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APPLICATION OF TWYMAN–GREEN INTERFEROMETER FOR EVALUATION OF *IN VIVO* BREAKUP CHARACTERISTIC OF THE HUMAN TEAR FILM

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ABSTRACT

The paper presents an interferometric method of assessing the *in vivo* stability of the precorneal tear film. To observe dynamic effects on a human cornea the Twyman–Green interferometer with television frame speed digital registration synchronized with a laser flash was used. The instrument was applied to the human cornea *in vivo*. The results of the experiment, both tear film distribution and its dynamics, are presented. The proposed interferometric setup can be used to evaluate the breakup characteristics of the tear film, its distribution, and to examine its dynamic changes. The breakup profiles and their cross sections calculated from the interferogram analysis are presented. The depth of recorded breakup, calculated on the basis of interferogram analysis, amounts to about 1.5 μm. The proposed method has the advantage of being noncontact and applies only a low-energy laser beam to the eye. This provides noninvasive viewing of human cornea *in vivo* and makes it possible to observe the kinetics of its tear film deterioration. © *1999 Society of Photo-Optical Instrumentation Engineers.* [S1083-3668(99)01601-9]

Keywords Twyman–Green interferometer; tear film breakup; tear film stability; contact lens.

1 INTRODUCTION

The main optical role of the tear film is to provide the cornea with a smooth optical surface. According to Lambert[1] the tear film contains three layers (lipids, aqueous component, and mucous) evenly spread over the corneal epithelium. The thickness of the tear film and its layers may be investigated by both invasive and noninvasive methods. The last mentioned are usually interferometric ones. Although the tear film thickness has been investigated intensively, there is still considerable uncertainty and controversy about it.[2]

The stability of the tear film is a complex function of these three components and the hydrophobic epithelial surface.[3,4] The stability of this tear structure is significant for normal wearing and functionality of the contact lens.[5–8] A well-fitted contact lens rests on a continuous tear film. It is also coated with a continuous tear film on the exterior surface of the lens. For comfortable wear and stable visual acuity it is necessary to achieve the prelens tear film exactly alike to the preocular tear film, both in structure and relative thickness.[9]

There are several methods of *in vivo* measurements of stability of the tear film during the interblink period. One such method, commonly used in clinical practice, is performed by placing a drop of fluorescein into the tear film. The observation of the fluorescing agent by use of a slit lamp with appropriate wavelengths helps to examine the integrity and thickness of the film. Although this technique is widely used, undesirable effects, such as altering the wetting and breakup characteristics of the tear film can occur.[10,11]

Another technique, based on a device utilizing the reflection of a grid pattern from the cornea to aid the observation of tear film breakup, was proposed by Mengher et al.[12] and modified by Cho.[13] A quick method for measuring the evaporation of tears from the ocular surface and evaluating tear dynamics and subclassification of dry eyes was reported by Tsubota and Yamada.[14]

Recent development in ophthalmologic measurement, based on interferometry and electronic imaging, creates an opportunity for a new type of diagnostic method. A special interferometric method, which uses light of low coherence length and the Doppler principle introduced by Fercher et al.,[15] allows us to measure intraocular distances along the visual axis of the human eye *in vivo*.

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Doane[16] proposed a noninvasive technique that permits a simultaneous examination of a large surface area of the tear film. This technique is based on thin-film interferometry (TFI) and allows recording of tear layer distribution and dynamic thinning processes during interblink periods. In Doane's setup for TFI, a dark spot was present in the middle of the interferometric images due to the illumination system. The spot made the observation of the central part of the corneal surface impossible. Doane applied a white light interferometry, used in thin-film measurements. This formed fringes caused by interference of the two wave fronts: one reflected from the outer surface of the tear film and the other reflected from the border between the tears and the corneal epithelium. As a consequence of the considerable differences of refractive indices on both borders (air–tear film, tear film–epithelium), the intensities of both reflected waves differ significantly and results in a relatively low fringe contrast.

A new approach for evaluation of the tear film stability on the human eye is reported in Refs. 17 and 18. The tear film distribution on the cornea is measured by the lateral shearing interference technique. Continuous recording and viewing of interferograms enables registration of the changes in disturbances of interference fringes during elapsed time. A fast Fourier transform (FFT) is applied to consecutive interferogram assessment of the tear film breakup time. Bigger fringe disturbances result in wider Fourier spectra. The noninvasive tear breakup time can be evaluated by comparing the value of the second momentum of the Fourier spectra calculated from consecutive interferograms.

