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Mechanisms, imaging and structure of tear film breakup

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Abstract

Tear film breakup (BU) is an important aspect of dry eye disease, as a cause of ocular aberrations, irritation and ocular surface inflammation and disorder. Additionally, measurement of breakup time, BUT, is a common clinical test for dry eye. The current definition of BUT is subjective; here, a more objective concept of “touchdown” – the moment when the lipid layer touches down on the corneal surface - is proposed as an aid to understanding processes in early and late stages of BU development. Models of BU have generally been based on the assumption that a single mechanism is involved. In this review, it is emphasized that BU does not have a single explanation but it is the end result of multiple processes. A three way classification of BU is proposed – “immediate,” “lid-associated,” and “evaporative.” Five different types of imaging systems are described, which have been used to help elucidate the processes involved in BU and BUT; a new method, “high resolution chromaticity images,” is presented. Three directions of tear flow - evaporation, osmotic flow out of the ocular surface, and “tangential flow” along the ocular surface - determine tear film thinning between blinks, leading to BU. Ten factors involved in BU and BUT, both before and after touchdown, are discussed. Future directions of research on BU are proposed.

Keywords

Breakup; Evaporation; Lipid layer; Marangoni flow; Osmolarity; Tear instabilit

1. AIMS AND SCOPE

Breakup (BU) is an important but poorly understood aspect of the tear film and dry eye disorders. The aim of this review is to describe the wide range of imaging methods for studying tear BU and to propose mechanisms and structure for BU that incorporate current understanding of the fluid dynamics involved. Much of the information presented in this review is based on the authors’ experience and thus is descriptive rather than quantitative.

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The following areas are covered in this review. First, the definition, importance and some early theories and studies of BU are discussed. “Touchdown” is suggested to be an important concept in understanding BU, and a new proposal for the structure of a BU area is presented. Second, a new three-way classification of BU is proposed, namely, “immediate,” “lid-associated,” and “evaporative” types of BU. Example images of each type are given and a novel phenomenon of “afterimages” is described. Third, because BU is defined and studied using imaging methods, five different types of system for imaging BU are described. Multiple images of BU and its development are given, including some using a new “high resolution chromaticity” method. Fourth, it is emphasized that tear film thinning between blinks, leading to BU, is affected by three different directions of flow – evaporation, flow through the corneal surface (osmosis), and “tangential flow” along the corneal surface. Fifth, it is shown that multiple factors are involved in tear film BU and breakup time (BUT), and the role of ten different factors is discussed. Sixth, future directions of tear BU research are suggested.

Tear film instability, which is assessed by BUT, is one of two core mechanisms of dry eye.¹ In pioneering studies of the fluorescein-stained tear film, Norn described two types of BU and their corresponding BUT. In the first type², the position of BU generally varied from trial to trial. BU was not observed immediately after eye opening, and the normal BUT varied between 3 and 132 sec after a blink with a mean of 27 sec. Norn did not consider BUT to be of clinical interest, but it is now considered to be an important clinical test for dry eye.¹ This type of BU is related to *tear film* properties and is the subject of the current review. The importance of this type of BU is emphasized by its relation to the other core mechanism of dry eye – hyperosmolarity.¹ Evaporation is a major cause of both hyperosmolarity and BU³ and will be discussed extensively in this review.

A second type of BU⁴ was apparent immediately on eye opening and occurred repeatedly in the same corneal position. This type of BU was associated with *corneal* disorders, such as dendritic keratitis and corneal erosions, and will not be considered in this review. However, it should be noted that BU in dry eye can sometimes be observed immediately after a blink.⁵ Also, the position of tear-related BU can have a tendency to be repeatable.^{6,7} Finally, it may be noted that there is no clear distinction between tear film and corneal disorders. In dry eye, tear film characteristics such as hyperosmolarity cause inflammatory response in the cornea, which reciprocally releases substances such as inflammatory mediators into the tear film.^{8–10} Dry eye can increase corneal surface irregularity¹¹ and reduce corneal epithelial thickness.¹²

The mechanisms of BU have been studied in various ways, including application of surface chemistry principles¹³, histological studies¹⁴, and *in vitro* models.¹⁵ In this review, we emphasize how a variety of *in vivo* imaging studies can provide extensive and complementary information about mechanisms of BU. We stress that there is no single mechanism of BU, but that numerous factors are involved in the development of BU. Important mechanisms influencing BU include evaporation³, Marangoni flow (induced by surface tension gradients)¹⁶, and lid-associated thinning.¹⁷ This review is chiefly concerned with the pre-corneal tear film (PCTF), but studies of the pre-lens tear film, PLTF, and the

pre-conjunctival tear film will also be considered when they help to elucidate BU of the PCTF.

Both fluorescence breakup time (FBUT)^{2,18,19} and noninvasive breakup time (NIBUT)^{19–25} will be considered. Images from studies at Ohio State and Indiana Universities are shown for a variety of methods. The methods include fluorescence imaging for the aqueous layer with and without staining of the corneal epithelium, Shack-Hartmann aberrometry, retroillumination, combined fluorescence and tear film lipid layer (TFLL) imaging, and a new method - “high resolution chromaticity imaging.” (Studies followed the tenets of the Declaration of Helsinki and were approved by the respective institutional review boards. Informed consent was obtained from all subjects.) Studies elsewhere have provided additional important information from methods such as noninvasive BU²⁰ and videokeratoscopy^{26,27}, thermal imaging²⁸, Twyman-Green interferometry^{29,30}, lateral shearing interferometry^{27,31}, and Nomarski microscopy.³²

We note that the challenges to understanding tear film dynamics and BU have led to differing viewpoints on causes and mechanisms. Types of BU may include mechanisms that are evaporatively driven^{1,33,34}, glob-driven (or Marangoni-driven)³, or dewetting-driven.^{5,35} However, the role of the TFLL, for example, has generated rather strongly held opinions. Many believe that *in vivo*, the TFLL is a barrier to evaporation^{33,34,36–41}, while others, due in no small part to the inability to recreate a strong barrier function *in vitro*, do not believe there is a significant reduction of evaporation (“barrier function”) in a healthy tear film.^{42–44} More generally, active areas of investigation include the composition^{45–47}, mechanical properties^{48,49}, and structure of the lipid layer.^{50–53} In this review, we cannot definitively end these debates, but we present evidence from experiment and theory to support our interpretation of experimental observations of tear film dynamics and BU.

2. DEFINITION OF BREAKUP

According to the 2007 Dry Eye Workshop¹, “the tear film breakup time is defined as the interval between the last complete blink and the first appearance of a dry spot or disruption in the tear film.” The “first appearance of a dry spot” may relate to the appearance of a dark area in FBUT, whereas the “disruption of the tear film” may correspond to distortions of the tear surface seen in NIBUT. Lemp and Hamill¹⁸ added that, from trial to trial, the BU position should be randomly located, but as was noted in Section 1, BU can tend to re-occur in a similar location.^{6,7}

2.1. Limitations and subjective nature of the definition

A concern about the above definition is that events within areas of BU are poorly understood, so it is not certain whether “dry spots” actually occur. When evaporation contributes to BU, this causes tear hyperosmolarity and hence osmotic flow of water out of the cornea, helping to maintain some precorneal water.^{54,55}

A more serious concern is the subjective nature of the definition. In FBUT studies, there is typically no sudden drop in fluorescence, which would mark an obvious FBUT. Rather, fluorescence decays steadily after a blink before gradually leveling towards a constant

value^{56,57}, thus making a definition of BU (e.g., fluorescence falls to a critical value) rather arbitrary. While Lemp and Hamill¹⁸ reported that BUT is “a reproducible phenomenon,” Vanley et al.⁵⁸ found that it is “not closely reproducible”; it may be noted that Lemp and Hamill made measurements in succession on the same day, whereas Vanley et al. made measurements on different days and at different times, so the latter study better describes variations of BUT over longer periods. How dark a spot must be to identify it as BU is the clinician’s judgment and can vary among clinicians, so the training of observers and clinical experience are likely to have an impact. Observation methods vary, and may involve viewing the whole cornea or else scanning a slit back and forth across the cornea.⁵⁹ The clinical judgment of BUT may also be affected by other factors, such as the concentration of fluorescein in the tear film. Too little fluorescein dye may render tear thinning and BU difficult to observe⁶⁰ and too high a concentration of fluorescein can lead to the same result due to fluorescence quenching⁵⁷. According to Johnson and Murphy⁶¹, the quantity of fluorescein and saline instilled in the tear film greatly affects the variability between BUT measures.^{57,61} When fluorescein dye-impregnated strips are wetted with saline, these quantities are often unknown. Similarly, the volume of tears in the eye which dilutes the instilled fluorescein is unknown. Use of a yellow or green barrier filter in the viewing pathway is recommended to improve visibility of BU¹; without a barrier filter, FBUT may tend to be increased if poor visibility limits viewing of dark spots of BU. In NIBUT studies, distortions of the tear film surface vary throughout the blink cycle^{26,27} and can start to increase before “disruption in the tear film.”

In most FBUT or NIBUT measurements, only the time to BU is recorded, while potentially important information about the spatial extent of BU is ignored.^{6,23} An additional limitation is that, like all other signs and symptoms, BUT correlates poorly with other signs and symptoms.⁶² A further limitation is that BU is a poorly understood process, limiting the clinician’s ability to interpret BUT. Finally, it should be noted that whereas room temperature and humidity are typically reported, an important environmental factor which is typically not reported is the velocity of the air current near the subject’s eye; the best way of recording low air velocities is probably a three-dimensional ultrasonic anemometer (Dr. Peder Wolkoff, personal communication), but these anemometers are large and difficult to place near the subject’s eye.

2.2. “Touchdown” – an important concept in breakup

As the tear film thins after a blink, eventually the tear film surface will “touchdown” on the corneal surface. More specifically, the inner polar molecules⁶³ of the TFL touch the outer tips of the glycocalyx of the corneal epithelium. This is illustrated in Fig. 1, which shows how the moment of touchdown divides a post-blink interval into pre- and post-contact periods. In this example, pre-contact thinning is assumed to be mainly due to local evaporation, but it will be shown how other mechanisms also affect thinning. After touchdown, evaporation continues, causing hollowing of the corneal surface (Fig. 1C). Additionally, it is proposed that, in a BU area, there is active binding between the TFL and the glycocalyx of the corneal surface; binding between lipids and glycocalyx has been discussed by Cone.⁶⁴ (This binding may be influenced by changes in membrane-associated mucins in dry eye.⁶⁵) This binding lowers the surface energy in the BU area, compared to

the combined surface energies of the TFLL and corneal surface in the surrounding tear film. Therefore, because the tear film tends to a state of lower energy, the BU area will spread in a process analogous to the dewetting of a “low-energy” hydrophobic surface⁶⁶ partially covered in a thin layer of water.⁶⁶ This can cause a steep bank of tears surrounding the BU area. It is seen that the BU area has a rough surface, which may scatter light and degrade the optical image. Additionally, the surrounding bank of tears refracts light like a prism, adding to the image degradation.

After the next blink, the hollow in the corneal surface remains for some time, while the tears form a smooth surface over this hollow (Fig. 1D). There is thus a thicker region of tear film over the hollow, which causes a bright “afterimage” in the fluorescein stained tear film – Section 5.1.1 and Fig. 4. It should be emphasized that while touchdown is theoretically an objective definition of BU, the moment of touchdown may be hard to determine, particularly from FBUT studies. No sudden change is observed at touchdown, but, rather, it is a moment when new processes start to occur. In NIBUT studies, touchdown is the moment when the rough, scattering surface of the BU area starts to appear and spread, together with a surrounding bank (prism) of tears. Because the moment of touchdown is often uncertain, we will retain the typical clinical meaning of BU, e.g., a sustained dark area in a fluorescein image which continues to darken and expand, rather than fading away (see Fig. 5 for examples of “sustained” and “transient” dark spots).

3. IMPORTANCE OF BREAKUP

3.1. Breakup time is an important clinical test of tear film function

Together with the Schirmer test, staining tests, and history, FBUT is one of the preferred diagnostic tests for dry eye used by eye care practitioners⁶⁷; since that report, osmolarity testing has become another common test⁶⁸. Abelson et al.⁶⁹ found a normal mean FBUT of 7.1 sec, which was reduced to 2.2 sec in dry eye; they recommended a cutoff for dry eye diagnosis of 5 sec, while previous studies proposed a cut off of 10 sec.^{70,71} It may be noted that a larger cutoff value increases sensitivity (detection rate) but reduces specificity (probability of correct negative finding).¹ It should also be noted that binary classification based on a cutoff value loses information in the original BUT value (e.g., less than 10 sec might mean 2 or 9 sec); it has been argued that, to estimate dry eye severity, continuous data (e.g., BUT) rather than binary data (pass/fail) should be included in an overall score.⁶⁹

A useful derived measure is the Ocular Protection Index (OPI), which is the ratio BUT/IBI, where IBI is the interblink interval. If the OPI is less than one, BU tends to occur before the following blink, exposing the cornea to the risk of surface damage.⁷² A modified OPI method⁷³ is based on measuring the area of BU under natural blinking conditions, and may have improved ability to distinguish dry eyes from normals.

