methodology to determine the level of redness of the eye using images of the bulbar conjunctiva

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INTRODUCTION

The estimation of conjunctival hyperemia is a standard procedure during clinical evaluation in ophthalmology. Conjunctival redness or hyperemia is evaluated on the dilation of blood vessels in the conjunctiva area, and it can be symptomatic of different kinds of inflammations or infections. Its evaluation is commonly investigated for the contact lens effects [1,2] and for the so-called dry-eyes syndrome [3]. The hyperemia estimation is performed on images of the patient's eye acquired using a slit lamp. The slit lamp, also called slit bio-microscope, is an optical instrument used in ophthalmology forthe observation of eye tissues. It allows for visualizing the bulb and the ocular annexes, the corneal layers, the vitreous and the anterior chamber, the crystalline lens and the iris. Allof this information can be acquired posing no risks for the patient .

Hyperemia is commonly evaluated using qualitative grading scales, where the con- junctival hyperemia is compared to a set of standardized pictures (template images or references). These reference pictures are images artificially generated using software, painted, or real photographs. The clinician establishes the score by matching the pa- tient conjunctiva to the reference one that he considers the most similar. This measure is performed for each eye independently.

There are many grading scales proposed in literature for the hyperemia estimation. Davies (1978), Mandell (1989), and Woods (1989) proposed descriptive grading scales; Koch et al. (1984) and Schnider (1990) proposed art illustrations for the evaluation of singlecondition grading scales; Annunziato et al. (1992) and Efron (1999) extend the previous evaluation to multiple conditions; Courtney & Lee (1982), Lupelli (1998), McMonnies & Chapman Davies (1987), Price et al. (1982), Begley (1992), and Lofstrom et al. (1998) introduce the use of photographic references. The latest proposed reference images were generated using computer graphic (Jenvis, 2009). Each scale is associated with a different set of reference images and each of them is focused on a different aspect of the hyperemia estimation; for instance, the conjunctival redness in the Efron Grading Scales for Contact Lens Complications [1] is expressed through five images depicting 1–5 grading ranging from normal to severe.

A critical issue of these assessments is their reproducibility: the template matching evaluation is an intrinsic operator-dependent procedure and thus it is affected by subjectiv- ity. The reproducibility of the image acquisition and moreover the hyperemia evaluation are determined by the experience of the operating clinician, and it can be affected by several experimental conditions, leading to imprecisions and biases Generally, the automation of the clinical exam provides a support for the clinical laboratory or clinical practice assessment through faster and more reliable evaluation and reducing the reliance on the operator expertise for the evaluation

Several authors have already proposed semi-automated grading systems for the hyper- emia quantification, offering semi-supervised pipelines to process the patient's images [7]. All of these methods facilitate the clinician operations providing a series of automated processing steps, which, however, require the human intervention (e.g., a manual selection of the region of interest and color values) At the same time, they allow for intro- ducing new quantitative grading scales that are difficult to relate to the standard clinical assessments. These grading scales can provide a viable medical alternative to standard grading scales ensuring greater accuracy and different information for the clinician, but they can not be used as standalone results prior to a lengthy medical certification trial.Thus, despite the efficiency of the automated methods, their practical usage by clinicians re- mains limited.

The creation of a fully automated pipeline which starts from the acquired image until the hyperemia score prediction is still an open problem .Some authors have already proposed interesting results on this problem. Sánchez Brea et al. implemented a fully automated pipeline for the segmentation of the conjunctiva, but the predictions of their model were obtained only on a small part of the dataset on which there was a reasonably good agreement between experts' evaluations, discarding any source of issues, that might be instead encountered during clinical practice. Derakhshani et al. tried to predict the assessment of the vascularity of conjunctiva using a neural network approach: the images were rescaled to improve the computational efficiency, losing a great part of the information; moreover, the results reported in their work are not easily interpretable, since the final scores are obtained without a subdivision of the samples in train and test, alongside a best-model selection procedure on the data.