Recently, interferometric methods of measuring the tear film thickness were proposed.[19,20] The thickness of different layers of the tear film has been measured by these methods and the particular advantages and limitations of each method are discussed. The thickness of the aqueous layer measured by the method presented in Ref. 19 had about 3 μm.

The Twyman–Green interferometer (TGI) can also be helpful as an instrument for noncontact measuring methods. It allows the simultaneous examination of fine corneal topography, its irregularities, and the dynamic breakup characteristics of the tear film with high accuracy.[21,22]

Rottenkolber and Podbielska[23] proposed a new technique for measuring ophthalmologic surfaces such as aspheric contact lenses and the human cornea by means of moiré deflectometry. Although the proposed method offered accuracy comparable to interferometry, the interpretation of the fringe patterns obtained is more difficult.

This paper shows the possibility of the use of interferometry for the observation of the state of the tear film. Breakup characteristics like the profile, depth, width, and wideness can be measured by analysis of the interferogram obtained from the TGI. On the other hand, long sequences of recorded interferograms can be used for evaluation of the dynamic changes in the tear film, which helps to evaluate the noninvasive tear breakup time. Interferometry makes it possible to perform measurements with high accuracy. In the presented case, the accuracy is higher than the wavelength of the light used for experiment, that is 633 nm. Currently in use are techniques based on the reflection of the grid pattern from the cornea, which gives accuracy much less than 10 μm.

The profile of the breakup is an important parameter that has great influence on the acuity of vision, which has been proved by the analytical model study presented in Ref. 24. The phase of a wave front passing by a disrupted tear film is different from the wave front passing by a smooth tear cover. Usually, the point-spread function of the disrupted tear film is wider and the modulation transfer function shows a worse transfer of the middle range of the spatial frequencies.

2 EXPERIMENT

This paper gives examples of the sequence of interferograms obtained from the setup presented in Figure 1. It also provides precise and detail information about the topography of the tear film overlaying the cornea. In order to obtain interference fringes of high contrast in a two-beam interferometer with variable optical path difference, certain conditions have to be fulfilled. First of all, a spatial and temporal coherent light source, for example a laser, has to be used for illumination. The quick movement of the eye globe and the tear flow require a short exposure time. Moreover, the Twyman–Green interferometer is extremely sensitive to the position of the eye globe; slight movements of the subject's head along the optical axis change the form and density of the interference pattern. A specially designed head holder (not shown in Figure 1) had to be used to immobilize the subject's head. The examined eye remained free during the experiment and some effort had to be taken to keep it fixed. Earlier the subject was trained to fixate, which helped to obtain stable interference images.

A 3 mW HeNe laser at a 632.8 nm wavelength was used as the light source. An electromechanical shutter synchronized via a shutter driver with a charge-coupled device (CCD) camera chopped the laser beam at the frequency of 25 Hz. The vertical blank signal from the CCD camera was separated in a frame grabber card (Matrox Pulsar) (FGC) installed in the PC. Then, the signal was sent to the shutter driver. This connection ensured laser illumination at the beginning of each camera frame. The shutter remained open for 1 ms (exposure time), which enabled us to take stable images and limit the light energy absorbed by the eye. Neutral den-

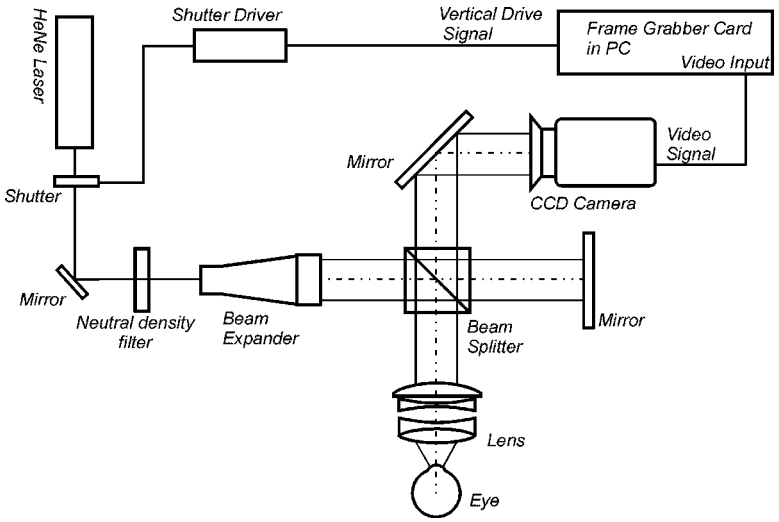

Fig. 1 Diagram of experimental setup for recording interferograms of the cornea surface.

