

# Evaluation of regional bulbar redness using an image-based objective method

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## Abstract

• **AIM:** To develop an image-based objective method to precisely evaluate regional ocular bulbar injection.

• **METHODS:** Six healthy adult volunteers were photographed in four orientations (superior, inferior, nasal and temporal sides) with and without stimulating eye drops. Six line segments (covering 30°) were drawn 4mm away from the limbus on each image using ImageJ software. The graph peaks, which were derived from the areas under the line segments and corresponded to the cross-sectional grey-level of the vessels, were analyzed to obtain peak area, peak height/width (PH/PW), and peak numbers. Different-sized areas were selected to calculate the pixels based on the edge-detection algorithm. Also, conjunctival and superficial scleral vessels were analyzed separately.

• **RESULTS:** This method had a smaller coefficient of variation, especially for PH/PW, in all four orientations. Hyperaemia parameters changed the least after challenging in the superior region. Moreover, 95% of the PH/PW ratios were greater than 0.87 in conjunctival vessels and less than 1.00 in superficial scleral vessels. PH/PW significantly increased in conjunctival vessels and changed less in superficial scleral vessels.

• **CONCLUSION:** A new method of objectively assessing bulbar injection based on ocular surface images was developed. This method can be used to quantify ocular regional injection and to distinguish the superficial scleral and conjunctival vessels.

• **KEYWORDS:** ocular surface; hyperaemia; image analysis; ImageJ

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## INTRODUCTION

Bulbar redness is a common sign of ophthalmic diseases in the clinic, such as conjunctivitis, ocular xerosis, glaucoma, scleritis and keratitis, which can cause vessel hyperaemia in conjunctivae and sclera, leading to eye redness. The assessment of the ocular surface redness is a routine procedure in clinical practice. The most commonly used method is subjective observation and description. Given the influence of external and internal factors, the results of such evaluation are often unreliable, varying from doctor to doctor. In recent years, some objective and subjective methods have been reported for the assessment of bulbar redness. Although these methods improved inter- and intra-observer reproducibility, they still need substantial improvement.

Assessment of bulbar redness encounters significant difficulties due to the wide variation of repeat assessments between observers or for the same observer on different occasions<sup>[1-3]</sup>. For example, Fieguth and Simpson<sup>[2]</sup> examined the relationship between physical image characteristics and the clinical grading of images of conjunctival redness and found that clinical grading of redness images was highly variable. Whether grading through *in vivo* observation or conjunctival photographs, using psychophysical or physical attributes or both, variable outcomes and lower reproducibility have often been observed<sup>[3-6]</sup>. A general approach for evaluating bulbar hyperaemia is needed in clinical practice. Thus, objective, highly reproducible and fully automated methods of analysis would clearly be of great benefit to many medical staff and patients.

Image analysis has been used to evaluate bulbar redness in previous studies. In 1987, the first photographic scale for the assessment of ocular responses to contact lens use was introduced by McMonnies and Chapman-Davies<sup>[7]</sup>. Several image analysis methods have been introduced to objectively evaluate bulbar hyperaemia, which include separating the image sets (colour, greyscale, and binarised) or the image channels (red, green, and blue) to calculate physical attributes (chromaticity, fractal dimension, pixel count or percentage of pixel coverage, and/or vessel parameters)<sup>[2,5,8]</sup>. Some of these studies detected very small changes (*e.g.* vessel hyperaemia) in the region of interest (ROI). However, few of them evaluated the regional changes in hyperaemia that may occur in different ocular diseases, since most of these studies were

conducted on contact lens wearers [1,3,7-11]. In many eye diseases, the ocular hyperaemia might be unevenly displayed on the ocular surface. The evaluation of bulbar redness requires that information about the congestive region, the degree of congestion and its changes over time be described objectively, automatically and simply.

Generally, bulbar redness includes ciliary injection that involves branches of the anterior ciliary vessels and conjunctival injection that mainly affects the posterior conjunctival blood vessels. The anterior conjunctival vessels, deriving from the branches of anterior ciliary vessels, and the posterior conjunctival vessels communicate each other and form a vascular network around cornea [12]. To differentiate the vascular changes in this network region can facilitate the clinical diagnosis of red eye.