All of these methods introduced the usage of modern machine learning and deep learning frameworks for the processing of the features extracted from the original images, after a semi-automated segmentation of the region-of-interests (ROIs). Deep learning convolutional neural network (CNN) image segmentation models have shown promisingresults in medical applications in the last few years. We can find classical or tailored deep learning architectures in many medical research fields, including the opthalmologic one Despite the growth in computational power availability, which allows for enlarging the application of even more complex deep learning models, their usage necessarily requiresthe manual annotation performed by experts

SUBJECTS AND METHODS

Image Acquisition The study included six volunteers (age 22-30 years, four males and two females), who never wore contact lenses and did not have any ocular diseases during the previous 6 months.

The purpose of this study was explained to all participants, and informed consent was obtained. The Ethics Committee of Zhongshan Ophthalmic Center approval was obtained to carry out this study.

The photographs were taken by a TOPCON SL-D7 slit lamp connected with a Nikon D200 camera. The eye drops, named Jinshile Eye drops (Approval Number Z20053110), manufactured by Changchun Three-nine Bio-pharmaceutical Company and had some efficacies for myopia in children. Because of the main component of the eye drops was traditional Chinese medicine and had some stimulating effect. So we could use this eye drops to stimulate the eye surface to achieve the purpose of bulbar redness without any damage to the human body. Photographs were taken before and 10 and 20min after the eye drop challenge in the examination room in the morning. Under a 45-degree illumination, photographs were taken of the bulbar conjunctiva of each eye in four orientations. In this way, 24 photographs were collected from every participant for further analysis. One participant was photographed six times in all four directions to calculate the intraclass correlation coefficient (ICC).

Analysis of the Photographs The original file type of the photographs was JPEG with a size of 2 896伊1 944 pixels and a resolution of 72dpi. ImageJ software (http://rsb.info.nih.gov/ij) was used to analyse the photographs. First, the RGB split function was used to divide the photographs into red, green and blue channels, and the green component of the picture was extracted because the green component of the picture provides the highest signal-to-noise ratio.

The horizontal diameter of the cornea was set to 11.75mm, and vertical was set 10.75mm, which can convert to 1 923 and 1 759 pixels, respectively. Then, a circle with diameters at 1 923 (superior and inferior photographs) or 1 759 pixels (nasal and temporal photographs) was drawn on the picture. Next, the circle was moved to match the limbus, and the calculated centre of the circle was used to locate the centre ofthe cornea. Based on the cornea centre on each photograph, seven points, with 5-degree intervals and 4mm away from thelimbus, were calculated and labelled in the conjunctiva. Finally, these points were connected with line segments that were used to evaluate the vessel parameters (Figure 1). By analysis in ImageJ, the plot profile under the seven-point linesegments formed a line graph denoted by pixels. Then, the line graphs were inverted by subtracting each pixel value from 255 to ensure the peaks pointed upwards for ease of reading. Finally, we obtained graphs of the greyscale pixelsof vascular sections that were used for the evaluation ofbulbar vessels. The dark vessels in the images correspond to the peaks of graphs.

To select real vascular peaks, we used three criteria: 1) 5% of the average of greyscale value was set as a baseline; 2) the greyscale value of each point was subtracted from the following point. If there were at least 5 sequential points of ascending values (the differences >0) and 5 sequential points of decending values (the difference<0), we decided it was a peak; 3) if some data were difficult to interpret, we went back to the photograph to decide upon the presence or absence of a peak (to exclude pigmentation, nevus, *etc.*). In this way, we obtained a curve with the parameters peak height (PH), peak width (PW, width at half-height), peak area (PA) and peak number, which can be used to describe hyperaemia in the conjunctiva [14,15]. The peak number represented the number of conjunctival vessels. Peak height corresponded to the bright levels of congestive vessels, and peak width corresponded to the appropriate diameter of the vessel *in vivo*. To avoid one or several measurements of larger variation caused by a large vessel, abnormally large values were removed based on the Pauta criterion: if the repeated measurement data satisfied 讦x_i-$\bar{x}$讦>3啄, then x_i was considered an abnormal value and was removed (where x_i is the *i* th measurement value, $\bar{x}$ is the mean of all measurement values, and 啄 is standard deviation). Generally, about 0-2 abnormal values of each group were removed.