sity filters varied the beam intensity at the input to the beam expander. To remove irregular variations in intensity across the beam, a spatial filter was applied.

A collimating lens for illumination of the cornea is one of the most important optical elements of this interferometer. Pentacon objective with a focal number $f=50/1.8$ was selected from a number of photography lenses. The minimal axial wave aberration and F numbers of the lens were taken into consideration. In our method, a collimating lens has two important functions: first, it illuminates the cornea; second, it flattens the spherical wave front reflected from the cornea (Figure 2). In order to obtain a quasiplane wave front of the object beam, after this wave front is reflected by the cornea and passes back through the collimating lens, it is necessary to keep a proper distance between the collimating lens and the cornea. It means that the collimated spherical wave front falls on the cornea in such a way that the focus of the wave front coincides with the center of the central corneal curvature. Because interferometry indicates only relative optical path changes, the eye–objective position can be found by searching for a minimum number of interference fringes in the continuously recorded interferograms (Figure 3). As indicated in Figure 3, the minimum number of fringes are observed when parameter δs is the smallest (middle position of the cornea in Figure 3). When the cornea is in the position marked II in Figure 3, the radius of the central corneal curvature and the focal point are coincident. Positions of the cornea marked I and III introduce fringes caused by defocus. The operator observes the recorded images on an analogue monitor and adjusts eye–objective distance by searching for the minimum density of fringes in the interferograms.

Kowalik et al.[25] described how the translation and tilt of the eye affect the fringe pattern and topography evaluation. They also estimate the influence of aberrations of the collimating lens on the measured wave front.

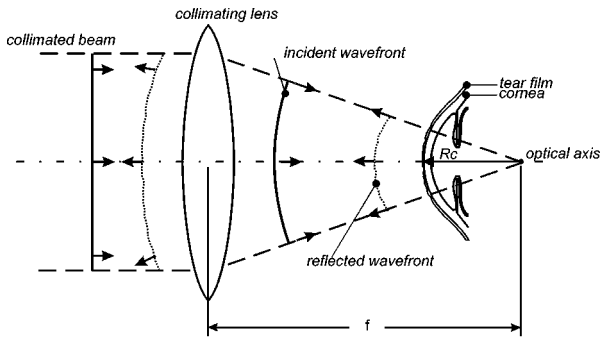

Fig. 2 The propagation of the wave fronts through the collimating lens.

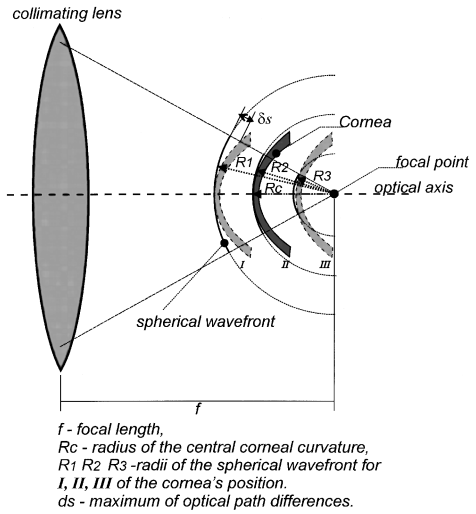

Fig. 3 The positions of the cornea (I, II, III) vs collimating lens and its influence to introduced optical path differences δs.

The diameter of the examined area of the corneal surface depends directly on the aperture of the lens, that is, the greater the F number of the lens, the smaller the observed region of the cornea. In our setup the central area of the cornea with a diameter of about 4.5 mm can be measured.

The CCD camera with the TV frame speed registered the interferograms. A VHS video recorder was connected to the camera for dynamic registration of the sequences of interferograms that were simultaneously displayed on an analogue monitor. The recorder was connected to the computer via a FGC. Long sessions of approximately 10 min of continuous recording was performed. Then, the selected interferograms were captured by the FG for further analysis.

