 4.02, p = 0.06), LipiView® maximum (F = 0.41, p = 0.53) or LipiView® minimum (F = 0.77, p = 0.39).

NIKBUT

Because there was no significant difference between visits A and B, the NIKBUT was averaged and considered the baseline result. This was then compared to visit C to determine the effect of lubricants on NIKBUT (Table 3). There was no significant difference between baseline and Visit C for NIKBUT-1 ($F = 0.28$, $p = 0.61$) or NIKBUT-average ($F = 0.77$, $p = 0.39$).

The association between clinical parameters and fern grading

Table 4 lists the correlations between the variables measured. There was no correlation between the tear fern pattern and any of the clinical variables. Similarly, comfort was not correlated with any of the clinical variables measured in this study. Objective tear fern grading significantly correlated (r = 0.56; $p<0.001$) with subjective tear fern grading (Figure 3)

Agreement between objective and subjective analysis

The Agreement between the objective and subjective grading was determined using a modified Bland-Altman plot (Figure 4). Tear ferning micrographs obtained from all the visits were used for the analysis of the agreement. The central dotted line indicates the linear regression line for the difference between the objective and subjective tear fern grading. The line passes along the no difference ('0' difference) mark, indicating negligible expected difference between the two-grading method with varying tear fern grading. The thin shaded zone indicates the linear regression area for 95% limits of agreement.

DISCUSSION

This study detailed inter-observer and day-to-day repeatability of tear ferning patterns in healthy participants when using subjective and objective grading methods. Given that tear LLT thickness and tear break up time are critical parameters for tear film quality, their association with tear ferning grading was determined in healthy participants and how these features change with artificial tear supplements was further investigated. This study has also reported an objective tear ferning grading system which is highly correlated with subjective grading.

This study found that there was no significant difference in the grading of tear fern patterns between observers when examining healthy participants with the Masmali subjective grading scale [5]. In addition, this tear fern pattern appeared to be stable between days. This is in agreement with an earlier report by Masmali et al. [12], who showed good diurnal repeatability, with no significant difference in the tear fern pattern in young healthy participants. The average minimum and maximum LLT found in this study was repeatable and their mean differences at each visit remained low, ranging between 1.1 to 6.6 nm. A similar trend was observed for NIKBUT with a mean difference of 0.5 to 0.7 seconds. This study also showed that LLT and NIKBUT as determined by the LipiView® and the Oculus® keratograph, respectively, was highly repeatable when measured on two separate days. The LLT results are in agreement with earlier results reported by Zhao et al. [13] and Markoulli et al. [14], where they found average LLT in healthy eyes to be 53.53 ± 14.59 nm, similar to the result of this study 62.61 ± 14.54 nm. Average NIKBUT showed borderline correlation with the maximum LLT ($r = 0.33$, $p=0.05$). This finding supports earlier reports that a thicker LLT masks the underlying aqueous layer and prevents evaporation, leading to higher break-up time and enhanced stability [21].

This study found that subjective tear fern grading did not correlate with other tear parameters measured in this study. A similar trend was seen with objective analysis where the correlation coefficient (r) with other tear parameters ranged between -0.07 to -0.21. This is in contrast to an earlier study by Kaur et al. [22] which showed that tear ferning highly correlates with NIKBUT. However, the difference is probably due to the fact that they used 7-point Ronaldo ferning grading system which is significantly different to the 5-point Masmali grading system [5] used in this study. Subjective and objective tear ferning also showed poor correlation to the OSDI questionnaire, whereas previous work supports a strong correlation between the ferning and the McMonnies Dry Eye Questionnaire [8, 12]. Reasons for this disagreement may be due to the inclusion of healthy participants rather than those with dry eye as the OSDI questionnaire has been proven to be reliable in dry eye diagnosis and has significantly positive correlation with the McMonnies Dry Eye Questionnaire [23].

Tear parameters following instillation of tear supplements remained unchanged. Masmali, et al. [5] demonstrated that subjective tear fern grading was specific to the level of dry eye present, suggesting that an improvement in dry eye should be reflected in the improvement of the fern grade. The reason behind the insignificance found in the tear fern grade with the use of lubricants in the current study is not clear. Tear ferning may not be sensitive enough to demonstrate the subtle changes in the tear film stability made with the temporary lubricant use. Further investigation into the change in the tear ferning with use of a greater volume of ocular lubricants, treatment over a longer period of time and lubricants with different constituents (such as lipids, pH levels) may provide more in-depth understanding. Especially, exclusively selecting participants with established dry eye disease and following them with dry eye treatment may further provide better insight and findings of significance. This study found that average LLT marginally increased with tear supplements, however the difference had borderline significance (p=0.06). Interestingly, there was no improvement in other LLT parameters and tear break up time with the tear supplement, supporting the notion that artificial tear supplements may provide palliative treatment to ocular symptoms but may have very little impact on the composition and stability of the tear film.