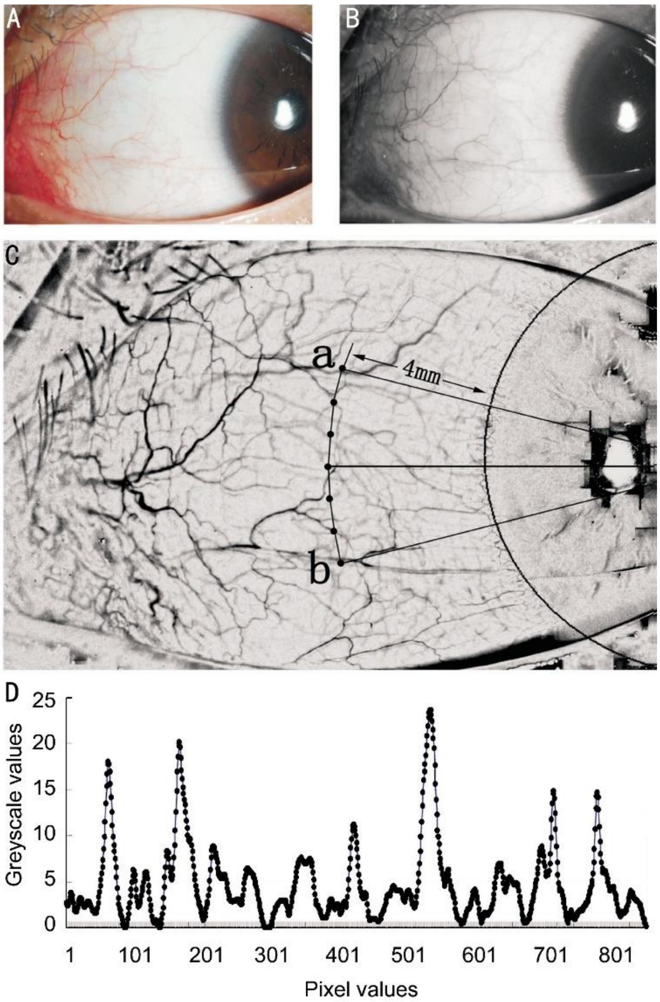

Figure 1 Image processing and data analysis A: The initial-state image was taken by a digital camera and saved as a JPEG image (2 896伊1 944 pixels); B: The green component was extracted from the initial state image; C: After subtracting the background, six line segments (from a to b) were drawn according to the method described in the main text; D: The plot profile of the pixel values under the line segments was obtained and used to describe the vessel condition. The peak number represents the number of conjunctival vessels. The peak area (PA), peak height (PH) and peak width (PW, width at half-height) describe the level of vascular congestion.