3 EVALUATION OF THE INTERFEROGRAMS

Recording sessions of the dynamic changes of the tear film during long opening of the eye were performed on the volunteers. The interferograms were recorded for further analysis on a VHS videotape. During this recording, blinking has to be prevented and the eye has to be kept in the same position. The four interferograms presented in Figure 4 were selected from an approximately 15 s sequence of these recordings. The time interval between the presented interferograms is approximately 200 ms. All of them present the development of breakup on the healthy eye. Blinking was prevented for approximately 15 s to cause evaporation of the tear film, which led eventually to the breakup. Usually, the interference fringes become disturbed on the whole observed region before breakup appears. The shape of presented breakup can be found in one from hundreds of cases. Smooth and continuous fringes that surround the breakup enabled calculation of its depth.

Experimentally collected fringe images had different contrast depending on the experiment parameters. To improve the original data prior to fringe analysis, image preprocessing procedures had been used. OPTIMAS[26] is a powerful software program for image processing and further fringe analysis. All steps described below were carried out by use of this program.

The FGC digitized the chosen interferograms directly from the VHS video recorder. An area of 256×256 pixels that covers the breakup was selected from the interferograms. To remove the high-frequency interference fringe pattern overlying the investigated one, fast Fourier transform based filtering was applied. Then, 3×3 kernel median filtering[27] was used to reduce the pepper-and-salt noise. Finally, brightness and contrast corrections based on the histogram of the image improved its quality (Figure 5).

A procedure of tracing the fringe center was then applied to the enhanced interferograms. First, a line perpendicular to the fringes is drawn manually.

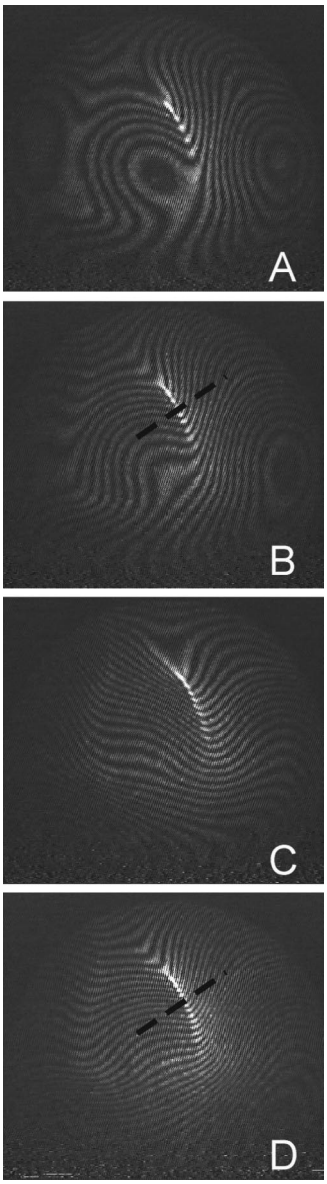

Fig. 4 (A)–(D) Four interferograms of the cornea covered by the tear film (recorded with an interval of 200 ms) present the process of tear film breakup dynamics.

Then, the minima of intensity along the line are found, which correspond to the dark fringes [Figure 6(a)]. The number of fringes has to be equal to the number of detected minima. Then, the minimum intensity is traced along each fringe. The results of the tracing are shown in Figure 6(b). Detected centers of the dark fringes are automatically ordered [Figure 6(c)]. One fringe order corresponds to the 633 nm optical path difference (the 633 nm HeNe laser used for experiment). The differences in height between neighboring fringes are $\lambda/2$ because the light passes the distance between lens and eye twice. On this basis, the three-dimensional (3D) surface plot was performed. Figures 7(a) and 7(b) present the 3D views of the breakups calculated from the interferograms in Figures 4(A) and 4(D).