3.2. Breakup is a core mechanism of dry eye

In the report of the 2007 Dry Eye Workshop¹, tear film instability, measured by BUT, was considered to be one of the two core mechanisms of dry eye. The other core mechanism, hyperosmolarity, is caused by evaporation.¹ As an example, if evaporation thins the tear film

(aqueous layer) in an area to half its original thickness, the osmolarity will be doubled, provided that any tear solutes, such as salts, are not lost from the aqueous layer (e.g., by diffusion out of the area). High osmolarity can be caused either by evaporative dry eye (EDE) or by reduction of aqueous tears in aqueous deficient dry eye (ADDE) or by a combination of both EDE and ADDE.¹ It may be noted that while osmolarity is measured clinically using tear samples from the meniscus,⁶⁹ the osmolarity of the PCTF may reach much higher values.^{9,54,55,74} Such hyperosmolarity is a major cause of the disruption and dysfunction in corneal epithelial cells in dry eyes.¹⁰

Evaporation is also a major factor in BU.^{1,3,9,54,55} (The role of evaporation will be discussed in Sections 7.2 and 8.4.) Thus, the two core mechanisms, instability (BU) and hyperosmolarity are closely related to each other by the common factor of evaporation, and both can provide complementary information about tear film characteristics in dry eye.

3.3. Relation to symptoms of ocular discomfort

“Symptoms of discomfort” are part of the defining characteristics of dry eye.¹ A distinction may be made between “immediate sensation” which varies with time after eye opening up to BU, and “chronic sensation” which is a more continuous symptom not specifically related to one blink cycle. Immediate sensation is closely related to both hyperosmolarity⁹) and BU⁵⁶ and is discussed here. Chronic sensation may be related to the effects of BU in multiple blink cycles; it may be caused by increased sensitivity of sensory receptors or by release of substances such as inflammatory mediators from the corneal epithelium which stimulate corneal neurons.^{10,75}

The most frequent adjectives used to describe immediate sensation during extended eye opening were “stinging” and “burning.”⁷⁶ Methods have been developed to record the increase in immediate sensation when an eye is kept open as long as possible after a blink.^{56,77} The rate of increasing discomfort was found to be highly correlated (Spearman’s $r = 0.70$) with the rate of developing tear BU.⁵⁶

Corneal sensory nerves are sensitive to both cooling⁷⁵ and hyperosmolarity⁷⁸; both these stimuli can result from evaporation, which causes tear thinning and BU.⁴¹ The sensory response to BU may stimulate blinking; Brown⁷⁹ proposed that “the usual response (to BU) was an immediate blink which rehydrated the dry areas.” However, in some dry eyes, he stated “when sensation was reduced, blinking in response to drying was delayed” causing rapid desiccation of the superficial epithelial layers.

The role of BU in stimulating blinking is supported by the significant correlation between BUT and blink rate.⁸⁰ More recently, Wu et al. showed a linear relationship between corneal stimulation and the blink response.⁸¹ In dry eyes, rapid BU is a probable cause of the observed increased blink rate⁸² and reduced “maximum blink interval” (maximum time that subjects can keep their eyes open without feeling uncomfortable).⁸³ It is possible that blink rate is controlled by the “chronic sensation” discussed above as much as the “immediate sensation” during BU. Additionally, the fact that blink rate is dependent on tasks such as reading and conversation shows that blinking is also controlled by other mechanisms such as cognitive state, and blink rate may be determined by a combination of neural inputs.^{82,84}

Finally, it may be noted that another consequence of the sensory response to BU is the stimulation of reflex tears, which can be observed in BUT studies (Fig. 2B4).⁸⁵ The blink and tearing responses are correlated with each other and show a dose-response relationship to surface stimulation, which is not surprising since the stimulus for both responses can arise at the ocular surface.⁸⁶

Some sensory nerves, the mechano-nociceptors, respond to noxious mechanical forces.⁷⁵ It is possible that, after touchdown, when there may be binding between the lipid layer and the corneal surface (Fig. 1C), the lipid layer may apply forces to the corneal surface, causing irritation and stimulating blinking.

3.4. Optical aberrations and scatter

“Visual disturbance” is one of the defining characteristics of dry eye.¹ Visual disturbance may be considered a patient-reported symptom, but it may also be measured objectively using optical systems.²⁶ It may also be studied by visual testing; decline of visual acuity within 10 to 20 sec after eye opening was found to be over 70% in dry eye patients, but it was not significant in normals.⁸⁷

After a blink, optical quality of the eye may improve somewhat for a few seconds, but then it declines near the time of BU.^{26,27,88–90} The improvement of optical quality after a blink is probably due to the “leveling” effect of surface tension which tends to smooth out any initial irregularities in the tear surface.⁵⁴ Optical deterioration before BU may be caused by local evaporation causing distortions in the tear surface, as in Fig. 1A. After touchdown (Fig. 1B), surface distortion is increased both by the exposed rough surface of the cornea and the surrounding tear prism (Fig. 1C). Optical aberrations related to BU will be discussed further in Section 6.

4. EARLIER THEORIES OF BREAKUP

4.1. Surface physical chemistry models

An early model and much referenced paper by Holly¹³ was based on ideas from surface physical chemistry; the model involved diffusion of lipids through the aqueous layer, causing the mucus layer to become hydrophobic and thus generating BU. A critical review of this and other models based largely on physical chemistry of the mucus layer and corneal surface has been published by Peng et al.⁵⁵ A limitation of such models has sometimes been imperfect *in vivo* experimental evidence; for example, Holly’s assumption of initial tear thickness was higher than current estimates⁹¹, and his assumption of thinning rate from evaporation was lower than is now thought possible³, so he rejected the possibility that evaporation should be considered as a cause of BU. With more recent experimental studies, the evidence for a major role of evaporation in BU has become much stronger⁵⁴ and will be discussed further in this review.

More recent theoretical approaches by Sharma have calculated that the healthy corneal epithelium, with its glycocalyx, is nearly as wettable as in a hypothesized situation where the corneal epithelium is coated with a well-hydrated non-bound mucus layer. This may be interpreted to mean that a mobile hydrated mucus layer is not needed for wettability³⁵; this

result agrees with Tiffany's wettability measurements.^{92,93} In subsequent papers, Sharma goes on to calculate that damaged ocular surface cells without a glycocalyx may preferentially attract cell debris and other less- or non-wettable objects and thus become dewetting sites that promote TBU.^{35,94} More recent theoretical and *in vitro* approaches have been used as well.⁹⁵ However, despite the fact that the argument is reasonable, we are unaware of direct *in vivo* observation of BU in human eyes that is clearly dominated by this mechanism; it may be that it cooperates with other mechanisms to cause BU. It may be noted that microscopic observation of the corneal surface cells⁹⁶ involves elimination of the air space in front of the cornea, so it is impossible to observe BU and the corneal surface at the same time. On the other hand, it is possible to observe BU and the lipid layer simultaneously⁹⁷, and this is one reason why we emphasize the relation of BU to the lipid layer rather than to the corneal surface. We believe that BU that is driven primarily by evaporative^{3,54} or by Marangoni-driven mechanisms⁹⁷ (L Zhong, C. F. Ketelaar, RJ Braun, C.G. Begley and P.E. King-Smith, 2017, submitted) has been observed, and we focus our attention on those cases in this review.

4.2. Some experimental findings

The schematic diagram of localized BU in Fig. 1 is based on the assumption that evaporation through the TFL plays a major role in tear film thinning until touchdown. While Mishima and Maurice³⁷, in a classic study, concluded that the TFL reduced evaporation rate by a factor of about 15, Brown and Dervichian⁹⁸ found that meibum, spread on a beaker of saline, produced no detectable reduction in evaporation rate, and their finding has been confirmed a number of times. This has led to the suggestion that the key function of the TFL is not evaporation resistance but "to form viscoelastic films capable of opposing dilation of the air-tear interface"⁹⁹, or "to allow the spread of the tear film and prevent its collapse on the ocular surface"¹⁰⁰.

According to these ideas, BU may occur when the mechanical properties of the TFL are defective and allow dilation of the air-tear interface. Thus, there has been debate about whether the more important function of the TFL is evaporation resistance or mechanical stability. Bhamla et al.¹⁰¹ helped to resolve these conflicting ideas by showing that meibum spread on saline prevented evaporation, but only in a few small areas, which presumably are not sufficient to cause detectable reduction of evaporation from a beaker of saline.⁹⁸ That study confirms that meibum can form a barrier to evaporation when spread on saline (and therefore presumably in the eye); however, meibum does not spread on saline as well as on the normal *in vivo* tear film, so only small patches of meibum are effective barriers to evaporation. A conclusion is that the spreading of meibum on saline is less effective than in the normal tear film, presumably because of altered mechanical properties. Thus, both evaporation resistance and mechanical properties are important for the TFL.

Pfister and Renner¹⁴ created experimental dry spots in the rabbit cornea for examination by scanning and transmission electron microscopy. Small dry spots showed disintegration of the surface plasmalemma, with separation of cells from each other and from surrounding surface cells. Brown⁷⁹ and Torens et al.¹⁰² reported that BU in dry eye is sometimes associated with elevations on the corneal surface.

Brown⁷⁹ also noted BU when eyelashes were in contact with the cornea. An explanation for this finding is that sebum from the eyelashes displaces meibum and hence disrupts the evaporation barrier of the TFL. ¹⁰³ This interpretation raises the possibility that sebum may enter the lipid layer at other times, causing disruption of the TFL, evaporation and BU. Mixing of sebum with meibum at the air/water interface results in expansion of the mixed films and a shift of their isotherms to higher surface pressures, thus indicating a strong incorporation of sebum in the meibomian films. ¹⁰⁴ In contrast, squalene, a major component of sebum but not of meibum, has been found to overlay on meibum films without the ability to support TFL surface properties. ¹⁰⁵ In accordance with the suggestion that sebum may enter the TFL from the eyelids, Butovich¹⁰⁶ found that squalene could be detected in tears sampled from the meniscus. If it occurs, contamination of meibum by sebum could explain higher rates of tear film evaporation and BU in the presence of a seemingly intact tear film lipid layer.

5. CLASSIFICATION OF BREAKUP

BU is a complicated and still poorly understood process, so a complete classification of BU is probably not possible at present. In the classification proposed here, we have tried to distinguish three types of BU, which depend mainly on different mechanisms and can be differentiated from each other quite readily.

BU has sometimes been classified by shape of the BU area. Bitton and Lovasik¹⁰⁷ described three distinct patterns – “dots” with a circular shape, “streaks” having a linear shape, and “pools” with an irregular shape differing from the other two shapes. Yokoi and Georgiev⁵ proposed a somewhat similar classification with four types of BU – “spot break” with a circular shape, “line break” with a linear shape, “area break” of a large area, and “random break” differing from the other three shapes. A limitation of classification based on shape is that different shapes may occur simultaneously in some cases of BU, e.g., “dots,” “streaks,” and “pools” are all visible in Fig. 8E. Another limitation is that the shape of a BU area may change with time, e.g., between “spot breaks” in Fig. 2A1 and “line breaks” in Fig. 2A3. Shape of BU is potentially an important factor for classifying BU, but it is often related to the structure of the TFL⁹⁷, which is poorly understood. We have therefore chosen to use two other characteristics – time course and location of BU - in this classification scheme.

5.1. Immediate breakup

A major distinction in the classification of BU is shown in Fig. 2, which illustrates large differences in the time course of fluorescence BU between two subjects. The upper row illustrates BU occurring immediately after a blink. For comparison, the middle row illustrates a much slower development of BU in a different subject, with no dark spots just after a blink. Both sequences were recorded after instillation of 1 μ L of 0.1% fluorescein – a very small amount. As discussed in Section 6.1, this low initial fluorescein concentration in the tear film was below the concentration needed for self-quenching ^{38,60,108}, which indicates that the immediate BU in the upper row was not due to evaporation and quenching, but was probably due to other mechanisms, such as decreased wettability of the cornea⁵ or divergent flow of aqueous tears out of the BU areas (see Section 7). Additional evidence that

this immediate BU was not due to evaporation is that it was too fast; for example, with a relatively high evaporation rate of $20 \mu\text{m}/\text{min}$ ³⁴, evaporative thinning in 0.2 sec would be $0.067 \mu\text{m}$ and so only about 2% of an initial thickness of $3 \mu\text{m}$ ¹⁰⁹, whereas the observed dimming of the darkest areas in Fig. 2A1 was about 80%. This immediate BU in Fig. 2A1 perhaps corresponds to the “spot break” of Yokoi and Georgiev⁵, which they describe as a type of “short tear film BUT dry eye”. (Other aspects of Fig. 2 will be discussed in Section 5.3).

If immediate BU is not due to evaporation, then it must be due to some other mechanism, such as reduced wettability of the cornea⁵ or divergent flow of tears out of the BU area. For the example in Fig. 2, upper, we favor the second hypothesis. If BU is due to non-wetting, the original BU area, in Fig. 2A1, might be expected to remain dark (non-wetted); however, some BU (dark) areas move upwards so that the final BU area in Fig. 2A3 does not overlap with the original area in Fig. 2A1. It may also be noted that the pattern of dark spots after the next blink, Fig. 2A4, differs from that in the first blink (Fig. 2A1); this indicates that dark spots are associated with something mobile in the tear film, e.g., the TFL, rather than non-wettable areas of the cornea, which would be expected to remain more fixed in position.

The simultaneous fluorescence and TFL image in Fig. 3A⁹⁷ shows that areas of immediate BU can correspond to thick structures in the TFL, which will be called lipid “globs.” Figs. 3B and C show unstained color images of a glob at two times after a blink⁵⁴; initially, colors show that the glob is thick, but it then thins as it spreads upward, forming a “tail” in a way similar to the BU areas in Figs. 2A1 to 2A3. These observations could form the basis of one type of immediate BU. However, in the example of Fig. 16, BU areas after a partial blink seem to correspond to areas of non-wetting of the epithelial surface. If immediate BU occurs repeatedly in the same location, then this may possibly be a signal that dewetting is the cause of BU in those cases. Related to this, Yokoi and Georgiev⁵ have proposed a “non-wetting dry eye,” whose pathophysiology is considered to be a decrease in epithelial wettability.

