

# Direct observation and validation of fluorescein tear film break-up patterns by using a dual thermal-fluorescent imaging system

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**Abstract:** The fluorescein tear film break-up test is a common tear film stability test for dry eye diagnosis. This test requires applying fluorescein sodium drops to a tear film to observe the tear film break-up. However, this test is limited by using the fluorescein sodium drops, which can induce reflex tearing and reduce the reliability of the diagnosis results. This paper proposes that tear film evaporation accelerates on the fluorescein tear film break-up area (FTBA), resulting in a lower temperature area (LTA) on the tear film. A dual modality system was established to capture the thermal and fluorescent image of fluorescein-stain tear films for 48 participants. Observations showed that the LTA and FTBA were highly correlated in their location ( $r = 0.82$ ) and size ( $r = 0.91$ ). This is first study to show that the FTBA and LTA are essentially the same region. This study demonstrated the feasibility of using the noncontact thermograph method to evaluate tear film stability without using a fluorescein sodium drop.

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**OCIS codes:** (110.6820) Thermal imaging; (170.3880) Medical and biological imaging; (170.4470) Ophthalmology.

## References and links

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## 1. Introduction

Dry eye syndrome is one of the most common eye diseases affecting functional visual acuity [1] and reducing the quality of a patient's life [2]. Dry eye syndrome is caused by a poor quality tear film and inflammation of the eyelid [3] resulting from a lower tear production rate and / or a short tear film stable time. The ocular surface becomes dry and lacks lubrication, eventually damaging the ocular surface [4].

The fluorescein tear film break-up time (FTBUT) test is one of the most common tests for dry eye syndrome. This test evaluates the tear film stability. In clinics, one of the main measures for determining dry eye is defined as a FTBUT shorter than 5 s. The FTBUT test first involves application of fluorescein sodium drops to the ocular surface and subsequent measurement of the time required for the first random dark spot to appear.

The dark spot is an area of increased concentration of fluorescein caused by tear film evaporation. This area shows a greater reduction in fluorescent intensity because of self-quenching at high concentration, and appears as a dark color under cobalt light excitation [5–7]. The dark area is the fluorescein tear film break-up area (FTBA). The FTBA is commonly caused by a poor quality tear film, and is a sign of an unstable tear film structure.

The FTBUT test is a well-established test for evaluating dry eye syndrome [8, 9]; however, it has several limitations. The test requires instilling fluorescein sodium drops, which is not a fully noncontact procedure. The other problem relates to the standardized fluorescein sodium concentration. Instilling liquid fluorescein sodium drops changes the total tear amount, which can affect the actual tear film break-up time [10]. The fluorescein sodium drops can also cause reflex tear secretion, which might not be acceptable for all patients because of fluorescein allergies. These drawbacks reduce the repeatability and the accuracy of the FTBUT [11]. A fully noninvasive measurement method for tear film break-up is required.

Mapstone has initiated a noncontact measurement of the ocular surface using thermography [12] and this approach has been effectively used to determine the temperature of the ocular surface. Morgan used thermography to measure dry eye temperature [13]. Numerous researchers have recently used ocular surface thermography to estimate the stability of the ocular surface [14–16]. A significant correlation exists between FTBUT (tear film stability) and decreased ocular surface temperature [17]. The ocular surface temperature is irregularly distributed in the dry eye, compared with the normal eye [18]. An unstable tear film is typically associated with rapid tear film evaporation [19], and therefore, the ocular surface temperature decreases could be related to tear film break-up.

The lower temperature area (LTA) is the cool region on the ocular surface caused by tear film evaporation. LTA might be associated with the unstable region of the tear film or the tear film break-up area, but evidence for this explanation is limited. This paper proposes that the evaporation typically causes tear film breakup, resulting in the LTA on the tear film. Therefore, a dual modality noninvasive tear-imaging system was developed to assess objectively the characteristics of the tear film in real time. This dual modality system simultaneously observes fluorescein tear film break-up by using thermographic and fluorescent cameras to identify the relationship between the LTA and FTBA.

## 2. Method

All procedures in this study were conducted according to the tenets of the Declaration of Helsinki of the World Medical Association, and approval was received from the Institutional Review Board of Far Eastern Memorial Hospital, Taiwan. Informed consent was obtained from each participant after explaining the research purpose and procedure.