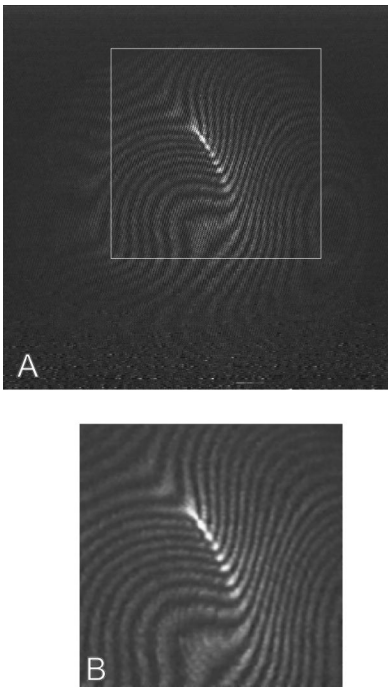

Fig. 5 The interferogram from Figure 4(B) before image enhancement (A). The area of 256×256 pixels selected for further processing is visible. Below the same area after image enhancement (B).

The power of the laser light that reached the cornea in the object beam of the interferometer was about 0.4 mW. If we take into account that the diameter of the illuminated area on the corneal surface was about 4.5 mm, the power density on the corneal surface amounted to about 20 W/m^2. Thus, the laser light illuminating the cornea during the single pulse had an energy of $25 \times 0.001 = 0.025$ J/m^2. 25 Hz repetition of laser pulses was used and illumination time of the cornea within 1 s of measurement was 0.025 s. The longest measurement we made lasted about 30 s, so the total illumination time then was $30 \times 0.025 = 0.75$ s and the total illumination energy amounted to 19 J/m^2. The expanded laser beam in front of the cornea formed a space angle that can be calculated as $\pi r^2 / R^2$ (r, the radius of the illuminated area on the corneal surface; R, the radius of the corneal curvature) and was equal to 0.3 sr. Finally, the total energy density of the expanded laser beam illuminating the cornea during one measurement reached the maximum value of about 63 J/m^2 sr. This is below the allowed value for this wavelength (633 nm) and this exposure time.

4 OBSERVATIONS AND DISCUSSIONS

Figures 7(a) and 7(b) present a three-dimensional view of the observed tear breakup, calculated by the interferogram analysis from the second and the last frame given in Figures 4(B) and 4(D). Interferogram analysis showed that the increase in the number of fringes on consecutive pictures was caused

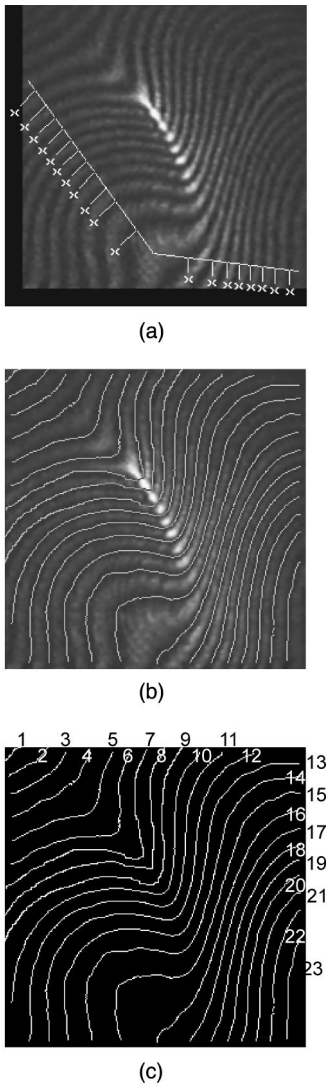

Fig. 6 Consecutive steps of interferogram analysis. Finding the fringe centers (a); fringe tracing (b); and fringe ordering (c).

by the eye tilt. The two cross sections of the breakup along the line indicated by the dark lines in the Figures 4(B) and 4(D) are shown in Figure 8. The depth of rupture did not change in the elapsed time (400 ms).

The characteristic profile of these cross sections can be observed on each interferogram of this sequence. The depth of the breakup presented on the cross sections stays the same and amounts to about 1.5 μm. However, its width and length has changed during the elapsed time (400 ms). Since the normal tear film is reported to have a thickness of approximately 7 μm, there is no evidence in this case that can help to indicate that 1.5 μm is the partial breakup or breakup of reduced thickness of the tear film. On the other hand, the tear film thickness over the cornea measured by the recently introduced method is based on the interferometry averaged 3 μm.[18] It is evident for anyone who observed the breakup process that the formation of a single