In this investigation, we attempted to establish an objective method of quantifying bulbar redness by analyzing digital images to identify surrounding ocular surface vessels in four zones around the cornea and directly calculate the vessel parameters to assess the degree of hyperaemia.

### 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.

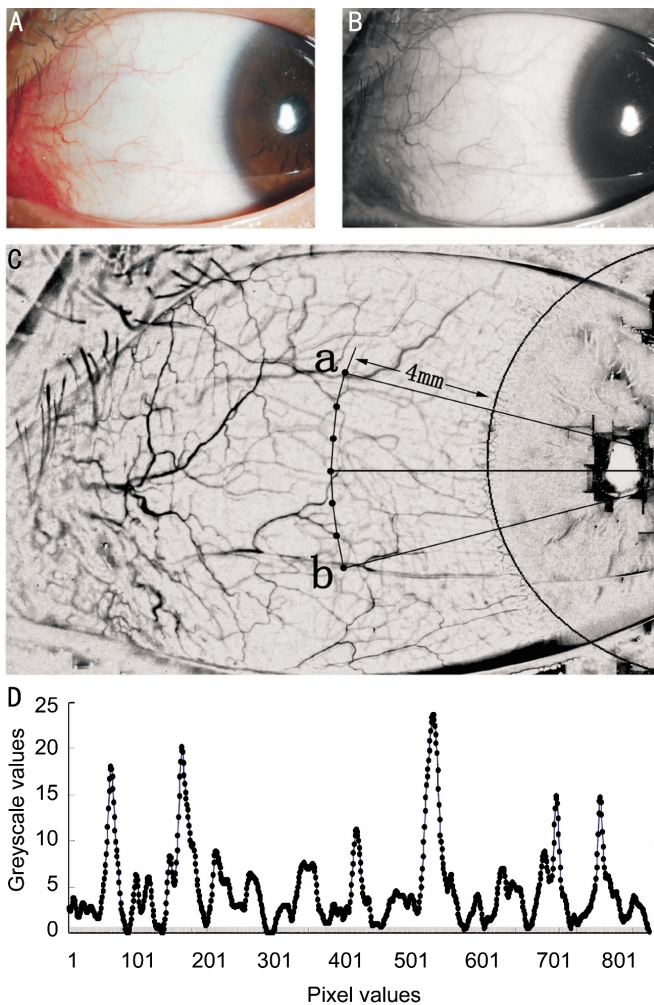
The photographs were taken by a TOPCON SL-D7 slit lamp connected with a Nikon D200 camera. The eye drops, named Jinshile Eye drops (Approval Number Z20053110), manufactured by Changchun Three-nine Bio-pharmaceutical Company and had some efficacies for myopia in children. Because of the main component 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 2 896×1 944 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<sup>[5]</sup>, which was confirmed in our analysis (data not shown). Then the outcome images (2 896×1 944 pixels) were modified in greyscale ranging from 0 to 255 to perform further analysis. To enhance the conjunctival vascular image, the background of the pictures was subtracted [13]. By comparison with a ruler photographed by the same camera, we determined that one pixel was equivalent to 6.11μm.