5.1.1. Afterimages—Fig. 4 illustrates “afterimages” of BU areas which occur after a blink following BU. Fig. 4A is a repeat of Fig. 2A3, showing immediate BU just before a blink. Fig. 4B was recorded after the next blink showing that the dark areas of BU (b) are replaced by light areas, which will be called afterimages (a). It is proposed that afterimages correspond to thicker tear film between the smooth tear surface and the hollow in the corneal surface left by the BU area (Fig. 1D). The upward movement of BU areas in Figs. 2A1 to 2A3, may have applied mechanical shearing forces to the corneal surface in BU areas, causing irritation and triggering the next blink soon after 8 sec. These mechanical forces may have contributed to hollows in the corneal surface, generating the afterimages in Fig. 4B. (Other images in Fig. 4 will be discussed in Sections 5.2 and 5.3)

5.2. Lid-associated breakup

BU can occur at any position on the corneal surface⁷ and is usually associated with the structure of the TFL.⁹⁷ However, one characteristic type of BU occurs just beneath the upper lid and may not be associated with particular TFL structures. Fig. 5 shows an

example of lid-associated BU at five different times after a blink. A very small amount and low concentration of fluorescein was instilled (1 μ L of 0.1%), so little self-quenching is expected, at least initially^{38,57}; therefore, dark regions typically correspond to thin tear film without much quenching from increased fluorescein concentration.

A dark band is already seen below the bright meniscus of the upper lid in Fig. 5A at 1 sec after the blink. This dark band corresponds to the meniscus induced thinning proposed by McDonald and Brubaker¹¹⁰; the surface tension in the concave surface of the meniscus generates a low pressure, sucking aqueous tears from the surrounding tear film and generating a “black line.” In the next few seconds, up to 9 sec after the blink (Fig. 5B), the subject raised his upper lid, extending the width of the dark area; because the upper lid velocity was low, the deposited tear thickness was correspondingly thin.¹¹¹ At 9.5 sec (Fig. 5C), the subject made a small partial blink, which covered most of the thin area except for a narrow dark strip near the bottom. Two small dark areas in this narrow strip have been labeled “s” for “sustained,” because these areas remained until the end of the recording (Fig. 5E); these areas may reasonably be considered to be BU, although the moment of touchdown is uncertain. However, another dark area, labeled “t” for “transient,” disappeared by the end of the recording, and so might not be classified as BU. This shows a common characteristic of BU; some dark areas become sustained BU whereas others fade away – a sort of an “all or none” mechanism. BU involves opposing processes causing tear thinning (e.g., evaporation, Section VIII.F) and thickening (e.g., leveling, Section 8.4), so in “sustained” cases, thinning processes may exceed thickening, leading to BU, whereas in “transient” cases, thickening may be stronger and BU does not occur.

A second example of lid-associated thinning is shown in the middle row of Fig. 2 (for very low fluorescein concentration). The irregular shape of the dark region, and particularly the downward arcuate region at the right, may correspond to the effect of meibum spreading out from meibomian glands and forming semicircular patches of the TFL. ¹⁰³ As the meibum spreads out, it would carry the underlying aqueous with it, causing thinning from divergent flow of tears. The examples in Fig. 5 and the middle row of Fig. 2 show that, while meniscus-induced thinning is an important contributor to lid-associated BU, additional mechanisms make a contribution. A third example of lid-associated BU is shown in Fig. 4C, together with afterimages after the following blink in Fig. 4D.

5.3. Evaporative breakup

The preceding two sections illustrate special types of BU, namely immediate BU and lid-associated BU. Additional special mechanisms of BU may be the effects of corneal elevations^{16,79,102}, eyelashes in the tear film⁷⁹, bursting bubbles⁹⁷, and lipid droplets in the TFL (Fig. 10). In all these cases, evaporation plays a role. However, in many cases, special mechanisms such as the above are not involved and evaporation is presumably the main mechanism of BU.³

Examples of evaporative BU from one subject are shown in the two lower rows of Fig. 2. The middle row shows images using a very small amount of fluorescein (1 μ L of 0.1%), which would be expected to show the effect of divergent flow but little effect of self-quenching; the bottom row was recorded after instillation of much more fluorescein (1 μ L of

5%), which is sufficient to show effects of quenching as well as divergent flow.^{38,57,108} In the bottom row of Fig. 2, for high fluorescein concentration, slight evidence of BU is already present after 1 sec in Fig. 2C1, and BU is much more pronounced (compared to low concentration) at longer times (Figs. 2C2 and 2C3). This is consistent with attributing most of the fluorescence dimming at high concentration to evaporation, which increases fluorescein concentration and self-quenching.^{38,54,57,60} It is uncertain to what extent the BU seen in the low concentration case (Figs. 2B3 and 2B4) is due to divergent flow rather than quenching.

Another example of evaporative BU is given in Fig. 4E, with afterimages after the next blink shown in Fig. 4F. Thus, Fig. 4 illustrates that afterimages can occur after all of the three types of BU discussed here – immediate, lid-associated, and evaporative.

6. IMAGES OF BREAKUP OBTAINED BY DIFFERENT METHODS

In this review, the importance of different imaging methods for studying mechanisms of BU is emphasized. Five different types of methodology for imaging the tear film are illustrated in Fig. 6 and will be reviewed in this section. Note that both the tear and corneal surfaces are rough, so both can contribute to spatial variations in tear film thickness. This may be expressed by the equation

$$h(x, y, t) = a(x, y, t) - c(x, y, t) \quad (1)$$

where h is tear film thickness, x and y give position on the tear surface, t is time, a is the height of the tear (air) surface and c is the height of the corneal surface (see enlarged section on the right of Fig. 6). Both tear thickness and tear surface shape are important in determining tear film dynamics and BU; however, current methods of tear film imaging are limited to measuring either thickness or tear surface, suggesting a need for a combined system which measures both these properties (Section 9.1.3). It should be noted that a clean contact lens may theoretically be assumed to have a smooth surface, so $c(x, y, t)$ would be constant in Equation 1; thus, for the PLTF on a clean contact lens, measurement of tear film thickness, $h(x, y, t)$, also provides precise information about tear surface shape, $a(x, y, t)$. Accumulation of deposits¹¹² alters the smooth reflection from the lens surface, so tear film thickness would no longer provide precise information about tear surface shape; it should be noted that lysozyme deposits on an Acuvue contact lens already reached about 4% of the “plateau” value after only 15 minutes of lens wear.¹¹³

Note also that interferometry, in which interference is generated within the optical system, is discussed in Section 6.2, whereas interference between reflections from different surfaces in the tear film is the subject of Section 6.3. Most tear surface reflection methods (Section 6.2) image the tear surface, but OCT images both the tear and corneal surfaces; the latter is illustrated by a dashed line in Fig. 6. The “refraction” methods in Section 6.4 correspond to methods that use reflected light from the retina as a secondary light source. Tear surface reflection and refraction are measures of the distortion of the tear *surface*, whereas

fluorescence, tear film interference, and thermography are measures of *bulk* properties of the tear film.

It should be noted that, when the distorted surface of the tear film surface has slope (s) compared to a smooth surface, it can be shown that the deviation of light by reflection (Section 6.2) will be $2s$, while the deviation by refraction (Section 6.4) is $(n-1)s$, where n is the refractive index of the tear film – about 1.34.¹¹⁴ Thus, the deviation by reflection is nearly 6 times greater than that from refraction. Therefore the rough surface of a BU area may be better observed by reflection (e.g., Figs. 7 and 8) than by refraction (Fig. 13A).

A major distinction is between invasive and noninvasive methods. Fluorescence from instilled fluorescein is the most common clinical test¹¹⁵, while several noninvasive methods have been developed.¹¹⁶ A limitation of fluorescence imaging is that fluorescein instillation reduces BUT measured by a noninvasive method, presumably by altering the tear film^{21,117}, although that conclusion was not confirmed by Cho et al.¹¹⁸ Fluorescence images from the whole cornea are subject to artifacts such as lens fluorescence and reflection from the iris (Figs. 2, 4, and 5). It should be noted that BUT from all methods are affected by environmental conditions of humidity, temperature and air velocity^{55,119}, BUT may vary between observers in subjective methods¹¹⁹, and FBUT can depend on the amount and concentration of fluorescein.^{57,61}

Table 1 summarizes some characteristics of imaging methods discussed in this section.

6.1. Fluorescence

Some characteristics and disadvantages of this common method are mentioned in the above section, but two advantages deserve comment. First, an important characteristic of fluorescence imaging is the phenomenon of self-quenching. When fluorescein is very dilute (e.g., 0.01% in the tear film), the efficiency (ratio of emitted to absorbed photons) is high and independent of fluorescein concentration. When fluorescein is concentrated (e.g., 1% in the tear film) the efficiency is greatly reduced by quenching.^{38,57,108} At high concentrations, efficiency is inversely proportional to the square of the concentration³⁸, so doubling the concentration (e.g., by evaporation) reduces efficiency by a factor of four.

It follows from the above considerations that when very low concentrations of fluorescein are used, increase in fluorescein concentration due to evaporation will not affect efficiency, and thus, fluorescence will be unaffected by evaporation (if the amount of fluorescein per unit surface area does not change). However, this low concentration condition can be used to observe divergent flow of tears out of an area; this flow will carry fluorescein with it, reducing fluorescence. For comparison, with high fluorescein concentration, both self-quenching (from evaporation) and divergent flow can reduce fluorescence. Thus, a comparison of low and high concentration cases can demonstrate the effect of evaporation and quenching on fluorescence dimming and BU^{54,57}; the bottom row of Fig. 2 (high concentration) illustrates the increased dimming of fluorescence due to quenching compared to the middle row (low concentration).

A second advantage of fluorescence imaging is as follows. If a fluorescence video imaging covers both the cornea and some surrounding conjunctiva, the (high contrast) conjunctival image can be used to track eye movements.⁵⁷ The images can then be aligned after correction for the effect of eye movements, providing a steadier video recording in which the effects of tear movement are easier to visualize and measure. Also, images can be averaged, after correcting for eye movements, to improve the signal-to-noise ratio.

6.2. Reflection from the tear film surface

6.2.1. Distortions in a reflected pattern—In these methods, the tear surface is used like a convex mirror, and the virtual image of a reflected pattern is viewed. The pattern can be either a rectangular grid²⁰ or concentric rings such as the Placido disk of a videokeratometer.^{23,25,123} As a clinical NIBUT test, compared to FBUT, this method has the advantage of being noninvasive and, also, it can provide objective results^{23,25}; Downie²⁵ reported that an objective NIBUT measure was significantly less variable than FBUT measured by clinicians. However, because resolution is limited by the spacing of the grid or rings, it does not provide as much spatial information about BU as some other systems, including fluorescence imaging. Arnold et al.¹²⁴ have described a modification of a videokeratometer for simultaneous examination of BU and the tear film lipid layer; one camera is focused on the tear surface and records the lipid layer, while a second camera records tear surface distortion and BU by focusing about 4 mm behind the tear surface where the virtual image of the Placido disk is located.

6.2.2 Defocus microscopy—Fig. 7 illustrates how defocus microscopy¹²⁵ can be used to demonstrate the rough surface and concave structure of tear film BU. The principle of the method is illustrated in Fig. 7A, where light is reflected from a hollow (h) on the tear surface (t), which is behind the plane of focus (f) of the camera system. The hollow acts like a concave mirror so light is concentrated at the plane of focus, making the image brighter (and also smaller). This is illustrated in Panel B, which shows that areas of BU appear bright, indicating that they have a generally concave surface. It can be shown that, in this “too far” condition, bumps on the tear surface have the opposite effect from hollows, causing dimming; thus, the numerous hollows and bumps of the rough BU surface cause light and dark regions, producing a speckled appearance, which is not apparent in the smoother surrounding tear film. When the tear surface is in perfect focus (Panel C), the BU regions have about the same brightness as the surrounding tear film (consistent with theory), and details of the rough surface structure are lost. When the tear film is too close (Panel D), the BU regions appear dim (again consistent with theory) and the rough surface is again visible. These images support the proposed concave and rough surface of BU in Fig. 1C.

Fig. 8E is another example of defocus imaging showing three types of BU – dots (d), streaks (s), and pools (p) – described by Bitton and Lovasik¹⁰⁷. Three thick lipid droplets (l), in Panel A, move upwards with the TFLM until they appear to stick to the corneal surface in Panel C, and generate three dot BU areas in Panels D and E. It is proposed that when the droplets touch down on the corneal surface, they trigger a “binding-spreading” mechanism between the inner polar molecules of the TFLM⁶³ and glycocalyx (Fig. 1C), which causes

the BU area to spread, which squeezes aqueous outward to help form the surrounding tear prism.

Panel B is a repeat of Panel A with contrast increased five times to show details of the TFL. A thinner (slightly darker) area of lipid (t) resulted in the “streak” BU (s) in Panels D and E. A proposed explanation is that evaporation was increased in this thin TFL area, causing tear film thinning and BU, followed by binding-spreading to generate a steep surrounding tear prism. The width of the streak (s) in Panel D increases by about 100 μm in the 4-sec interval until Panel E. Thus, the width expands at a rate of 25 $\mu\text{m}/\text{sec}$, implying that, on average, each edge spreads outwards at about 12.5 $\mu\text{m}/\text{sec}$.