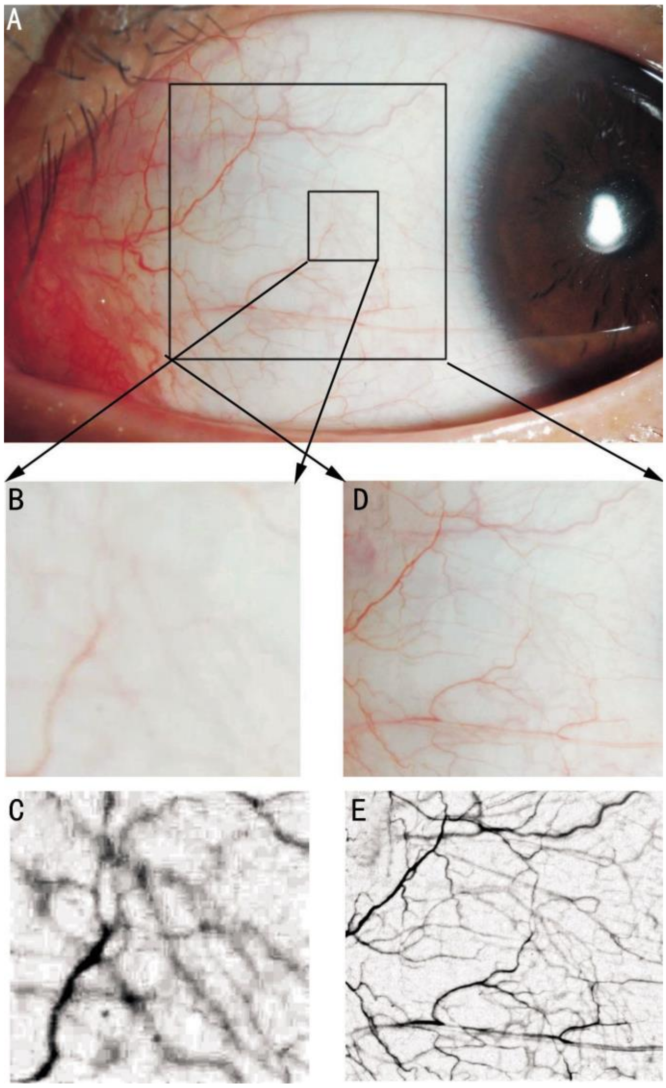

Figure 2 Evaluation of ocular surface redness using the method of pixel counting in the ROI From an initial-state photograph (A), areas of different size (B: 600伊600 pixels; D: 1 200 伊1 200 pixels) were chosen. The background that was automatically assigned by ImageJ was subtracted from the green component of the picture. Then the pictures were binarised, and the pixel values of the foreground (blood vessels) were counted (C and E).

To compare this method with calculated pixel values (*i.e.* the black pixels in the binarised image), a ROI was also used to analyse the photographs as reported previously [5,16]. A rectangle 4mm away from the limbus with an area of 600伊600 pixels (Figure 2) and one of 1 200伊1 200 pixels in the horizontal direction or a rectangle of 1 600伊900 pixels in the vertical direction in original images without eye drop challenge was chosen to calculate the pixel count of the ROI. The pixels were counted as previously reported [5,16]. Briefly, the ROIs were preprocessed by splitting and extracting the green channel, background subtraction, and binarisation, which were done in ImageJ. The total pixel value of the ROI from different images was used to calculate the mean and standard deviation.

Statistical Analysis The SPSS program (version 13.0, SPSS Inc., USA) was used for statistical analysis. Comparisons between two groups were performed by Student's *t* test, and comparisons among multiple groups (the hyperaemia in four orientations of the eye) were performed by one-way ANOVA. The ICC was calculated by a two-way mixed effects model. Differences were considered significant if $P<0.05$.

RESULTS

General Characteristics of Conjunctival Vessel Figure 1 displayed our method of calculating the greyscale values under the broken line graph of a nasal conjunctiva photograph. Each peak in the line chart corresponds to the vessel one by one along the line segments drawn on the photograph. There were around 24 peaks/vessels in the target areas of the four direction photographs, with the mean peak height of 9.28 pixels and the mean peak width of 11.50

Table 1 Vascular parameters in four conjunctival zones (n=12 eyes)

	Vascular No.	Average diameter (μm)	95% *CI*
Temporal vessels	297	74.23	24.44-146.64
Superior vessels	284[b]	70.39	24.44-134.32
Nasal vessels	299	72.85	30.55-171.08
Inferior vessels	290	63.52	24.44-171.08
Total	1170	70.27	24.44-152.75

[b]ANOVA tests: P<0.01.

Table 2 The calcu ation of various parameters by different methods (n=12 eyes)

	Superior		Inferior		Nasal		Temporal	
	Mean±SD	CV	Mean±SD	CV	Mean±SD	CV	Mean±SD	CV
PA[a]	131.82±45.44	0.34	108.00±33.96	0.31	163.43±47.16	0.29	142.26±54.51	0.38
PH/PW	0.75±0.24	0.32	0.79±0.20	0.26	0.91±0.22	0.24	0.73±0.18	0.24
S. pixel count[b]	3.60±1.39	0.39	2.56±0.75	0.29	3.58±1.22	0.34	4.27±1.90	0.47
L. pixel count[b]	10.28±2.81	0.27	7.99±2.65	0.33	13.31±3.61	0.27	12.73±5.06	0.40