This study consisted of 48 participants, including 27 women and 21 men, with a mean age of  $41.8 \pm 12.6$  years (range, 21–61). Participants with signs of ocular surface abnormalities, previous ocular surface surgery, and fluorescein allergies were excluded from this study. None of the participants received any eye drop instillations for at least 6 hours before the measurement. All the examinations were performed between 10 a.m. and 4 p.m. in the same room, at a stable temperature and humidity, with no air drifts.

### 2.1 Measurement procedure

A standard procedure for the fluorescein stain of FTBUT test was applied in this study. A 2-microliter volume preservative-free solution consisting of 2% fluorescent dye was applied to the bulbar conjunctiva by using a micropipette. Blinking several times allowed the fluorescein sodium to cover the ocular surface area uniformly. A dual thermograph and fluorescein camera measurement system was aligned with the eye, and the thermal and fluorescein images were subsequently recorded. The participants were instructed to close their eyes for 9 s, blink their eyes, and open their eyes for 6 s during the recording. The tear film was measured for 6 s after a complete blink, using a short measurement time to avoid reflex tearing.

### 2.2 Equipment

A dual modality system (Fig. 1) was established to observe the fluorescein tear film break-up, which included a custom made thermograph (Fig. 1(A)) and a conventional fluorescent camera. (Fig. 1(B)) The dual modality system simultaneously observed the dynamics of the thermal and fluorescent characteristics of the fluorescent-stain ocular surface (Fig. 1(C)).

Ocular surface thermography (IT-85, United Integrated Services Co., Taiwan) was used to capture the thermogram; this system was designed to capture the ocular surface thermogram. The system recorded 30 frames per second at a resolution of 320 (H) x 240 (V) pixels. The difference in noise equivalent temperature was  $0.07\text{ }^{\circ}\text{C}$  and the Germanium lens transmitted an infrared spectrum of 8 to  $12\text{ }\mu\text{m}$ .

A fluorescent camera (PowerShot G12, Canon Inc., Japan) with a cobalt light filter (500 long-pass filter) was attached to the side of the thermograph. Cobalt light excited the fluorescein-stain tear film. A Germanium filter transmitted the infrared light to the thermography camera and reflected the fluorescence to the fluorescent camera. The camera system adjusted the images by correcting the position of the fluorescent camera and the Germanium filter. The system measured a standard target for calculating the pixel length to the millimeter, after adjusting the thermal and fluorescent images in the same unit.

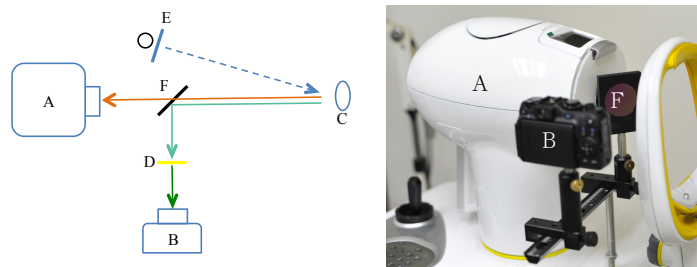


Fig. 1. Dual modality measurement system was established to capture the thermal image and fluorescent image of fluorescein-stain tear films. A. Thermography camera. B. Fluorescent camera. C. Fluorescein-stain tear film. D. Long-pass filter (500 nm). E. Cobalt light source. F. Germanium filter.

### 2.3 Image processing

Image processing enhanced the LTA of the thermographic image and the FTBA of the fluorescein image. The image processing steps are shown in Fig. 2. The initial fluorescent and thermal images were obtained immediately after blinking (a, e), and at 5 s after blinking (b, f). The images were subtracted between the initial and 5 s after the blinking for both the fluorescent image (c) and the thermogram (g). The images were transferred to binary images; the black area was the FTBA (d), and the black area was the LTA (h) before the center position of each LTA and FTBA was calculated, respectively.