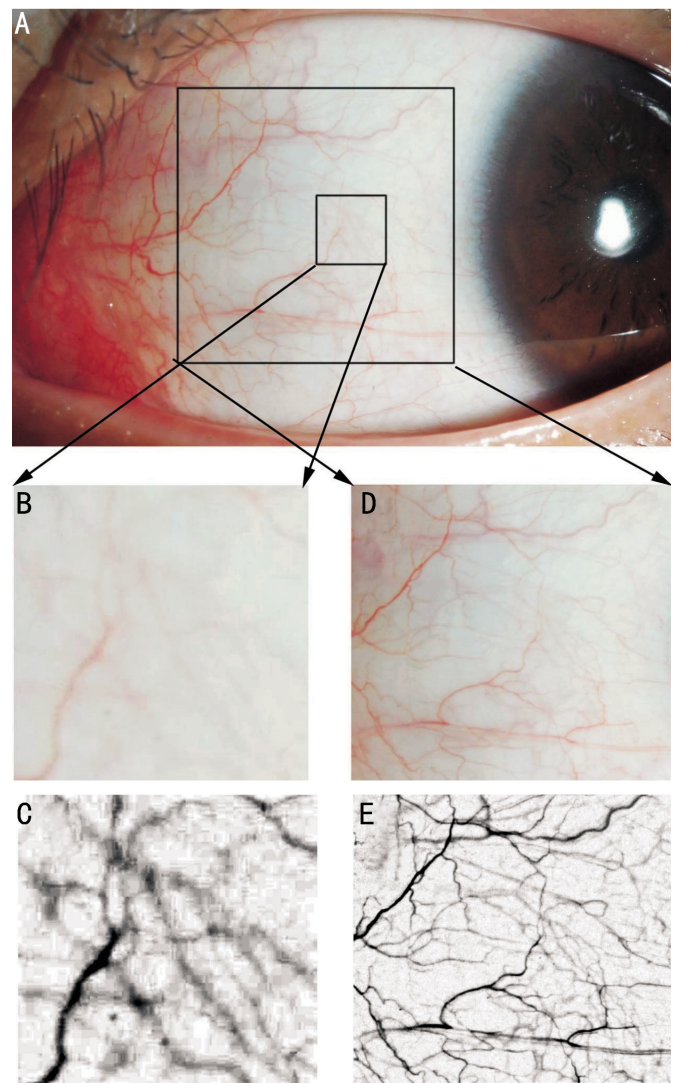
The horizontal diameter of the cornea was set to 11.75mm, and vertical was set 10.75mm, which can convert to 1 923 and 1 759 pixels, respectively. Then, a circle with diameters at 1 923 (superior and inferior photographs) or 1 759 pixels (nasal and temporal photographs) was drawn on the picture. Next, the circle was moved to match the limbus, and the calculated centre of the circle was used to locate the centre of the cornea. Based on the cornea centre on each photograph, seven points, with 5-degree intervals and 4mm away from the limbus, 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 line segments 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 pixels of vascular sections that were used for the evaluation of bulbar 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 descending 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  $|x_i - \bar{x}| > 3\delta$ , 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  $\delta$  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 (2 896×1 944 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.

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 600×600 pixels (Figure 2) and one of 1 200×1 200 pixels in the horizontal direction or a rectangle of 1 600×900 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.



**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: 600×600 pixels; D: 1 200×1 200 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).

**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 <sup>b</sup>	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

<sup>b</sup>ANOVA tests:  $P < 0.01$ .

**Table 2 The calculation 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 <sup>a</sup>	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 <sup>b</sup>	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 <sup>b</sup>	10.28±2.81	0.27	7.99±2.65	0.33	13.31±3.61	0.27	12.73±5.06	0.40

<sup>a</sup>PA: Peak area; PH: Peak height; PW: Peak width; S. pixel count: Small area pixel count; L. pixel count: Large area pixel count; <sup>b</sup>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<sup>[7,16]</sup>. 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 from photographs taken before and 10 or 20min after eye drop challenge were calculated to show the hyperaemia in the four orientations of the conjunctiva (Figure 3). At baseline, the PH/PW value of nasal conjunctiva was significantly higher than the other three orientations ( $P < 0.01$ ), and the differences among the other three directions were not statistically significant. Ten minutes after application of eye