[a]PA: Peak area; PH: Peak height; PW: Peak width; S. pixel count: Small area pixel count; L. pixel count: Large area pixel count; [b]the mean and SD are divided by 10^4.

pixels. When comparing one photograph of each eye in the same orientation in the same person, we found that all parameters in the two eyes showed no significant differences (P>0.05). So the 12 photographs in the same orientation from the 6 individuals were analyzed together without distinguishing the sides of eyes. We also found that the ICC, calculated by using PH/PW, was 0.92 (95%*CI*: 0.783-0.978), which suggests there were no significant alterations during image taking and processing.

A total of 1 170 vessels were detected in the four orientations, with the fewest vessels in the superior zone (Table 1). The blood vessel diameter ranged between 18.33 and 226.07μm, with a mean value of 70.27μm.

Comparing Our Method with the Method of Regional Pixel Counting The same photographs were analyzed to compare our method with previously reported methods of counting the numbers of absolute pixel values of the ROI[7,16]. ROIs of two sizes were set to perform pixel counting (Figure 2). Table 2 displays the data of the pixel counts of conjunctival vessels and the coefficient of variation (CV) of each data set. In all photographs of all four orientations, our vascular parameter counting revealed a lower CV compared to the pixel counting in the corresponding region. Since both PH and PW are associated with vascular hyperaemia and the absolute pixel values would vary case by case, we used PH/PW in our further analysis.

Bulbar Hyperaemia Changes in the Four Orientations We analyzed photographs of the participants in various situations and directions. The PH/PW values fromphotographs taken before and 10 or 20min after eye drop challenge were calculated to show the hyperaemia in the fourorientations of the conjunctiva (Figure 3). At baseline, the PH/PW value of nasal conjunctiva was significantly higher than the other three orientations (<0.01), and the differences among the other three directions were not statistically significant. Ten minutes after application of eye drops, PH/PW increased in all four orientations (P<0.01), with a weaker and slower change in superior conjunctiva (P< 0.01). Twenty minutes after eye drops, the PH/PW of all four zones of the bulbar conjunctiva was lower than that at 10min (P<0.05). Vessel numbers mildly increased after challenge, though without significant differences(P>0.05).

Difference Between Conjunctival and Superficial Scleral Vessels As there are different areas between conjunctival and superficial scleral vessels, we examined 5 conjunctival vessels and 5 scleral vessels in each of the four orientations under slit-lamp biomicroscopy, and PH/PW in photographs was calculated. The results revealed significant differences between conjunctival and scleral vessels. In the initial state, 95% of the PH/PW values of conjunctival vessels were greater than 0.87 and less than 1.00 in the scleral vessels (Figure 4A). The mean PH/PW of conjunctival vessels was 1.41, and the mean PH/PW of scleral vessels was 0.61 (P< 0.05, Figure 4B). When the conjunctiva was challenged by eye drops, PH/PW significantly increased in conjunctival vessels at 10min (P<0.01), but no increase was observed in scleral vessels (Figure 4B).

DISCUSSION

Bulbar redness, varying in location and severity, is a common sign of ocular diseases. It is important to appropriately describe the hyperaemia, which can offer effective information for guiding diagnosis and treatment. With the development of computer-based image analysis, a few new approaches have become available to evaluate ocular hyperaemia. Schulze *et al*[5] described methods that used three physical measures (fractal dimension, percent pixel coverage and colourimetric information) to determine the accuracy of four bulbar redness grading scales. In the current study, we emphasised points of clinical application (*i.e.* the observation area and dilation of vessels) by using techniques of image analysis with colour extraction and counting the greyscale pixels of vascular sections. Our results show that