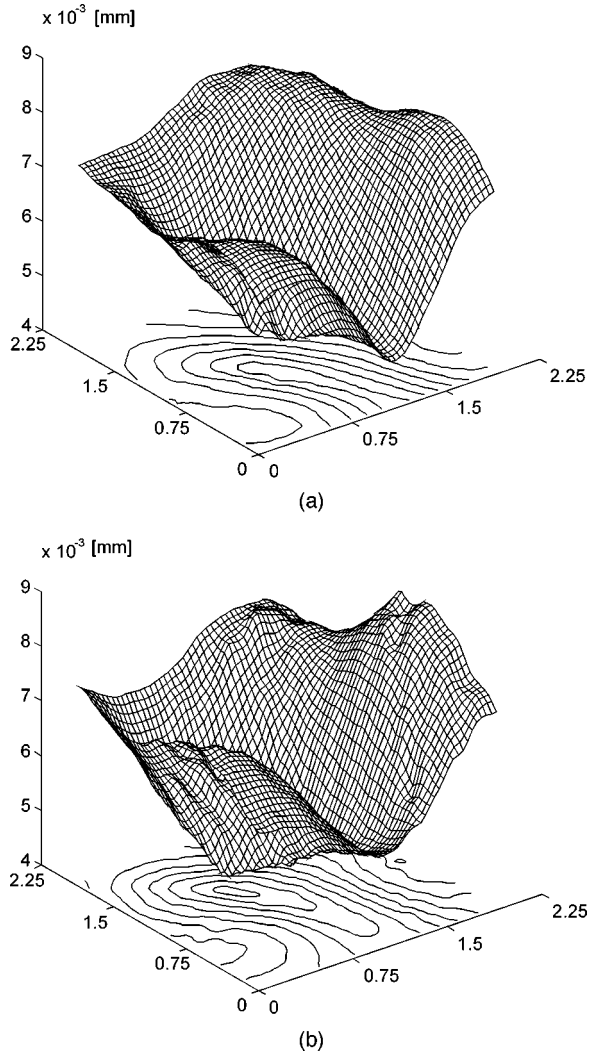

Fig. 7 (a), (b). The 3D presentations of the tear film breakup calculated by interferograms analyses, which are given in Figures 4(B) and 4(D).

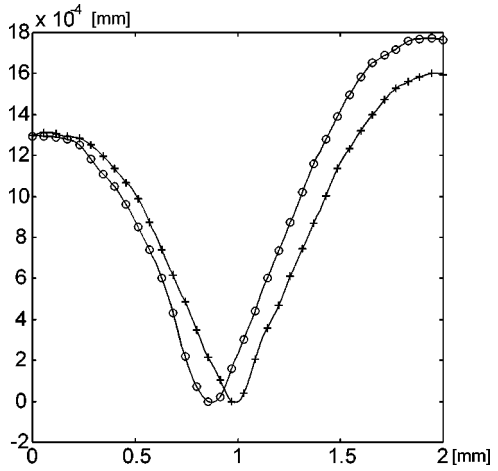

Fig. 8 The cross sections of breakups calculated on the basis of the interferograms presented on Figures 4(B) and 4(D) and made along the black lines indicated on them. Solid line with the ''+'' measuring point mark presents the cross section of the breakup, which corresponds to Figure 4(B), and the ''○'' measuring point mark corresponds to Figure 4(D).

breakup is immediate. The presented breakup formed after approximately 15 s and was observed for the next few seconds. It is more probable that the thickness of the tear film decreased to 1.5 μm due to evaporation from the thickness of 3 μm rather than from that of 7 μm.

5 CONCLUSIONS

The presented interferometric setup, with fast recording of interferograms, enables us to obtain a sequence of images that show the dynamic processes on the corneal surface, like tear film breakup. Apart from the high accuracy of measurements of the corneal topography and its irregularities, interferometry also allows the observation of dynamic processes of the tear film deterioration. All measurements were implemented with natural tear fluid and under the dynamic conditions that exist in the eye. The analysis of interference fringe patterns allows us to find the fine topography of the tear film breakup, which has great influence on the vision. Analysis of the cross sections of the breakup could be used to determine the analytical model of the function, which describes the breakup's profile. Analysis of the interferograms allows calculating the depth of the breakup, which amounts to about 1.5 μm. The proposed method gives possibilities of precise quantitative diagnostics of the state of the tear film.

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