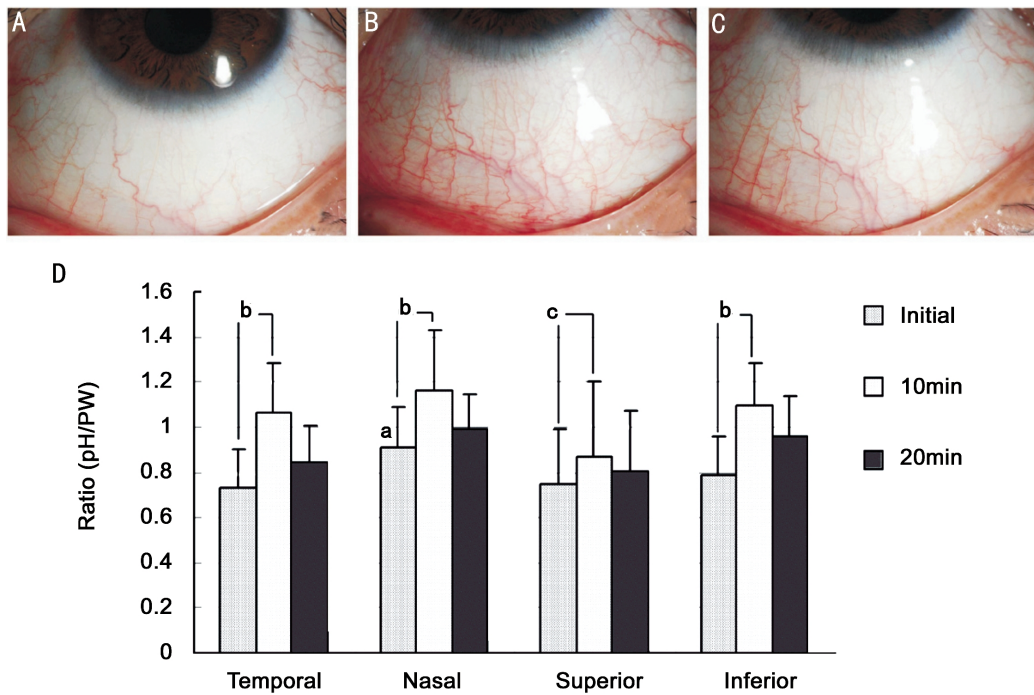
drops, PH/PW increased in all four orientations ( $P < 0.01$ ), with a weaker and slower change in superior conjunctiva ( $P < 0.01$ ). Twenty minutes after eye drops, the PH/PW of all four zones of the bulbar conjunctiva was lower than that at 10min ( $P < 0.05$ ). Vessel numbers mildly increased after challenge, though without significant differences ( $P > 0.05$ ).

**Difference Between Conjunctival and Superficial Scleral Vessels**

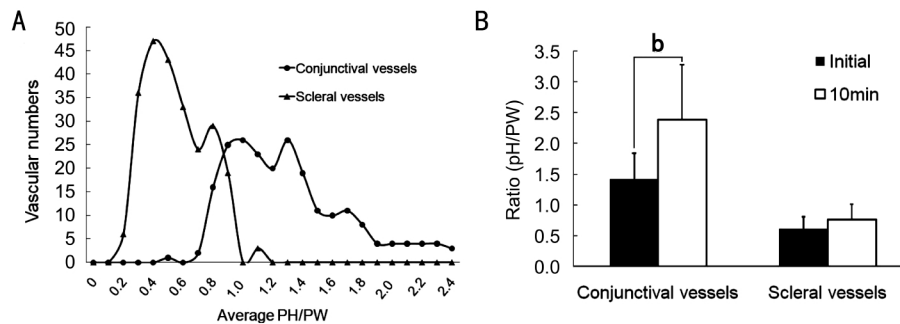
As there are different areas between conjunctival and superficial scleral vessels, we examined 5 conjunctival vessels and 5 scleral vessels in each of the four orientations under slit-lamp biomicroscopy, and PH/PW in photographs was calculated. The results revealed significant differences between conjunctival 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* <sup>[5]</sup> 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 ( $^a P < 0.05$ , ANOVA test). Tenmin after challenge, the PH/PW ratio of all four zones was increased significantly (superior  $^c P < 0.05$ ; other zones  $^b P < 0.01$ , 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 ( $^b P < 0.01$ ).

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 (*i.e.* 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<sup>[5]</sup>. 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 (*e.g.* 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<sup>[8]</sup> 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<sup>[14]</sup>. In the current study, blood vessel diameter was between 18.33 and 226.07 $\mu\text{m}$ , with a mean value of 70.27 $\mu\text{m}$ . This is consistent with Guillon's results (diameter between 53.3 and 57.8 $\mu\text{m}$ ) but is larger than Shahidis' reported vessel width (between 8.7 and 24.3 $\mu\text{m}$ , with a mean value of 15.5 $\mu\text{m}$ )<sup>[8,15]</sup>.

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<sup>[20]</sup>. 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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