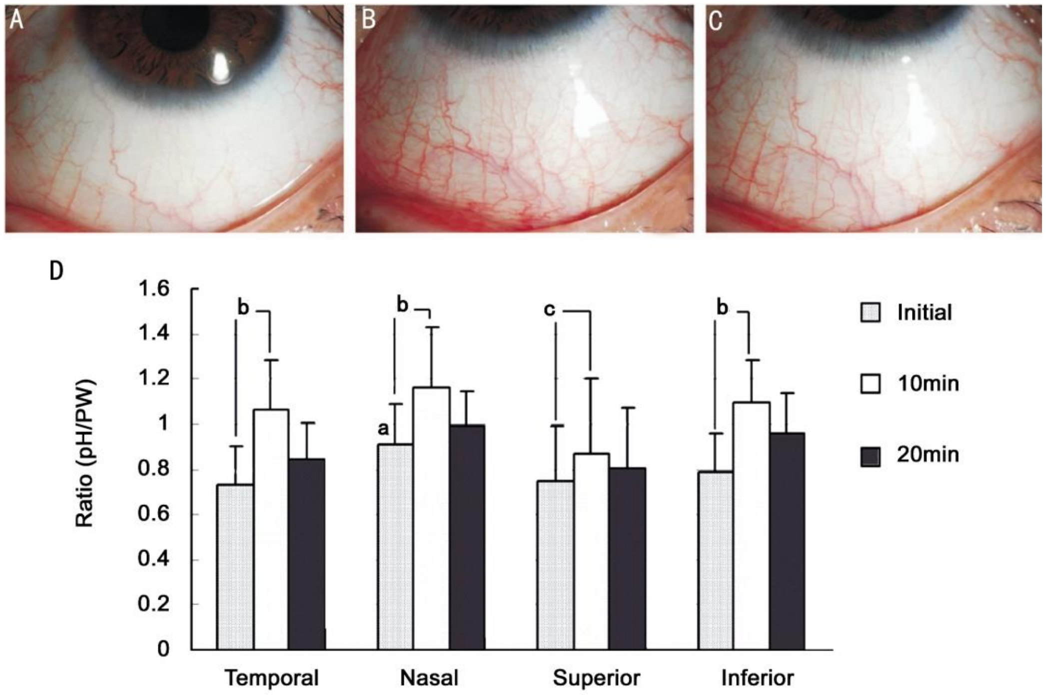

Figure 3 Evaluation of ocular redness after challenge with eye drops Photographs of the inferior bulbar conjunctiva were taken before eye drops (A) and 10 (B) and 20min after eye drops (C); D: Average PH/PW ratio of different ocular zones at each time point. In the initial state, the PH/PW ratio of nasal conjunctiva was higher than that of the other three directions ($^aP<0.05$, ANOVA test). Tenmin after challenge, the PH/PW ratio of all four zones was increased significantly (superior $^cP<0.05$; other zones $^bP<0.01$, Student's t test). At 20min, the PH/PW ratio of all four zones decreased slightly compared to 10min.

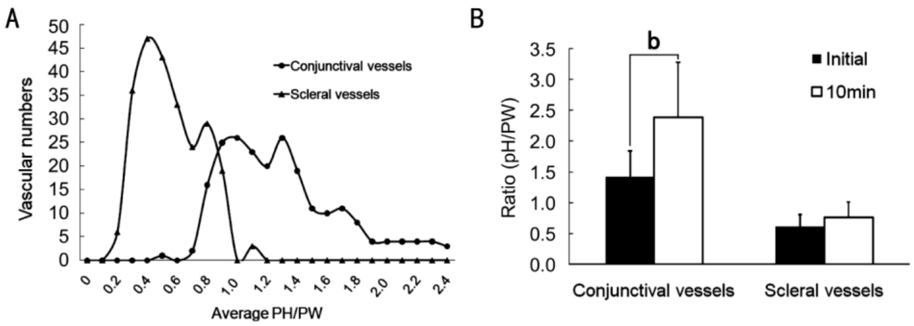

Figure 4 Comparison of PH/PW ratio between the conjunctival and superficial scleral vessels Conjunctival and superficial scleral vessels were chosen for quantitative analysis (5 vessels for each photograph, 240 vessels in each condition). A: The profiles of vascular numbers with varying average PH/PW value. Ninety-five percent of conjunctival vessels had a PH/PW value more than 0.87, and 95% of scleral vessels had PH/PW less than 1.00; B: In the initial state, the average PH/PW value of conjunctival vessels was 1.41, and the average in superficial scleral vessels was 0.61 ($P<0.01$). Eye drop challenge led to a significant increase of PH/PW in conjunctival vessels, but not in scleral vessels ($^bP<0.01$).

for the superficial ocular vessels at 4mm from the limbus on the superior, inferior, nasal and temporal sides, quantification of their parameters, especially PH/PW, is a reliable and objective way to assess bulbar hyperaemia.