While the dots (d) and streaks (s) have sharply defined boundaries, the superior edge of the pool at the right of Panels D and E has a blurred boundary with a broad gradient (g) between rough BU inferiorly and smooth tear surface superiorly. This blurred edge moves upwards by about 1 mm in the 4-sec interval between Panels D and E. Thus, this edge velocity is about 250 $\mu\text{m}/\text{sec}$ and so is some 20 times faster than the spreading velocity of the sharp edges of the streak (s) estimated above. It is proposed that the more rapid velocity of this blurred boundary corresponds to evaporative thinning of a shallow gradient of tear film, hence exposing the rough corneal surface; for example, the above edge velocity of 250 $\mu\text{m}/\text{sec}$ would be observed if the tear film was thinning from evaporation at a rate of 0.25 $\mu\text{m}/\text{sec}$ and the gradient of tear thickness was 1 mrad (1 mrad = 3.44 minutes of arc). This type of rapid spreading of BU may be described as “evaporative spreading” to distinguish it from the slow “binding-spreading” mechanism described above and in Fig. 1.

6.2.3. Interferometry—Optical interference effects can be used in two ways for study of the tear film. First, interference can occur between reflections from different surfaces in the tear film, e.g., between outer and inner surfaces of the TFL, which generates the colored appearance of Fig. 3B. Second, interference between two beams can be generated within the optical system, as discussed in this section. Four types of interferometer will be discussed.

Nomarski microscopy (or differential interference contrast microscopy) generates images based on interference between reflections between pairs of neighboring points on the tear film surface, e.g., separated by 2.3 μm ³²; these points were close enough together that a double image was avoided. The direction joining the two points is called the *shear direction*. Interference between these two reflections depends on their relative phase which, in turn, depends on the relative height of the two surface points. Therefore, the image shows the surface slope of the tear film. Hamano and Kaufman’s³² Nomarski image of BU is perhaps still the best available to show both the rough surface of the BU area and the surrounding tear prism – it is a higher resolution image similar to the BU in Fig. 8E. This method gives precise surface shape along a line in the shear direction. However, the surface shape in the orthogonal direction is not directly derivable, and requires further assumptions.

Twyman-Green interferometry is based on interference between light reflected from the tear film surface and a “reference” beam, which is combined in the interferometer.^{29,30} An interference pattern of stripes is generated, and distortions in the pattern correspond to distortions of the tear surface. This is a very sensitive method, capable of detecting

distortions of the tear surface of a small fraction of a wavelength. However, it is difficult to implement, because it is very sensitive to eye misalignment and movement, thus requiring brief exposures to avoid blurring; Nomarski microscopy is much less sensitive to movements along the optical axis, e.g., during blinking.¹²⁶ Micali et al.³⁰ introduced sophisticated technology and analysis methods, but it is not yet clear whether their method can demonstrate the rough surface of BU and its surrounding tear prism. Because Twyman Green interferometry depends on tear surface height, whereas Nomarski microscopy depends on tear surface slope (the spatial derivative of surface height), the former method may be more suitable for the broad and shallow slope distortions before BU, whereas the latter may be more suited to the narrow and steep slopes after BU; however, either method may be adapted for imaging before or after BU, Section 9.1.1. Twyman-Green interferometry can provide a precise two-dimensional description of a surface; this is an advantage over Nomarski microscopy, for which surface shape is not directly derived in the orthogonal direction (see above).

Lateral shearing interferometry is similar in principle to Nomarski microscopy in that it involves interference between reflections from pairs of points on the tear surface.^{27,31,127} However, the separation between pairs of points on the tear film is much greater, e.g., about 150 μm .³¹ An interference pattern of stripes is formed, and distortions in the pattern correspond to tear film distortions at one or both of the contributing points on the tear film. An advantage of this method is that it is not as sensitive to eye misalignment and movement as Twyman-Green interferometry. However, interpretation is ambiguous and more difficult because, as noted, distortions in the interference pattern may correspond to distortions at either location on the tear film. Thus, this method is more suited to an overall measure of surface quality²⁷ than to detailed imaging of BU. A comparison of tear surface studies using lateral shearing interferometry, Shack-Hartmann aberrometry and high-speed videokeratoscopy has been made by Szczesna et al.²⁷ They found that the best precision, as measured by lowest coefficient of variation, was obtained with high-speed videokeratoscopy, with lateral shearing interferometry being a close second. However, the most sensitivity to tear film buildup was found with lateral shearing interferometry, followed by Shack-Hartmann aberrometry. They also found that lateral shearing interferometry correlated significantly with the other methods, but Shack-Hartmann aberrometry and high-speed videokeratoscopy did not significantly correlate with each other.

Optical coherence tomography (OCT) provides information about both the surface shape and thickness of the tear film. An application of surface shape imaging is the study of the tear menisci.¹²⁸ However, for studies of BU, a more important application is imaging of tear film thickness. OCT is currently limited by an axial resolution of about 1 μm .^{120,129,130} The minimum PCTF thickness that can be measured is somewhat greater than this, because the weak reflection from the corneal surface tends to be masked by the stronger reflection from the tear surface.¹²⁰ Thus, the tear thickness resolution is not sufficient to show the details of the tear prism surrounding BU (seen in Figs. 10–12). Werkmeister et al.¹³⁰ showed a B-scan (section) of a BU area. OCT can also be used “en face” to provide an image (rather than a section) of tear film thickness¹²⁰; this method has yet to be applied to tear film BU.

6.3. Tear film interference

6.3.1. Low resolution tear film interference—The PCTF has three reflecting surfaces or interfaces: the outer (air) surface, the interface between the TFL and aqueous layers, and the corneal surface. The reflection from the outer surface is much stronger than from the other two surfaces, so the main interference effects correspond to the TFL, and the whole thickness of the PCTF. Examples of low resolution TFL interference images, based on a modification of Doane's¹³¹ optical system, are given in Figs. 3B, 3C, 7, and 16. Low resolution TFL interference images are not frequently used as a primary means of studying BU, but they can be used to show BU (Fig. 16) and to study the relation between BU and the TFL.

It is difficult to record full-thickness fringes from the PCTF because the contrast of these fringes is small and tends to be masked by higher contrast fringes from the TFL.^{121,131} King-Smith et al.¹²¹ developed a method for removing the lipid layer contribution from the image – an example is given in Fig. 9A. The image is noisy because the fringe contrast was low and has been increased 8 times. Three areas of BU (b) are seen inferiorly. For the wavelength of 850 nm, the thickness difference between neighboring bright fringes is 320 nm. The lateral spacing between fringes is variable; the maximum gradient of tear thickness, corresponding to the narrowest fringes, in this and other PCTF images was found to be about 5 mrad (a thickness difference of one cycle or 320 nm in about 64 μ m), except near areas of BU when the fringes were not resolved. This gradient of tear thickness has contributions from the surface slopes of both the tear and corneal surfaces (Fig. 6); thus, the tear surface slope may be somewhat less than the above gradient in tear thickness. Fig. 9B shows fringes for the PLTF, which have much higher contrast and are shown without contrast enhancement. Except where the fringes are disturbed by contact lens deposits, the spacing between fringes is similar to the PCTF in Fig. 9A, implying similar tear slopes. A superior area of BU (b) is observed; as for the PCTF, fringe spacing near BU is narrow, implying a steep tear slope. Similar PLTF images have been recorded by Hamano³² and Guillon.¹³²

Derivation of the PCTF thickness distribution involves solving two problems. First, when a slope in tear thickness is observed in a direction, e.g., from left to right, it is often not obvious whether the slope in that direction corresponds to increasing or decreasing thickness. Second, when there are no BU areas, or the fringes near a BU area are too narrow to resolve, absolute thickness cannot be determined from a single image. A possible solution to these problems might be to reconstruct the surface shape from later images in the video recording; as the tear film thins from evaporation, tear film interference should produce oscillations (as a function of time) of reflected intensity¹³³, which should continue until BU occurs. Because of the low contrast of PCTF fringes, this approach has not yet been implemented, but it might be used with higher contrast fringes at longer wavelengths (see Section 9.1.2).

6.3.2. High resolution chromaticity images—High resolution images of the tear film have been obtained by confocal microscopy^{102,134} and also by microscopy using a stroboscopic white light source.⁵¹ An advantage of the latter system is that color can be recorded, providing additional interference information compared to specular microscopy.

To help observe whole thickness PCTF fringes, it may be noted that, when the TFL is thin (less than a quarter wavelength thick), the TFL has little color¹³⁵, whereas whole thickness PCTF fringes tend to have stronger color appearance in a thickness range from about 100 to 1000 nm.¹³⁵ Thus, by emphasizing color and eliminating intensity information, it is possible to enhance details of the PCTF in the tear prism surrounding BU.

Fig. 10 illustrates an application of using color information to study BU areas and their surrounding tear prisms. Panel A shows a recorded image made with a high resolution microscope⁵¹, but with the monochrome camera replaced with a color camera. Panel B shows a “chromaticity image” where color information has been emphasized and intensity information has been eliminated. This was achieved by calculating three “chromaticity” values of the form, (for red chromaticity) $r=R/(R+G+B)$ where R, G and B are the recorded intensities of red, green and blue pixels. It can be seen that chromaticities are independent of intensity; e.g., doubling red, green and blue intensities does not change chromaticities. The chromaticity plot, Panel B, is then generated by plotting the three chromaticities (rather than the recorded intensities in Panel A); the contrast in Panel B has been greatly increased to show details.

Regions of BU (b) are recognized by steep surrounding tear prisms (p), as in Fig. 8E and Hamano and Kaufman's³² image. Unexpectedly, regions of BU have an orange appearance, which is presumably related to the rough surface (e.g., microplicae) and structure (e.g., glycocalyx) of the exposed epithelial surface (a pale orange color can be seen in the recorded image, Panel A). The observed BU pattern seems to be related to the distribution of lipid droplets (d) seen in the recorded image (Panel A). These lipid droplets may trigger BU by a binding spreading mechanism (Fig. 1C) in a similar way to those observed in Fig. 8; one lipid droplet, indicated by a black arrow, was not associated with BU, presumably because the PCTF was too thick (about 1000 nm) for it to touch the epithelial surface and trigger BU. The slope of the surrounding tear prism is inversely related to the spacing of interference fringes; the slope is particularly steep – up to about 60 mrad (i.e., 600 nm in about 10 μ m) – in the region to the lower right of the central BU area, but the slope is less around other BU areas. The interference fringes can be seen faintly in the recorded image (Panel A), but are greatly enhanced in the chromaticity image (Panel B). A limitation of this method is that it has not been possible to study whether the BU region is concave, as deduced from Figs. 7 and 14.

Fig. 11 shows another pair of “recorded” and “chromaticity” images. Within the BU area of the recorded image (A), there are some dark structures, which may correspond to gaps (g) between surface epithelial cells; an interpretation is that tear hyperosmolarity has caused osmotic flow out of these cells, causing them to shrink and pull apart. Pfister and Renner¹⁴ demonstrated a similar detachment of superficial cells in experimental dry eye. In contrast to Fig. 10A, the BU area is considerably brighter than the surrounding tear film, presumably because the reflectance of the tear/cornea surface has been increased. Reflectance of a surface in air is given by Fresnel's Formula, $R=(n-1)^2/(n+1)^2$, where n is the refractive index of the surface¹³⁵; thus, reflectance is an increasing function of refractive index. Evaporation increases tear osmolarity (Section 7.1), and the resultant hyperosmolarity causes osmotic flow of water out of the corneal epithelium (Section 8.5), hence increasing the osmolarity of

the latter. The refractive index of solutions and cells is increased by hyperosmolarity.¹³⁶ Thus, the increased reflectance in the BU area may be related to hyperosmolarity of tears and epithelium caused by evaporation and consequent osmotic flow out of the epithelium; however, it should be noted that the rough surface of the BU region may modify the observed reflectance.

As seen in Fig. 10A, the BU area has a more orange color than the surrounding tear film. In this example, the slope of the tear prism is fairly uniform – about 25 mrad -- and less than the slope of about 60 mrad, around the small BU island in Fig. 10.

High resolution chromaticity images in Fig. 12 show development of BU over a 9.4 sec interval. (Because the high resolution microscope is only occasionally in focus and the field of view is small compared to eye movements, this was a rare example of repeat images of a BU area.) In this example, the edge of the BU area spreads at a rate of about 4 $\mu\text{m}/\text{sec}$. This is smaller than the edge spreading rate of about 12.5 $\mu\text{m}/\text{s}$ derived from Figs. 8D and E, implying considerable variability in spreading rate. As in Section 6.2.2, Fig. 8, this spreading may be interpreted as the effect of binding-spreading between the inner polar molecules of the TFL and the corneal surface (glycocalyx).

6.4. Refraction by the tear film surface – retinal reflection methods

6.4.1. Retroillumination—Retroillumination is a method of imaging the optical aberrations of the eye using light reflected from an extended area of the retina. The intensity of the observed image indicates deviations of the *slope* (in a horizontal direction) of the tear surface height from a smooth, spherical surface; the principle of the method is described by Himebaugh et al.¹³⁷ An example of retroillumination is given in Fig. 13A, with a simultaneous fluorescence image in Fig. 13B. The shape of the surface in the BU area can be derived by integration (in the sense of calculus) of the retroillumination image^{54,137} and is shown by the red curve in Panel C. The solid blue curve plots the corresponding fluorescence intensity; dashed blue lines have been fitted by eye to the BU area and surrounding tear prisms, so intersections of these lines give an estimate of BU width, marked by vertical green lines. An important conclusion is that the surface shape of the BU area, given by the red curve between the two green lines, has a concave shape, as in Figs. 1C and 7. Further discussion of comparison of retroillumination and fluorescence images is given by Braun et al.⁵⁴

Retroillumination has the advantage of producing a continuous image rather than a sampled image as in videokeratoscopy and so can provide more detailed spatial information. Retroillumination does not record much information about the surface roughness in the BU areas, as in reflected images in Figs. 7 and 8; this may be because a rough surface may have less effect on transmitted than on reflected light, and also because the resolution of the retroillumination system may be lower. A further example of a retroillumination image is given in Fig. 15C.⁸⁹

6.4.2. Wavefront sensor - Shack-Hartmann aberrometry—Shack-Hartmann aberrometry is another method for studying ocular aberrations based on light reflected from the retina, but in this case using a point, rather than extended, retinal source. The light

emerging through the tear film is focused on a Shack-Hartmann lenslet array which, if there are no aberrations, should form a regular array of spots on a video camera.^{89,123}

Fig. 14A shows an example image where many spots fall in the expected positions on the superimposed grid. However, some spots are deviated from the grid, indicating distortions in the tear film surface. Spot deviation of one square of the grid would correspond to a deviation of the emerging ray or wavefront of 16.7 milliradians (mrad). Arrows show angular deviations of about $\alpha=15$, 10 and 2 mrad, from top to bottom. It can be shown that this corresponds to a slope of the tear film surface of $s=\alpha/(n-1)$ mrad, where n is the refractive index of the tear surface. The fluorescence image of Fig. 14B indicates that these spots are probably in a region of BU (confirmed below), so it will be assumed that the appropriate refractive index is that of the corneal epithelium ($n=1.401$).¹³⁸ Thus, for the uppermost arrow in Panel A with an angular deviation of 15 mrad, the corresponding surface slope is $s=15/0.401=37.4$ mrad.