This study reported on a novel computerised objective technique to analyse the grade of the tear ferning pattern. Neural computations of the human visual system utilises spatial variations in image luminance to estimate the organisation of a textural pattern [17]. The novel objective technique uses a similar computational algorithm to identify ferning patterns and grade them on a 0-4 scale, where 0 is highly dense ferning and 4 has no detectable ferning. The objective analysis showed high agreement with the 5-step subjective grading system proposed by Masmali et al [5] and its correlation with other clinical parameters were similar to the findings with subjective grading. The agreement between the subjective and objective technique was evaluated using a Bland-Altman plot, which showed a narrow zone of 95% limits of agreement along the central line indicating minimum proportional bias across the 0-4 grading between the two techniques. This indicates that the objective technique could be used as a highly repeatable and computerised platform by clinicians to determine unbiased tear fern grading. As an example, figure 5A shows that a highly dense tear ferning micrograph that may be misinterpreted as a low ferning pattern during subjective analysis. Whereas, the novel objective analysis will be able to identify the fine patterns indicating high tear ferning. Subjective tear ferning assessment has been used as a simple and easy method to comprehend the biochemical compositions of the tear film. It is believed that the varying concentration of tear mucin and proteins participate in the formation of a denser tear fern pattern. Pearce et al [24]. with the use scanning electron microscopy showed that sulphur, indicative of the presence of macromolecules such as mucins and proteins, is only found at the peripheral thin amorphous film of the tear fern droplet. This suggests that proteins and mucins may not directly play a vital role in the formation of the actual central ferns and their absence allows the salt constituents to precipitate and form denser, and finer ferns in the centre. They further suggested that during evaporation the solubility limit is reached quickly, whereby proteins and mucins recede to the periphery. Once the solubility limit is reached, a spontaneous fern growth proceeds inside the droplet leaving the amorphous macromolecule band intact. This study also found similar ferning sequences where an amorphous peripheral band was present in all the tear ferning droplets collected before and after tear supplements from healthy participants. This is also in agreement with the current findings that the addition of tear supplements does not alter the tear fern pattern, owing to the fact that addition of tear supplements may not significantly alter the *in vivo* protein, mucin and salt concentration in the tear. The invariance in these metrics appears to account for the lack of change in the resulting fern pattern.

Contrary to this view however, Pearce et al. [24] hypothesised that increased tear osmolality such as in dry eye may create an imbalance of inorganic salts during tear fern without the proper opportunity for macromolecules to spread to periphery, causing accelerated asynchronous desiccation. During this procedure, major crystalloids are replaced by smaller and unstructured patterns leading to limited or no ferning pattern. Traipe-Castro et al. [25] showed a similar phenomenon by accelerating the tear droplet evaporation rate with reduced air pressure. They confirmed that the same tear constituents can show inferior tear fern patterns when they are dried faster, supporting the importance of controlled drying and proper spreading of tear macromolecules during tear fern formation.

There are some potential limitations to the current study. Traipe-Salas et al. [26] divided the tear ferning area into 4 zones to analyse the pattern objectively. The subjective grading used in this study considered the whole ferning pattern, as was reported earlier by Masmali et al. [5], whereas the objective analysis considered the central zone. The sample size of this study was not designed to determine the sensitivity and specificity of the objective grading. This study also did not include participants with dry eye disease or Sjogren's disease. Further studies will be required to determine the performance of the novel objective analysis technique with the tear samples from such participants.

In conclusion, this study showed that tear film grading achieved by subjective and objective techniques, LLT and NITBUT are highly repeatable. None of the ocular clinical parameters showed significant correlation with the subjective and objective tear ferning grades. Lubricants had negligible effect on tear ferning grade, LLT or NITBUT. The novel objective analysis showed high agreement with the established subjective tear ferning grading, suggesting it may serve as a promising candidate for a repeatable and unbiased assessment of tear ferning.

Acknowledgement

JK was supported by an Australian Research Council (ARC) Future Fellowship.

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FIGURE LEGENDS

Figure 1: Tear ferning patterns observed with 10x objective (A: grade 0, B: grade 1, C: grade 4 tear ferning). Image captured using an inverted microscope (IX73 Olympus, USA).

Figure 2: Method used to determine the average local contrast of image contours. Top: Sample images showing flat-fielded photographs of tear-film ferning acquired at 10-times magnification (input images). Bottom: Contour images computed by determining the local anisotropic energy of the input images. Note: values superimposed on contour images show the computed values for average local anisotropic contrast.

Figure 3: Correlation between subjective and objective grading scores

Figure 4: Bland-Altman plot showing the agreement between the subjective and objective grading techniques in grading tear ferning patterns. The dotted central line represents the linear regression line for the mean grading differences between the two methods. The shaded zone represents the area of 95% limits of agreement by regression analysis. Tear ferning gradings for all the visits were included in this plot.

Figure 5: A tear fern micrograph demonstrating very fine ferning patterns. The fine pattern of figure A can be easily misinterpreted by clinicians as a low tear ferning (such as grade 4 by Masmali grading [5]) during subjective analysis. Figure B (inset area of Figure A) shows the enlarged version of the same micrograph confirming highly dense ferning that can be correctly identified by the computerized objective ferning technique