The evaluation of conjunctival hyperaemia is usually reported in two broad categories. One depends on the clinical grading scores, with or without reference images, that are available for both human beings and animal models and has been widely reported [1,2,17]. However, there is little consistency between observers because many factors, such as individual professional knowledge, operation of the slit lamp and the lighting situation, strongly influence the results. The other category is based on image analysis with the application of techniques such as photometric measurements, fractal analysis and pixel values [5,16,18,19]. Although these approaches can qualitatively and quantitatively evaluate the severity of bulbar redness, they neglect the varying region of ocular congestion and are not well developed for clinical implementation. This study presents a method that has several attractive qualities compared to previous methods. First, this method can quantify the congestion in four quadrants, which properly matches the clinical observation by slit lamp. Especially in elderly patients, who usually suffer degenerative changes (pinguecula) or pterygium, the superior and inferior conjunctival zones are the appropriate areas for quantitative analysis. Second, the PH/PW ratio directly describes the alterations of a group of bulbar vessels (about 24 blood vessels) with less influence from the background setting, vascular scrambling and pixel spots outside of vessels [5]. Finally, this method also revealed that conjunctival and superficial scleral vessels could be differentiated by the PH/PW ratio, because this method assesses the vessels that have specific anatomic locations. In ophthalmological practice, bulbar redness may be due to conjunctival hyperaemia (conjunctivitis, ocular xerosis), and superficial scleral hyperaemia usually appears in scleritis, keratitis and uveitis. Therefore, this method may be used to distinguish these two signs more clearly, improving the clinical diagnosis and treatment.

Guillon and Shah [8] reported a method of analysing video recordings of conjunctivae to characterise them, including the number of vessels, the vessel width, and the percentage of vessel coverage under the sampling tangent lines. Compared with their work, by using photographs captured with a digital camera, we set up a sample line around the common boundary of the anterior ciliary artery and posterior conjunctival artery, which covered the hyperaemia originating from these two vessels, distinguishing conjunctival vessels from superficial scleral vessels. In addition to counting PA and blood vessels, the PH/PW ratio was also calculated, and we found that this ratio was much better to estimate hyperaemia than any other measurement. This advantage may be attributed to the fact that the PH/PW ratio suppresses the diversity of vessel diameters. Meanwhile,the intensity profile was normalised, and the width at half-maximum height was computed to estimate the vessel diameter, as is commonly used to evaluate vessel width in retinal images [14]. In the current study, blood vessel diameter was between 18.33 and 226.07 m, with a mean value of 70.27 m. This is consistent with Guillon's results (diameter between 53.3 and 57.8 m) but is larger than Shahidis' reported vessel width (between 8.7 and 24.3 m, with a mean value of 15.5 m)

We used eye drops to stimulate ocular hyperaemia. The induced conjunctival hyperaemia was similar to that of the second or the third degree of the validated bulbar redness scale, with shorter dynamic range [20]. Therefore, whether this method is applicable to the evaluation of more serious ocular hyperaemia has yet to be tested. Regardless, this method can detect artificial signs other than the nature of a disease. Future studies are warranted to investigate the application of this method in assessing ocular hyperaemia caused by diverse factors.

This study introduced a new method that directly calculated the parameters of ocular surface vessels around the limbus to assess the degree of regional bulbar redness. The method can quantify vascular changes of the ocular surface in four orientations, enabling the evaluation of the whole situation of a congested eye in different ROIs and the distinction of scleral and conjunctival vessels. It offers advantages for objective observation of ocular surface redness, especially for the follow-up of disease treatment in individual patients.

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