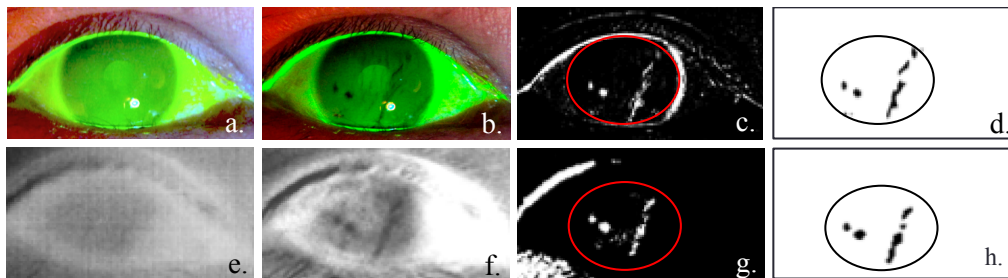


Fig. 2. The image processing steps for enhance the FTBA of the fluorescein images (a-d) and the LTA of the thermograms (e-h). The fluorescent image captured right after blinking (a.). The fluorescent image obtained at 5 s after blinking (b.). The subtraction of (a) and (b) is shown in (c). The binary image of (c) is (d). The same processing steps for the thermograms (e-h).

After the image processing steps, the first appearance of the FTBA and LTA was selected on the images obtained at 5 s after blinking, then the distance between the inner canthus and the FTBA and LTA was measured in the center, in the horizontal-center-distance, in the vertical-center-distance, and in the total-center-distances. The size of the FTBA and LTA was also recorded for further analysis, as shown in Fig. 3.

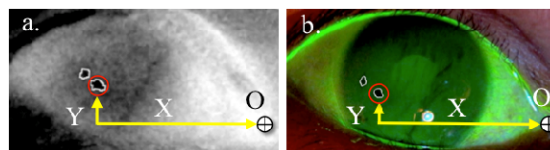


Fig. 3. The red circles indicate the first appearance of the LTA (a.), the FTBA (b.), respectively. O is the position of inner canthus. X and Y are the horizontal-center-distance, vertical-center-distance.

### 2.4 Statistical analysis

The data analysis was performed using SPSS version 20 (IBM, Chicago, IL, USA). Data were presented as the mean  $\pm$  standard deviation. Pearson's correlation coefficient was calculated to test the correlations among the horizontal-center-distance, vertical-center-distance, total-center-distances, and area of LTA and FTBA. The level of statistical significance was set at  $p < 0.001$ .

### 3. Results

The correlations of distances and areas between the LTA and FTBA are presented in Table 1. The position correlations between the LTA and FTBA were 0.82 in the horizontal-center-distance, 0.88 in the vertical-center-distance, 0.84 in the total-center-distances, and the area correlations between LTA and FTBA was 0.91. The scatter plot is shown in Fig. 4.

**Table 1. The correlation relation of the horizontal-center-distance (Horizontal), vertical-center-distance (Vertical), total-center-distances (Distances), and area between the LTA and FTBA (Area).**

	Vertical (mm)	Horizontal (mm)	Distances (mm)	Area (mm <sup>2</sup> )
LTA	9.6 ± 2.9	21.1 ± 5.7	23.4 ± 5.5	22.3 ± 15.4
FTBA	10.4 ± 2.7	20.2 ± 4.8	22.1 ± 4.3	16.6 ± 12.7
<i>r</i>	0.88*	0.82*	0.84*	0.91*

*r* = Pearson's coefficient correlation, \**p* < 0.001.

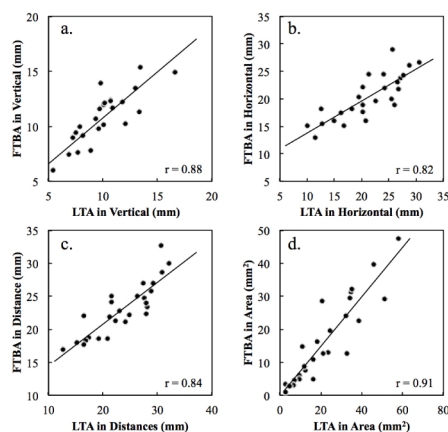


Fig. 4. The scatter plots of the FTBA (fluorescein tear film break-up area) and LTA (lower temperature area), in the vertical-center-distance (a.), in horizontal-center-distance (b.), total-center-distances (c.), and the area (d.).

Figure 5 shows a typical dual modality system measurement result. The dual modality system measured the fluorescent-stain tear film at the same time that the fluorescent camera recorded a series of FTBA images and the thermographic camera recorded the corresponding series LTA images. The FTBA and LTA exhibited relevant development.