The sampling distance on the cornea was $d=400\text{ }\mu\text{m}$, so the surface height difference between top and bottom of this lenslet would be $s*d=15\text{ }\mu\text{m}$. Because this value is much greater than tear film thickness¹⁰⁹, this confirms that the spot corresponds to a BU area. Adding the height difference contributions from the three lenslets indicated by arrows in Panel A, gives a total height difference of $27\text{ }\mu\text{m}$. Even after noting that the tear film below the lowest of these three lenslets may be a few μm thick, this indicates that this BU area is in the shape of a deep groove, with a probable depth of over $20\text{ }\mu\text{m}$, as in Fig. 1C. Rapid evaporation is the most probable cause of this groove, as indicated in Fig. 1.

Himebaugh et al.⁸⁹ distinguish two types of aberration seen by Shack-Hartmann aberrometry. The first type, macro-aberration, was illustrated in Fig. 14, and causes spot displacement, corresponding to disturbed slope of the tear film surface. The second type of aberration, micro-aberration, is illustrated in Fig. 15A. Micro-aberrations cause blurring, dimming, and disruption of individual spots, in addition to the displacement caused by macro-aberrations (which also occur in Fig. 15A). Micro-aberrations are caused by irregular variations of the tear surface within the 0.4 mm diameter of the lenslets. The fluorescence image of Fig. 15B and the retroillumination image of Fig. 15C indicate surface roughness on a scale comparable to lenslet spacing (Fig. 15A); this roughness presumably causes the microaberrations in Fig. 15A. We propose that micro-aberrations are caused by multiple small BU regions such as those near the three “dots” in Fig. 8D and also in Fig. 16. Scatter from the rough surface within a large BU area, such as in Fig. 14 and the “pools” in Fig. 8D, does not seem to contribute much to microaberration, as shown in Fig. 14A.

6.5. Thermal imaging

The preceding noninvasive methods have all used an external source of light or near-infrared radiation. An alternative approach is to use the thermal or long-wavelength infrared radiation which is emitted from the eye surface. Thermographic cameras can be used to record the temperature distribution over the cornea and changes over time.¹³⁹ Evaporation causes the tear film to cool and is probably the main source of cooling in evaporative dry eye.^{28,140} In those studies, subjects closed their eyes for 3–5 sec to warm the cornea, and then opened them for a 10-second recording. In that interval, the central corneal temperature fell

significantly more in dry eye patients than in normals. Advantages of this method are that it is noninvasive and that it is specific for studying the effect of evaporation. Limitations are that the procedure does not mimic a normal brief blink and that thermal diffusion limits the resolution of the images.

6.6. Combined optical systems

Recordings using multiple optical systems have been performed either sequentially, with images from different systems being made one after the other, or with images from two systems being recorded simultaneously. Fig. 14 is an example of sequential recording of a fluorescence image and Shack-Hartmann aberrometry, while a retroillumination image was added to the sequence in Fig. 15. Limitations of such sequential studies are that the tear film may alter in the interval between different images and only individual images rather than a continuous video recording is obtained. In this section, three examples of *simultaneous* images from combined optical systems will be considered.

Simultaneous fluorescence and lipid layer imaging provides information about the relationship between aqueous tear dynamics, including BU and the structure of the TFLL.⁹⁷ An example is given in Fig. 3A, which shows fluorescence and lipid images of “immediate breakup.” A general conclusion from the study of King-Smith et al.⁹⁷ was that localized fluorescence changes were typically correlated with lipid layer structures. However, the relationship between tear dynamics and lipid distribution was complex. In some cases, BU occurred in areas of thin lipid, probably because of increased evaporation through the thin lipid. In other cases, such as the immediate breakup in Fig. 3A, fluorescence dimming was associated with relatively thick lipid; this dimming may correspond to thinning of the tear film caused by divergent flow of the TFLL and aqueous layer.

Simultaneous fluorescence and retroillumination images can, respectively, provide information about tear film thickness distribution, and the surface structure of the tear film.⁵⁴ Spatial variations in tear film thickness, causing a corresponding pattern of fluorescence, can be due to variations in either the tear surface or the corneal surface. For example, a thinner area of tear film may correspond to a hollow on the tear surface or a bump on the corneal surface; the retroillumination image, which records tear surface structure, helps to distinguish between these two possibilities. This combination of imaging methods can provide further insights into mechanisms of BU; Braun et al.⁵⁴ observed that the sides of BU areas appeared steeper in fluorescence than in retroillumination images, and analyzed that observation in terms of the factors discussed in Section 8.

*Simultaneous fluorescence and thermal imaging*⁴¹ provides information about the relationship between tear dynamics, including BU, and evaporation which is probably the main mechanism of cooling in evaporative dry eye.²⁸ Su et al.⁴¹ found significant and large ($r = 0.82$) correlations between parameters of BU and of local cooling of the tear film; a similar correlation was found by Li et al.¹⁴¹. This finding supports evidence^{3,54,56} that evaporation is a major factor in most cases of BU.

7. THREE DIRECTIONS OF TEAR FLOW DETERMINE TEAR THINNING AND BREAKUP

Tear film thickness changes within a small area of tear film are determined by three directions of tear flow.³⁴ First, water may flow outwards into the air by evaporation causing tear thinning. Second, water may flow across the corneal surface, typically by osmosis¹⁴²; in the interblink interval, evaporation causes increased tear osmolarity and hence osmotic flow into the tear film, tending to oppose tear film thinning. Third, there may be “tangential flow” (flow along the corneal surface) across the boundary with surrounding tear film, with possible regions of both inward and outward flow; if there is more outward flow than inward flow, this will be called “divergent flow” and will contribute to tear film thinning. Thus, both evaporation and divergent flow can contribute to tear film thinning and BU, whereas osmosis tends to oppose the thinning caused by evaporation. In Section 8, a more detailed description of factors contributing to these three directions of tear flow will be given.

7.1. Evaporation

Evidence for the major contribution of evaporation to tear film thinning and BU has been discussed by Braun et al⁵⁴ (their Section 5). Mathers and Lane³⁶ also emphasized the importance of evaporation in tear film stability. The strong correlation between BU and local cooling, as seen by fluorescence and thermal imaging⁴¹, discussed in Section 6.3, also indicates the importance of evaporation as a cause of BU. In conditions of spatially-uniform evaporation, tear osmolarity increases and causes osmotic flow out of the cornea (Section 8.5), which reduces the tear thinning rate.⁵⁴ Additionally, a localized area of high evaporation, due to locally thin and/or abnormal TFL, forms a hollow on the tear surface that can stimulate and affect other directions of flow. The surface tension of the tear film in this concave surface causes a low pressure and hence an inward tangential flow of tears. This effect of surface tension tends to smooth the tear surface and so is called “leveling”(Section 8.6). The inward or convergent flow carries solutes such as salts and fluorescein with it by a process called “advection” (Section 8.7⁵⁴); this affects osmolarity and hence osmotic flow.

7.2. Osmotic flow

The rate of osmotic flow across the corneal surface may be expected to be proportional to the difference in osmolarity between the tear film and superficial epithelial cells.¹⁴² In conditions of uniform evaporation, the quantity of tear solutes per unit area should remain constant, so tear osmolarity would be inversely proportional to tear thickness.^{54,143} In conditions of non-uniform evaporation, advection and diffusion of solutes (Sections 8.7 and 8.8) also affect tear osmolarity and hence osmotic flow.^{54,55}

7.3. Tangential flow

While evaporation causes tear film thinning, and osmotic flow generally tends to oppose this thinning, tangential flow can either cause tear film thinning or oppose it; thus, tangential flow makes little contribution to average thinning rate but can add to the spatial variability of thinning rate. As discussed in Section 8.6, thinning due to localized high evaporation is opposed by both leveling and osmosis. In contrast, divergent flow of tears causes tear

thinning and so can contribute to BU. Unlike evaporation, divergent flow does not cause hyperosmolarity and osmotic flow out of the cornea. One cause of tangential flow is gravity, but because the tear film is so thin, this is typically a weak effect¹⁴⁴ and contributes little to tear thinning and BU. Two other causes of tangential, divergent flow make important contributions to BU and are considered here.

Marangoni flow is caused by gradients of surface tension in the TFL (Section 8.3). Surface tension is typically inversely related to lipid thickness.^{49,145} As a result, the thick “globbs” of lipid in Fig. 3A had a low surface tension and so were stretched out by the higher surface tension in the surrounding TFL. This divergent flow of lipid caused a corresponding divergent flow of aqueous tears, leading to the observed reduced fluorescence (thinner tear film) within the area of the globbs.

Pressure gradient flow is caused when tears flow from an area of high pressure to an area of lower pressure. The pressure in the tear film is due to the combined effect of surface tension and curvature of the tear surface; convex and concave surfaces give rise to high and low pressures, respectively. An example of pressure gradient flow is the leveling effect of surface tension (Section 8.6), which tends to smooth out convexities and concavities of the tear film. Pressure gradient flow contributes to tear film thinning and BU in generating the “black lines” near the lids (Fig. 5 and Section 8.2).

8. TEN FACTORS IN TEAR FILM BREAKUP AND BREAKUP TIME

Section 7 listed three directions of tear flow, which determine the thinning of the tear film, leading to BU. This section elaborates a number of factors that affect these three directions of flow. In addition, the initial thickness of the tear film deposited by a blink¹¹¹ is discussed because it is an important factor in BUT. Finally, factors involved in causing touchdown and after touchdown will also be considered.

8.1. Tear film deposition

For a given thinning rate of the tear film, BUT may be expected to be proportional to initial tear thickness; if the tear film is thinner in ADDE, then BUT should be correspondingly reduced. The PCTF is derived from the upper meniscus during the upstroke of a blink, by a coating process similar to painting a surface.¹¹¹ Analysis of the coating process involves a balance of internal drag in the fluid (viscosity) with the surface tension force in the deposition region, resulting in a prediction for the thickness of the PCTF. Thickness increases with eyelid speed, meniscus radius, and tear viscosity.¹¹¹ Therefore, as the eyelid slows towards the end of the upstroke, the deposited tear thickness is reduced¹¹¹; in addition to meniscus-induced thinning,¹¹⁰ this is partially responsible for the thinner tear film just below the upper meniscus (Fig. 5). Predictions of PCTF thickness distributions are improved when a pre-existing layer under the lids is included in the analysis.^{146–148}

The coating analysis is based on the assumption that the corneal surface is normally hydrophilic or wettable.⁹³ However, when BU occurs, the TFL may bind to the corneal surface (Fig. 1C), rendering it less wettable. It may therefore be expected that the coating process may not occur at all over some areas of BU, particularly at low upward speeds of the

lid.^{5,149} Fig. 16A is an unstained image of multiple small BU areas. In Fig. 16B, after a partial blink reaching the line between the two white arrows, some BU areas (b) remain in the region covered by the blink, presumably because these areas were less wettable.

8.2. Lid associated thinning – pressure gradient flow

Surface tension causes low pressure in the concave menisci, which sucks tears from the tear film next to the meniscus (Section 8.3); this causes tear thinning and the black line appearance in fluorescence images¹¹⁰ (Fig. 5). The thinning of the tear film can be simulated by computational modeling^{111,150}; Braun and Fitt¹⁵¹ showed that the effect of evaporation becomes dominant in the later stages of thinning, causing thinning to zero thickness (BU) to occur within a finite time.

8.3. Marangoni flow

Marangoni flow is caused by gradients of surface tension (Section 7.3); the TFLL is pulled towards regions of high surface tension, pulling the underlying aqueous layer along with it. Two examples of Marangoni flow have implications for BU. First, after a blink, there is an upward flow of the tear film lasting about 1–2 sec.^{147,152–155} This upward flow is driven by a surface tension gradient, with the highest surface tension superiorly¹⁵²; this surface tension gradient is thought to be related to a gradient in the surfactant between the TFLL and aqueous tears, e.g., polar lipids, immediately after a blink, due to depletion of surfactant as the tear film is deposited in the upstroke of the blink.^{91,152} This flow causes some redistribution of tears, with thickening superiorly and thinning inferiorly.¹⁵⁶ In this way, there is some modification of the initial PCTF thickness caused by tear film deposition (Section 8.1).

A second example of Marangoni flow is the thinning and “immediate breakup” caused by “glob” of lipid released during the upstroke of a blink (Figs. 2 [upper row] and 3). It is proposed that the tear film thinning under the glob is caused by low surface tension in the glob, causing glob expansion and divergent flow of the PCTF (Section 7.3). Holly¹⁶ proposed a similar mechanism contributing to BU.

Fig. 17 illustrates how divergent Marangoni flow in a glob, in conjunction with assumed uniform evaporation, causes a number of processes which will be discussed in following sections, including evaporation (Section 8.4), osmotic flow out of the cornea (Section 8.5), “leveling” of the tear surface (Section 8.6), advection of solutes (Section 8.7), and diffusion of solutes (Section 8.8). It should be noted that Yokoi and Georgiev⁵ provide a different explanation of this immediate BU in terms of non-wettability of the superficial corneal epithelial cells; they state that “decrease in epithelial wettability is considered to be its pathophysiology”.

8.4. Evaporation

As noted in Section 5.3, evaporation may be the most important cause of BU.^{3,36} It has been argued that measurements of evaporation rate using evaporimeters tend to be underestimates, because the chamber of the evaporimeter blocks the normal flow of air over the cornea³; this causes a layer of very humid air to accumulate in front of the tear film, reducing evaporation.

An alternative method of estimating average evaporation rates of the PCTF is to measure the rate of PCTF thinning.³⁴ As discussed in Section 7, PCTF thinning rate is determined by contributions from three directions of flow – evaporation, osmotic flow through the corneal surface, and “tangential flow” along that surface. Because tangential flow can be either divergent or convergent, it will make little contribution to the average PCTF thinning rate, while osmotic flow (Section 8.5) is small until BUT is reached and tends to oppose thinning.^{54,55,143} Thus, average PCTF thinning rate gives an estimate of evaporation rate. In a meta-analysis, Tomlinson et al.⁴⁰ found evaporation rates measured by evaporimeters of 0.81, 1.07 and 1.52 $\mu\text{m}/\text{min}$ in normals, ADDE and EDE, respectively. For comparison, Nichols et al.³⁴ found an average PCTF thinning rate of 3.79 $\mu\text{m}/\text{min}$, implying that rates measured by evaporimeters underestimate evaporation rates in natural conditions.³ While experimental artifacts, such as eye movements during the recordings, may cause random errors in the estimation of thinning rate, such random errors should not contribute systematically to the average rate. Some of these PCTF thinning rates could be quite high; rates up to about 20 $\mu\text{m}/\text{min}$ were observed.³⁴ These higher rates under more natural conditions were supported by evaporimeters, which use a flow of dry air over the tear film^{39,157} and have recorded higher evaporation rates than evaporimeters without air flow. While it is true that tear film thinning and evaporation rates cannot necessarily be directly compared, studies under more natural conditions of air flow over the cornea indicate that tear thinning by evaporation can take place at much higher rates than measured by goggle experiments.

Evaporation rate depends on both intrinsic and extrinsic factors. The main intrinsic factor is the evaporation barrier of the TFLL, which is discussed in Section 4.2. It has been proposed that the TFLL has a structure like that of the evaporation barrier in the skin¹⁵⁸, containing multiple lamellae with a dense array of saturated hydrocarbon chains which retard flow of water molecules⁵⁰; evidence for such lamellae in meibum was provided by X-ray studies.⁴⁸ Extrinsic factors are the temperature and relative humidity of the ambient air, and the speed of air currents over the tear surface.^{159,160}

Evaporation leads to several further processes related to BU, which are summarized in the right column of Fig. 17 and will be discussed in the following sections.

8.5. Osmotic flow out of the cornea and conjunctiva

As noted in the preceding section, evaporation reduces tear thickness and hence increases tear film osmolarity. In an area of uniform evaporation and no tangential flow, the total mass of solute per unit area remains constant, so osmolarity varies reciprocally with tear thickness. If PCTF thickness is reduced to a fraction (f) of its original value, osmolarity will be increased by a factor $1/f$; for example if thickness is halved, osmolarity will be doubled, etc. Because of this reciprocal relationship, osmolarity tends to increase rapidly as the tear film becomes very thin, near BU.^{54,55,143} It should be noted that when evaporation is non-uniform, the analysis becomes more complicated, Fig. 17, right; the tear surface becomes distorted causing leveling (Section 8.6), which in turn causes advection of solutes (Section 8.7); additionally, diffusion of solutes (Section 8.8) occurs.

Increased tear osmolarity causes an osmotic flow of water from the ocular surface to tears. This helps to counteract the thinning caused by evaporation. In the simple case of uniform evaporation, the tear film should thin to a constant thickness corresponding to a dynamic equilibrium between evaporation and osmotic flow rates.⁵⁴ This equilibrium thickness depends on the ratio of evaporation rate to water permeability of the ocular surface; larger values of this ratio, e.g., in EDE, cause smaller equilibrium thickness and hence an increased probability of BU. The water permeability of the conjunctiva is greater than that of the cornea, thus causing less evaporative tear thinning over the conjunctiva⁵⁴; it may be noted that osmotic flow through the conjunctiva makes an important contribution to overall water supply to the tear film and helps to limit the tear osmolarity in the meniscus.^{54,160}

8.6. Leveling of the tear surface

The surface tension of the tear film tends to smooth the tear surface by a process known as leveling. An elevation or convexity of the tear surface increases the underlying pressure, while a hollow or concavity reduces pressure. Leveling involves flow of tears from regions of high to low pressure – pressure-gradient flow (Section 7.2). Leveling therefore tends to flatten elevations and fill in hollows, smoothing the tear film surface.

As illustrated in Fig. 17 (left), leveling tends to oppose the thinning caused by divergent Marangoni flow in globs. Immediately after a blink, the Marangoni flow in globs dominates, causing rapid thinning, but later leveling tends to counter this initial thinning, while evaporation can cause continued thinning. Depending on the balance between leveling and evaporation, thinning may continue causing BU, or it may be reversed so that BU does not occur. As illustrated in Fig. 17 (right), local high evaporation tends to cause a hollow in the tear surface. In addition to osmotic flow, leveling tends to oppose the thinning from local evaporation, reducing the depth of the hollow.

8.7. Advection of solutes

Flow of aqueous tears (Fig. 17) carries solutes with it by advection. When there is a gradient of osmolarity, advection tends to alter osmolarity. For example, aqueous flow into a region of local evaporation (Fig. 17, right) carries not only water, which tends to reduce osmolarity, but also, by advection, solutes that tend to increase osmolarity. With further evaporation, these solutes contribute to increased central osmolarity and, hence, osmotic flow out of the cornea.

8.8. Diffusion of solutes

Tear film solutes, such as salts, diffuse from regions of high to low concentrations. This is illustrated in Fig. 17. The overall movement of solutes depends on both diffusion and advection. In the case of localized evaporation (Fig. 17, right), outward flow of solutes by diffusion opposes inward flow from advection; as indicated in Fig. 17, right, simulations indicate that diffusion tends to be smaller than advection at an early time when the tear film is still relatively thick, but later, when the tear film is thinner, diffusion is greater than advection.^{54,160}

8.9. Lipid droplets

Thick droplets of lipid in the TFLL⁵¹ may touch down on the corneal surface causing BU. Examples are given in the defocus image of Fig. 8 and the high resolution chromaticity image of Fig. 10. Touchdown of a droplet may trigger a binding-spreading mechanism (Fig. 1C). It is possible that elevations on the corneal surface may likewise cause touchdown.^{16,79,102}

8.10. Effects after touchdown

Before touchdown, the roughness of the tear surface is generally limited by leveling (Section 8.6). This is illustrated in Fig. 17, right, which shows how a hollow caused by local high evaporation tends to be smoothed out by inward aqueous flow from leveling. Before touchdown, the maximum slope of tear film thickness is about 5 mrad (Section 6.3.1; Fig. 9). (Possible exceptions are that higher slopes may be generated by globs [Figs. 2 and 3] and small imperfections such as bubbles.⁹⁷)

When touchdown is due to evaporation, osmotic flow starts to compensate for evaporation, so the corneal epithelium starts to thin. If there is a local region of high evaporation, this thinning will cause a concave region of BU, as seen in Fig. 1C. Because the epithelium cannot flow like the tear film, this thinning is not opposed by leveling, so high surface slopes can be potentially be generated, e.g., 37 mrad in Fig. 14 (condition of local anesthetic and extended eye opening). In non-invasive conditions, higher tear thickness slopes can be observed in the surrounding tear prism by high resolution chromaticity images – up to 60 mrad (Fig. 10). It is suggested that this steep slope of the tear prism is generated by a “binding-spreading” mechanism between the inner polar molecules of the TFLL⁶³ and glycocalyx, which squeezes out the aqueous layer between lipid and cornea to form the tear prism (Fig. 1C and Section 6.2.2).

The velocity of spreading of this steep tear prism is relatively slow – about 12.5 $\mu\text{m/s}$ in the low resolution images of Fig. 8 and 4 $\mu\text{m/s}$ in the high resolution chromaticity images of Fig. 12. When the edge of a BU area is shallow rather than a steep tear prism, as in the boundaries marked “g” in Figs. 8D and E, the velocity of the edge movement can be much faster – about 250 $\mu\text{m/sec}$ in that example. This rapid movement may correspond to the effect of evaporation of a thin wedge of tears (Section 6.2.2).

9. FUTURE DIRECTIONS

There is an important need for a more quantitative understanding of the processes involved in BU and BUT. This applies to the thinning of the tear film before touchdown (Figs. 1 and 17), as well as the processes after touchdown. In Section 7, three directions of tear flow contributing to, or countering, tear film thinning were discussed, namely evaporation, flow across the corneal surface (osmotic flow), and tangential flow along the corneal surface. The relations between these three directions of flow are complex. Section 7I discusses factors contributing to tangential flow, such as Marangoni flow, lid-associated thinning, and leveling; factors contributing to osmotic flow include evaporation as well as advection and diffusion of solutes, all of which affect tear film osmolarity.

Many factors involved in BU require better quantification. Local evaporation rate has been estimated from the rate of thinning of the tear film,^{3,34} but osmotic flow and leveling also affect this rate (Fig. 17); more importantly, thinning rates have generally been determined at only one central corneal position, but understanding the contribution of evaporation to BU requires study of spatial variation of tear thickness and thinning rate over an area of cornea (Fig. 17). Osmotic flow depends of tear hyperosmolarity and the osmotic permeability of the corneal surface, both of which need better quantification.⁵⁴ Lid-induced thinning and leveling both depend on the surface tension and viscosity of the tear film; whereas the variation between subjects in surface tension may be relatively small,¹⁶¹ viscosity at low shear rates may be increased up to 5 times in dry eye.¹⁶² Leveling depends on the compressibility of the TFLL; when the compressibility is very high, allowing free movement of the tear surface, the rate of leveling flow is some 4 times greater than when the compressibility is very low, forming a rigid “tangentially immobile” surface.⁵⁴ Thus, a knowledge of the in vivo viscoelastic properties of the TFLL⁵² is important for the analysis of leveling; information about the effect of in vivo compressibility of the TFLL on leveling, could be obtained by simultaneous imaging of whole tear film thickness and the TFLL (e.g., if the TFLL is tangentially immobile, it will remain stationary as leveling occurs by movement of the underlying aqueous layer).

Improved understanding of tear thinning leading to touchdown will therefore require improved imaging methods. More quantitative analyses of tear film images and video recordings are needed, together with further mathematical analysis and modeling of the tear film and BU. Molecular and structural aspects, particularly of the TFLL, need to be elucidated, together with their clinical implications.

9.1. Improved imaging systems

Possible developments of reflection systems (Section 6.2) and tear film interference systems (Section 6.4) are considered here together with further development of combination systems (Section 6.5).

9.1.1. Reflection from the tear film surface—*Nomarski Microscopy* (Section 6.2.3) was applied by Hamano³² over 30 years ago to study distortions of the tear surface. One image of BU was presented. To our knowledge, this method has not been used since then. A limitation of Hamano’s study is that the images were recorded with photographic film rather than digitally, and computer analysis methods were limited at that time. There is therefore scope to repeat this method with modern digital imaging and processing methods. Combination Nomarski images and lipid layer images will be discussed in Section 9.1.3.

Twyman Green Interferometry (Section 6.2.3) has been developed by Licznarski et al.²⁹ and Micali et al.³⁰ Those systems are suited for studying the shallow tear film slopes occurring before BU, but are less suited for the high slopes, up to 60 mrad, occurring after BU (Section 6.3.2); for example, the system of Micali et al.³⁰ is limited to a maximum theoretical slope of 22 mrad and less in practice due to problems such as eye misalignment. There is thus a need to develop higher resolution Twyman Green systems which would be able to record the

higher surface slopes after touchdown. As with Nomarski microscopy, discussed above, it may be possible to combine Twyman Green and lipid layer imaging systems.

9.1.2. Tear film interference—*Low resolution tear film interference* (Section 6.3.1) currently suffers from two problems for imaging the whole thickness of the PCTF. First, interference contrast of whole thickness fringes is low, so images are noisy (Fig. 9A). Second, the whole thickness fringes are often masked by higher contrast interference from the TFL, requiring special methods to remove the TFL contribution.¹²¹ Better whole-thickness fringes could be obtained by using a longer wavelength, such as 1600 nm, compared to 850 nm used in Fig. 9A. At this wavelength, contrast of whole thickness PCTF fringes should be increased by a factor of about 4.¹⁶³ Additionally, for typical TFL thickness of about 50 nm³³, the contrast of TFL interference should be reduced at this longer wavelength. Sensors based on indium-gallium-arsenide semiconductors can provide good signal-to-noise ratios at 1600 nm¹⁶³ and so would be suitable for whole thickness PCTF images. As noted in Section 6.3.1, while tear film thickness distribution cannot generally be derived from a single image of interference fringes, it might be derived from tracking oscillations in reflected intensity at any location, until BU occurs. Additionally, by adding a beam splitter in the viewing system, the TFL layer could be imaged simultaneously using a color camera.