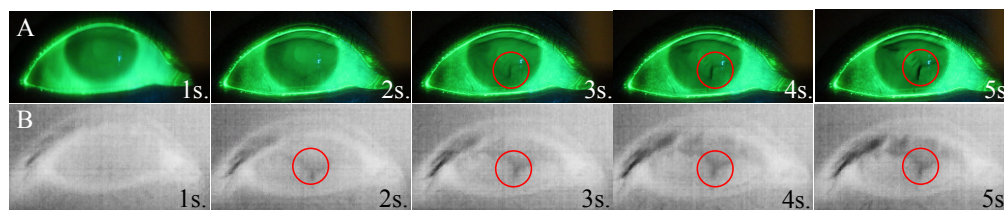


Fig. 5. A fluorescent-stain tear film is measured by the dual modality imaging system. (A) FTBA images (B) LTA images. The time interval between each image is 1 second. The FTBA is fully developed at 3 s; while the LTA can be observed at 2 s. Red circles represent the FTBA and LTA, respectively.

#### 4. Discussion

In this study, a dual modality imaging system was established for observing the dynamics of fluorescein tear film break-up. The system includes thermographic and fluorescent cameras. The dual modality imaging system continually monitors the fluorescein tear film break-up in real time.

The location and area of the LTA and FTBA were highly correlated. The correlation of the center to the inner canthus distance was compared between the LTA and FTBA, which revealed a strong correlation. The result suggested that the LTA and FTBA were matched in

their locations. In addition, the sizes of the LTA and FTBA were strongly correlated, and we also observed that LTA and FTBA both increased in size over time.

In this study we observed that the physical phenomenon of FTBA and LTA was similar although they were measured according to distinct image modalities in this study. The FTBA and LTA were caused by lower tear film stability. In clinics, the FTBUT test is used to test tear film stability; during this test, the tear film evaporates, becomes thinner, and eventually breaks up. During this evaporation and thinning process, the tear film is unstable, leading to tear film break-up which results in an LTA on the tear film. This can explain why the FTBA and LTA were observed in the same region in this study.

The measurement results of the tear film stability when using the fluorescent method and the thermograph method were also compared. In 22 of 48 cases, the FTBUT  $> 5$  s, ( $8.8 \pm 2.9$  s) and exhibited a stable tear film; the LTA did not appear on the tear film until 5 s after blinking. However, in the other 26 cases, the FTBUT  $< 5$  s, ( $2.8 \pm 2.1$  s) and exhibited an unstable tear film and the LTA appeared within 5 s after blinking. The results suggested that the appearance of the LTA is associated with low tear film stability.

This paper proposes that tear film break-up is related to tear film evaporation and results in a LTA. Several of the results supported this hypothesis. First, their locations were strongly correlated to each other. Second, they exhibited similar temporal behaviors and, therefore, their sizes were strongly correlated. Third, they possessed the same physical principle, which was caused by an unstable tear film and tear film evaporation. Fourth, they both exhibited tear film stability; participants with an LTA at 5 s exhibited a fluorescein tear film break-up. These results supported the hypothesis that the LTA is a tear film break-up area.

This study had limitations. The ocular surface thermogram might have been blocked by an eyelash, which might have reduced the observation area of the ocular surface. In addition, the experiment was sensitive to air drifts, heat sources, eye movements, and the camera focus setting. Because of the relatively small sample size in this study, the results should be confirmed in a large clinical trial before they can be used with confidence to evaluate tear film stability.

The traditional measurement of the fluorescein tear film break-up time is frequently conducted for dry eye evaluation, and certain test problems limit its sensitivity and specificity [20]. First, because alterations in tear volume can artificially lengthen TFBUT, instilling fluorescein must be performed with care to avoid inducing reflex tearing. Second, appropriate patient instruction is critical. If patients were not told to blink freely after TFBUT occurs, reflex tearing might occur and skew subsequent measurements. Third, large, uncontrolled volumes of fluorescein might also artificially lengthen TFBUT. Fourth, the test is thus examiner-dependent, with large variability that hampers clinical study. Thermography is a noncontact approach to observing tear film break-up that reduces the chance of reflex tearing by avoiding fluorescein sodium drops. The evaluation of tear film break-up by using thermography shows promise for becoming an established method for dry eye testing.

## 5. Conclusions

The FTBA was demonstrated to be strongly correlated to the LTA in location and size, and also the appearance of the LTA is associated with tear film stability. These results supported that the LTA can be used to evaluate tear film break-up. Thermography can be used to implement a tear film stability test without using fluorescein sodium drops. This approach can alleviate the discomfort and inconvenience of the traditional tear film break-up test.

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