Hyperspectral imaging is an alternative to the above tear film interference method for determining the thickness distribution of the tear film. It would involve measuring a reflection spectrum at every location of an image of the tear film. The spectrum could then be analyzed to determine the thickness of both the tear film and the TFL^{33,109,164} at every location. One method of hyperspectral imaging, “spatial scanning,” would be similar to a spectral-domain OCT system but without the reference mirror; the strong reflection from the tear surface would take the place of the reflection from the reference mirror. Another method of hyperspectral imaging, “spectral scanning,” would be to record a sequence of monochromatic images with a step changes in wavelength from image to image.

High resolution chromaticity imaging is currently limited by the fact that the small depth of focus of the microscope objective (about 1 μm), combined with axial eye and head movements, causes most images to be greatly out of focus; only about 1% of images, (e.g., 20 of 2000), are in reasonable focus and these are recorded at random times over a period of about 30 sec. For this reason, taken together with the effect of lateral eye movements and the small field of view of 0.2 mm diameter, development of BU, as in Fig. 12, was rarely seen. If axial eye movements can be monitored, e.g., optically, then this information could be used in a feedback system for automatic control of focus. Increasing the field of view to, e.g., 0.4 mm diameter, would reduce loss of image features from lateral eye movements; this increase in image area could be achieved while retaining sufficient resolution to image tear prisms (Figs. 10–12) by using a lower power objective with a higher resolution video camera. These changes would help provide continuous monitoring of the development of BU.

9.1.3. Combination systems—The TFL is a key factor in BU, both in controlling evaporative thinning of the tear film (Section 5.3 and Fig. 2 and in the tear thinning associated with globs (Section 5.1 and Figs. 2 and 3). Simultaneous imaging of the TFL

and either the tear surface or PCTF thickness can thus provide important information about BU mechanisms. Additionally, TFLM imaging might be used to improve tear surface or thickness images, by correcting for artifacts caused by the TFLM. For Nomarski microscopy, Twyman Green interferometry and low resolution tear film interference, an image of the lipid layer could be obtained by inserting a beam splitter at an appropriate position in the viewing optical path.

9.2. Quantitative analysis of tear film images

There is a need for more objective studies of BU based on quantitative analysis. For example, In Section 5, we distinguish between “immediate” and “evaporative” types of BU, but it is not clear whether there is a sharp dichotomy between these two types or whether these are simply extremes of a continuous distribution. An extensive, quantitative analysis of many examples of BU is therefore needed. For example, BU in an image could be quantified by the standard deviation of fluorescence intensity or by a measure of gradients in this intensity. In immediate BU, it would be expected that these measures would be relatively large as soon as a clear image is obtained after a blink, whereas, in evaporative BU, this measure would be small immediately after a blink and increase steadily over several sec. Further work is also needed to test the distinction between two types of immediate breakup – Marangoni driven, as in Fig. 2 (upper), 3, and 17 (left) compared to non-wettable cornea (Fig. 16).⁵

The surface shape in a BU area is still poorly understood. An example of a cross section of BU is given in Fig. 13C, but the resolution is not sufficient to show details of the rough surface of BU (Figs. 7 and 8), and the absolute scale of surface height is unknown. There is therefore a need for improved information about the surface structure of BU; this might be provided by higher resolution Twyman Green interferometry or by Nomarski microscopy (Sections 6.2.3 and 9.1.1). The development of surface shape before and after touchdown deserves study and should help explain why the slope of the surrounding tear prism is variable, e.g., Figs. 10–12.

As represented in Fig. 6, both the tear and corneal surfaces are rough, so both surfaces can contribute to variations in tear thickness. The roughness of the tear surface has been investigated in various ways (Sections 6.2 and 6.4), but the roughness of the corneal surface has been less studied. A possible method of study might be to use OCT after instilling a drop of artificial tears to thicken the tear film sufficiently so that reflections from the tear and corneal surfaces are clearly resolved and do not interfere with each other.¹²⁸ Liu and Pflugfelder¹¹ reported corneal topography measurements, showing increased surface irregularity in ADDE. Corneal topography is based on reflection from the tear surface, so it is not clear how much their finding corresponds to corneal surface irregularity rather than irregularity in PCTF thickness. If corneal surface roughness is increased in dry eye, then elevations on the corneal surface (Section 5.3) may trigger BU and help to explain reduced BUT.

Evaporative BU (Section 5.3) is caused by deficiency of the evaporation barrier of the TFLM. The thinning rate of the PCTF can increase up to about 20 $\mu\text{m}/\text{min}$ when the TFLM is defective, compared to a normal value of about 1 $\mu\text{m}/\text{min}$.³⁴ The thinning rate depends on

the evaporation resistance of the TFL, but also on the evaporation resistance of the adjacent air. Thinning rate measurements were performed in room conditions³⁴, with normal room air currents; it is possible that, in some cases, evaporation rate was limited more by evaporation resistance of air than that of the TFL. In outdoor conditions, subjects can be exposed to much higher air speeds, reducing the evaporation resistance of the air layer and potentially causing more rapid BU.⁵⁵ There is therefore a need to understand the relative importance of the evaporation resistances of TFL and air. For example, air currents generated by a fan could be used to study the effect of air velocity on tear film temperature, thinning rate and BUT.

9.3. Mathematical analysis and modeling of the tear film and breakup

Earlier mathematical modeling studies of the tear film have been reviewed by Braun¹⁴³, with further studies described by Peng et al.⁵⁵ and Braun et al.⁵⁴ Some aspects that require further analysis are considered here.

Improved imaging systems, discussed in Section 9.1, could provide information for more exact analysis of the factors in BU (Section 8). For example, an improved low resolution interference system for whole thickness fringes, discussed in Section 9.1.2, could provide better information about tear thickness distribution and also about tear surface irregularity (if it is assumed that corneal surface irregularity is small). This information could be used to quantify tear surface changes in terms of factors discussed in Section 8, such as evaporation, leveling, and osmosis.

The slope of the tear prism surrounding BU is described in Section 6.3.2. It is proposed that the steep slopes observed – up to 60 mrad (Fig. 10) may be due to a binding-spreading mechanism which squeezes out the aqueous layer between the TFL and corneal surface (Fig. 1C). Test of this proposal might involve simulations showing that such slopes are too steep to be generated by other mechanisms considered in Section 8, including localized evaporation, osmotic flow, leveling, advection, and diffusion of solutes. Modeling of the intermolecular forces between the TFL and the corneal surface, including glycocalyx, should also be considered.¹⁶⁵ The viscous drag of the surrounding tear prism should influence the rate of spreading. Additionally, the roughness of the corneal surface, including variation of cell to cell height⁹⁶, may impede the binding-spreading mechanism.

The development of tear BU can involve an “all-or-none” aspect of BU. For example, in Fig. 5D, 10 sec after a blink, three dark spots are indicated by arrows; by 60 sec after the blink, two are still present and have expanded, whereas one has faded away. Similarly, some of the dark areas (globes) in Fig. 2A1 have faded by Fig. 2A2, while others remain and develop. A possible explanation is that, for a given distortion of the tear surface, the flux (volume flow) of tears which causes leveling (Section 8.6) is proportional to the cube of tear thickness^{111,143}; for example, a hollow of a given shape with a central thickness of 2 μm will have 8 times the leveling rate of a central thickness of 1 μm . Thus, it is possible that leveling may be greater than the evaporation rate for the thicker tear film but not for the thinner film. The thicker film could then smooth out, whereas the thinner film would continue thinning, causing BU. Mathematical modeling could be applied to study this all-or-none effect.

As discussed in the preceding section, an important but poorly understood aspect of evaporative BU is the role of the pre-corneal air layer in restricting evaporation.⁵⁵ Evaporation can be modeled by the flow of current through an electrical circuit consisting of a battery discharging through two resistors in series, one for the TFLL and the other for the air layer in front of the eye.³ If evaporation is increased much above (e.g., 10 times) the normal rate, this implies that the sum of the two resistances is reduced by the same factor. However, it is not known how much the remaining resistance is due to the TFLL rather than the air layer. Modeling of the effect of the evaporation resistance of the air layer on tear film dynamics, in evaporative dry eye, could be used to determine whether the evaporation resistance of the TFLL ever becomes negligible, like a bare aqueous surface. If so, this would imply very high evaporation rates in windy, dry conditions.

To date, BU models have not included a dynamic lipid layer. The models could be improved by including the flow and deformation of the lipid layer during tear film movement and its effect on the evaporation and other processes in TBU. This effect could be important both in the processes leading up to touchdown, as well as the TBU spreading thereafter.

TBU models have also idealized the ocular surface itself to a planar semipermeable surface. The ocular surface is certainly more complex than that, with inherent roughness¹⁶⁶, the glycocalyx¹⁶⁷, various transport processes across the epithelial surface^{142,168}, signaling pathway activation and apoptosis¹⁶⁹, and epithelial cell sloughing and replacement.^{170,171} Models that can better incorporate interaction of TBU dynamics with the more detailed conditions at the ocular surface may shed new light on the interaction of hyperosmolarity and the cellular processes in the epithelium and the rest of the cornea.

The interaction of blinking and flow over the exposed ocular surface may be important in the conditions leading to the onset of TBU. This may be of particular importance in Marangoni-driven BU, which occurs rapidly following a blink. Currently, flows over the exposed ocular surface have been described by mathematical models during the interblink with stationary lid margins.^{74,172} Models that including blinking are likely to advance our understanding of TBU that occurs rapidly after a blink.

9.4. Molecular aspects and clinical implications

The importance of the TFLL in BU development has been emphasized in this review. Evaporative BU (Section 5.3) is caused by subnormal evaporation resistance of the corresponding area of the TFLL. Abnormality of the TFLL may also cause immediate BU by the deposition of thick globs of lipid during the upstroke of the blink (Section 5.1). The globs expand rapidly by Marangoni flow (Section 7.3), causing divergent flow of tears and immediate BU. It is thus important to understand the causes of deficiency in the TFLL.

With regard to defects in the evaporation barrier of the TFLL, Rosano and La Mer¹⁷³, in their study of evaporation resistance of lipid monolayers (e.g., fatty acids, alcohols, and esters), showed that only molecules with long saturated hydrocarbon chains provide a good barrier to evaporation. King-Smith et al.⁵⁰ proposed a model of the evaporation barrier the TFLL which is based on non-polar “lamellae” containing such dense arrays of long, saturated chains. This model is similar to the multi-lamellar evaporation barrier in the

skin¹⁵⁸ and is consistent with the demonstration of lamellae in meibum by Xray methods.⁴⁸ Archer and La Mer¹⁷⁴ calculated that a tiny fraction of defects in a monolayer, 0.1%, can reduce its evaporation resistance by a factor of 3. Breakdown of the TFL evaporation barrier may thus be due to holes or defects, which may be quite small, in what may normally be continuous lamellae. Experimental evidence for holes in the TFL leading to evaporative BU in vivo has been directly observed, as shown in Fig. 6 of King-Smith et al⁹⁷; the surrounding TFL structure was not revealed by those methods, however. The X-ray methods of Leiske et al.⁴⁸ have been interpreted to mean that there are localized crystallites with ordered arrangement of non-polar lipids; the theory of continuous layers of ordered non-polar lipids⁵⁰ is not experimentally confirmed at this time. The coarse-grained molecular dynamics simulations of Cwiklik et al,¹⁷⁵ do not show long-range order of non-polar lipids within the limits of their computations. Evidently, a better understanding of the structure of the TFL should elucidate the origin of subnormal evaporation resistance in evaporative dry eye. Such understanding of this complex issue is likely to come from efforts that make use of *in vitro*, *in vivo* and *in silico* methods.

The TFL may be influenced by a number of factors that change its composition and structure. Bacterial esterases can break down wax and cholesteryl esters into free fatty acids, alcohols, and cholesterol^{176,177}; these breakdown products may disturb the structure of the TFL and cause increased evaporation. Meibomian gland dysfunction involves keratinization of the meibomian ducts, with inclusion of keratins in meibum,¹⁷⁸ which may affect both the evaporative and viscous properties of the TFL. The possible contributions of sebum to BU was discussed at the end of Section 4.2.

Yokoi and Georgiev⁵ have emphasized a concept they call “tear film oriented therapy,” in which BUT and the BU pattern are used to diagnose the tear film abnormality, which can then be treated appropriately. This approach to dry eye therapy has yielded successfully treated subjects, and so it is likely that in at least some of their subjects the effort to address wettability of the corneal surface yielded a benefit. Evidently, the ability to distinguish between different times of BU depends on the interpretation of BU observations could aid in deciding between their course of treatment or perhaps others, and so emphasizes the importance of developing further understanding of BU mechanisms. Cases that may appear to be driven by lipid globs may be addressed by therapies that include applying heat to promote a more fluid and uniform lipid spreading (Lipiflow). Elevated osmolarity and longer BUTs may suggest evaporation driven BU and still different courses of treatment.^{103,104,106}

10. CONCLUSIONS

Tear film BU is an essential characteristic of dry eye. BUT is reduced in dry eye, so it is an important clinical test. BU often causes high PCTF osmolarity and may sometimes cause mechanical shear of the cornea; hence, BU stresses the corneal surface causing irritation and inflammation.

The definition of BU suffers from uncertainty (does the tear film form a dry spot?) and lack of objectivity (e.g., how dim should fluorescence be at BU?). It is proposed that a more

objective definition would be “touchdown” – the moment when the lipid layer touches the corneal surface. However, a better understanding of tear film dynamics is needed for a precise determination of the moment of touchdown.

A structure of tear film BU after touchdown is proposed. The BU area exposes the rough corneal surface, and it may become concave from the effect of evaporation. The BU area can be surrounded by a steep tear prism.

BU is important both as a clinical test and as core mechanism of dry eye, contributing to ocular surface disorder and inflammation. When (as commonly) BU is largely due to evaporation, it causes very high osmolarity, which can greatly exceed osmolarity measured from the meniscus. BU is associated with a rapid increase in corneal sensation, which may be related to either tear hyperosmolarity or shearing of the corneal surface. BU causes optical aberrations and scatter; the role of the BU area and the surrounding tear prism in optical effects is considered.

Early theories of BU were largely based on surface physical chemistry models but were limited by imperfect experimental evidence, e.g., about the thickness and evaporation rates of the tear film. In this review, it is emphasized that no single and simple theory of BU can explain all observations. Rather, BU is a complex process involving some ten factors before and after touchdown.

A three-way classification of BU is proposed, and example images are shown. “Immediate” BU may be seen as prominent dark areas of fluorescence as soon as a stable image can be obtained after a blink. It is often associated with thick “globbs” of lipid in the TFLM that drive rapid tangential flow due to the Marangoni effect. Immediate BU may also be caused by non-wettable areas of corneal surface, corresponding to previous BU areas. “Lid-associated” BU is seen under the upper meniscus; it is largely caused by divergent flow of tears into the low pressure volume of the meniscus. “Evaporative” BU occurs when the other types are not evident and is probably the most common cause. A novel phenomenon of “afterimages” can occur after a further blink in any of the three types of BU. Additionally, BU can show an “all-or-none” characteristic so that some dark spots in a fluorescence image continue to strengthen, forming obvious BU, whereas other dark spots fade and disappear.

Five types of imaging studies can be used to study BU. First, fluorescence imaging studies can take advantage of self-quenching – reduction of fluorescence efficiency for high but not low fluorescein concentrations. Self-quenching can provide information about the role of evaporation in tear film thinning and BU. Second, reflections from the corneal surface can be used to show irregularity in the tear surface by distortions in a reflected pattern, defocus microscopy, and interferometry. Third, interference effects in the tear film can be studied; some new, high-resolution, “chromaticity” images of BU and the surrounding tear prism are presented. Fourth, light may be reflected from the retina, and irregularity of the tear surface can be studied by distorted refraction of the emerging beam. Fifth, the thermal infrared radiation from the corneal surface can be imaged. Finally, combining these different methods provides important information about tear film characteristics and dynamics leading to BU.

Three different directions of tear flow can contribute to or counteract tear film thinning and BU, namely evaporation, osmotic flow through the epithelial surface and divergent flow of tears out of an area. Evaporation and divergent flow are the two processes that can contribute to tear thinning and BU, while osmotic flow opposes tear thinning. Divergent flow contributes to both immediate and lid-associated BU, while evaporation is probably the most common factor in BU over the corneal surface distant from the lid margin.

A quantitative understanding of BU involves contributions from at least ten factors. Tear film deposition is important in ADDE, while evaporation is obviously important in EDE. Evaporation causes hyperosmolarity, which causes osmotic flow out of the corneal surface and helps to counteract the thinning from evaporation. In the case of local high evaporation, a hollow is generated in the tear surface; this causes low pressure and inward flow of tears, again helping to counteract the thinning from evaporation. This inward flow of tears carries tear solutes with it, while solutes diffuse in the opposite direction. Lipid droplets in the TFLM may speed BU by touching down on the corneal surface. After touchdown, it is proposed that the BU area may spread by a “binding-spreading” interaction between the polar lipid interface and the glycocalyx.

Future directions of study should involve improved imaging systems, both for reflections from the tear surface and for tear thickness interference. More quantitative analysis of BU is needed, e.g., how common is immediate compared to evaporative BU and how do they relate to other clinical findings? There is scope for further mathematical analysis and modeling of processes leading to BU, e.g., the role of the pre-corneal air layer in restricting evaporation. Because the TFLM is key to an understanding of the role of evaporation in BU, a better understanding of its structure is needed, together with the possible role of esterases, keratins and sebum.

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Fig. 1. Schematic diagram of tear film “touchdown” on the corneal surface (not to scale). This divides the post-blink interval into pre- and post-contact phases. In this example, it is assumed that tear thinning, leading to touchdown, is due to local high evaporation. See text for details.

Fig. 2.

Fluorescence images of BU, recorded with the optical system of King-Smith et al.⁵⁷ Time after a blink is given except in Panel D, which was after a second blink. The gain of the camera system was kept constant for each row (but varied from row to row). The upper row is from a 45-year-old male, dry eye, after instillation of 1 μ L of 0.1% fluorescein; this subject blinked a second time at just over 8 sec after the first blink. The middle row is for a 26-year-old female, dry eye, after the same fluorescein instillation, while the bottom row is for the same subject as the middle row but after instillation of 1 μ L of 5% fluorescein. F= lens fluorescence; r= reflections of the two light sources; IBI= inter-blink interval. Scale bars 1 mm.

Fig. 3.

The role of the lipid layer in immediate BU. A. Simultaneous recording of immediate BU in a fluorescence image (after instillation of 5 μ L of 2% fluorescein) and of “globs” of thick lipid in the TFL. 33-year-old white female, dry eye (Modified from King-Smith et al.⁹⁷). B and C. Unstained color images of a glob at different times after a blink. 22-year-old normal white female. Right panel is a color key of lipid thickness (Modified from Braun et al.⁵⁴). Time after the blink is given for each image. Scale bars are 1 mm.

Fig. 4. “Afterimages” of BU in fluorescence images. The upper row shows examples of BU areas (b). The lower row shows afterimages, a, from these BU areas after the next blink. A. Immediate BU, repeat of Fig. 2A3. (f=lens fluorescence; r=reflections of light sources). B. Afterimages of BU areas. C. Lid-associated BU. D. Afterimages after a partial blink covered about a quarter of exposed cornea. E. Evaporative BU. F. Afterimages after a partial blink covered about a half of the exposed cornea.

Fig. 5. Fluorescence BU near the upper lid, after instillation of 1 μ L of 0.1% fluorescein, at indicated times after a blink. 34--year-old normal white male. S=sustained dark area; t=transient dark area. Scale bar is 1 mm.

Fig. 6.

Five different types of methodology for imaging the tear film (not to scale). The corresponding section number for each type of method is given. Enlarged section on the right shows the relation between tear thickness (h), corneal surface (c) and tear (air) surface (a).

Fig. 7.

Effect of focus on appearance of BU (natural image without fluorescein). A. Optical effect of reflection from a hollow (h), on the tear film surface (t), when the tear film is too far, i.e., behind the plane of focus (f) of the optical system. I=incident rays; r=reflected rays. B. Appearance of BU ((b), when the tear surface is too far away. C. Appearance when the tear film is in focus. D. Appearance when the tear film is too close. See text for details.

Fig. 8.

Unstained tear film images of the rough surface and steep banks of BU areas (Panels D and E). Three thick lipid droplets (l) in Panel A move upwards until Panel C, where they become fixed to the cornea and generate BU “dots” (d) in Panels D and E. Panel B shows part of Panel A with contrast increased five times to show details. A thinner, (slightly darker) patch of lipid (t) in Panels A and B, eventually gives rise to a “streak” BU (s) in Panels D and E. p=“pool” BU area; g=a gradient between the rough surface in BU and the smooth surface of the surrounding tear film. 22-year-old Asian female, dry eye. Time after blink is given at lower right. Scale bars, 1 mm. Based on King-Smith et al.³

Fig. 9. Low resolution whole-thickness interference fringes. A. PCTF, 27-year-old, white male. Contrast increased 8 times. Method of King-Smith et al.¹²¹ B. PLTF (contrast not increased). Wavelength for both images, 850 nm. B=BU areas.

Fig. 10.

A. High resolution recorded color image of the tear film, recorded 27 sec after a blink. Contrast has been doubled. B. “Chromaticity image” emphasizes color information while eliminating intensity information. B=BU areas; d=lipid “droplets” (black arrow points to droplet outside BU areas); p=interference fringes in the surrounding tear prisms. An approximate scale of PCTF thickness is shown at right. 54-year-old white, normal female. See text for details.

Fig. 11.

A. High resolution recorded color image of the tear film, recorded at least 17 sec after a blink. Contrast has been doubled. B. Chromaticity image. B=BU area; g=apparent gaps between superficial cells, p=tear prism surrounding BU. 62-year-old, white, normal female.

Fig. 12.

A. High resolution chromaticity image recorded 11.3 sec after a blink. B. Corresponding area recorded 20.7 sec after the blink, i.e., 9.4 sec later. Because of eye movements between the two images, only overlapping areas are shown. B=BU area; p=surrounding tear prism. 49-year-old, normal, white female.

Fig. 13.

A. Retroillumination image. B. Simultaneous fluorescence image. C. Analysis of intensity distributions along the corresponding bars in A and B. The blue line is the fluorescence intensity distribution and the red line is the integrated retroillumination image. A horizontal blue dashed line has been fitted by eye to the fluorescein BU area, while sloping blue dashed lines have been fitted to the surrounding tear prism. The intersections of these lines give an estimate of the BU width and this has been marked by vertical green lines. 25-year-old white male, dry eye. See text for details. Data from Braun et al.⁵⁴

Fig. 14.

A. Shack-Hartmann aberrometry. The spots show images from the lenslets of the Shack-Hartmann array. The grid shows the expected positions if there is no optical aberration. Arrows show how three spots are deviated from their expected positions. B. Fluorescence image obtained after the same blink. The pupil had been dilated with tropicamide and anesthetized with proparacaine. Time after blink is given at the lower left. 30-year-old white male, normal. Scale bars, 1 mm. Based on Himebaugh et al.⁸⁹

Fig. 15.

A. “Micro-aberrations” within the circular outline in Shack-Hartmann aberrometry. B. Fluorescence image after the same blink. C. Retroillumination image after the same blink. All images obtained after the same blink. Time after blink is given at the lower right. 45-year-old white female, normal. Scale bars, 1 mm. Based on data from Himebaugh et al.⁸⁹

Fig. 16.

A. Unstained image of the PCTF, showing multiple BU areas, 24 sec after a blink. B. Immediately after an additional partial blink which reached the curved line between the two white arrows. B=areas of BU which still remain in the region covered by the partial blink. 51-year-old, normal white female. Contrast doubled.

Fig. 17.

Left column - "immediate breakup," driven by Marangoni (surface tension gradient) flow. Density of shading represents tear osmolarity. A. Immediately after a blink, the high concentration of polar lipid in a "glob" lowers its surface tension. The surface tension gradient causes rapid divergent flow of aqueous tears. B. At a slightly later time, the divergent flow caused by lipid spreading is balanced (on average) by an inward flow of tears into the low pressure region in the concave, central hollow. C. Expansion of the lipid layer has ceased, so the low pressure in the central region now generates an inward flow of tears. This carries solutes (salts, etc.) by advection. Evaporation in the thin central region has increased tear osmolarity slightly, as well as osmotic flow of water out of the cornea. When touchdown is completed somewhat later than the initial rapid thinning, there will be some outward diffusion of solutes. Right column - "evaporative breakup." Density of shading represents tear osmolarity. D. Soon after a blink, local evaporation has generated a hollow in the tear surface as well as increased osmolarity. The hollow causes leveling involving inward aqueous flow and advection of solutes. Increased local osmolarity causes osmosis and outward diffusion of solutes. E and F. At later times, central osmolarity, aqueous flow, advection, osmosis and diffusion tend to increase.

Table 1

Imaging methods for studying mechanisms of tear film breakup

Method	Review section	Typical image size	Typical pixel size	Studied parameter	Sampling ^d	References or figures
Fluorescence, high fluorescein concentration	6.1, fluorescence	Exposed ocular surface	25 μ m	e_{rh} ^b	Continuous	57
Fluorescence, low fluorescein concentration	6.1, fluorescence	Exposed ocular surface	25 μ m	r_{rh} ^b	Continuous	57
Distortions in a reflected pattern	6.2, tear surface reflection	10 mm	25 μ m	Tear surface quality (slope variation)	Discrete	20
Defocus microscopy	6.2, tear surface reflection	6 mm	6 μ m	Tear surface curvature	Continuous	Fig. 7
Nomarski microscopy	6.2, tear surface reflection	1.5 mm	(5 μ m?)	Tear surface slope	Continuous	32
Twyman-Green interferometry	6.2, tear surface reflection	6 mm	6 μ m	Tear surface height	Continuous	30
Lateral shearing interferometry	6.2, tear surface reflection	4 mm	10 μ m	Tear surface height difference between two positions	Continuous	27
Optical coherence tomography, OCT	6.2, tear surface reflection	2.5 mm	10 μ m	Tear surface height and tear film thickness	Continuous	120
Low resolution tear film interference	6.3, tear film interference	9 mm	15 μ m	Tear film thickness	Continuous	121
High resolution chromaticity images	6.3, tear film interference	0.2 mm	0.5 μ m	Tear film thickness	Continuous	Figures 10 to 12
Retroillumination	6.4, tear surface refraction	6 mm	15 μ m	Tear surface slope	Continuous	89
Shack-Hartmann aberrometry	6.4, tear surface refraction	6 mm	7 μ m	Tear surface slope	Discrete	122
Thermography	6.5, thermal imaging	Exposed ocular surface	70 μ m	Tear temperature	Continuous	28

^a,"Continuous" means that all pixels in the image provide information. "Discrete" means that only some pixels provide information, thus limiting spatial resolution.

^b Parameters: e, fluorescence efficiency (reduced by quenching). r, reduction factor of fluorescence caused by the absorption of light in the outer tear film which reduces the illumination of the inner tear film. f, fluorescein concentration in the tear film. h, tear film thickness.