High Resolution Microscopy of the Lipid Layer of the Tear Film

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ABSTRACT Tear film evaporation is controlled by the lipid layer and is an important factor in dry eye conditions. Because the barrier to evaporation depends on the structure of the lipid layer, a high resolution microscope has been constructed to study the lipid layer in dry and in normal eyes. The microscope incorporates the following features. First, a long working distance microscope objective is used with a high numerical aperture and resolution. Second, because such a high resolution objective has limited depth of focus, 2000 images are recorded with a video camera over a 20-sec period, with the expectation that some images will be in focus. Third, illumination is from a stroboscopic light source having a brief flash duration, to avoid blurring from movement of the lipid layer. Fourth, the image is in focus when the edge of the image is sharp – this feature is used to select images in good focus. Fifth, an aid is included to help align the cornea at normal incidence to the axis of the objective so that the whole lipid image can be in focus. High resolution microscopy has the potential to elucidate several characteristics of the normal and abnormal lipid layer, including different objects and backgrounds, changes in the blink cycle, stability and fluidity, dewetting, gel-like properties and possible relation to lipid domains. It is expected that high resolution microscopy of the lipid layer will provide information about the mechanisms of dry eye disorders. Illustrative results are presented, derived from over 10,000 images from 375 subjects.

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I. INTRODUCTION

The role of evaporation in dry eye disorders is well established.[1] Tear hyperosmolarity, one of two "core mechanisms" of dry eye disorders,[1] is related to an increase in the ratio of evaporation rate to tear secretion rate.[2] In evaporative dry eye (**EDE**), evaporation rate is increased with normal tear secretion. In aqueous deficient dry eye (**ADDE**), tear secretion rate is reduced with normal evaporation. Therefore, the ratio of evaporation to tear secretion rate, and, hence, osmolarity, is increased in both EDE and ADDE, and evaporation is important in both disorders.[2,3] Correspondingly, hyperosmolarity may be the best marker of dry eye severity.[4] It is an important cause of ocular surface inflammation and damage.[5,6] The osmolarity of the precorneal tear film may reach much higher levels than reported values obtained from the tear meniscus.[5,7,8]

The other core mechanism of dry eye is tear film instability, which is assessed by tear film breakup time (**TFBUT**) measurements.[1] We have argued that breakup is closely related to the thinning rate (reduction of tear thickness) of the tear film, which can be observed between blinks,[9] and that this thinning is largely due to evaporation.[8,10,11] Thus, there is evidence that evaporation plays an important role in both hyperosmolarity and tear film instability and in both EDE and ADDE. Correspondingly, tear evaporation rates have been reported to be increased in dry eyes.[12,13]

Evaporation is controlled by the lipid layer of the tear film. Evaporation rate of the rabbit precorneal tear film was increased by a factor of 15, from 0.47-7 μm/min, after the lipid layer was washed away.[14] Correspondingly, evaporation from the human tear film has been related to lipid layer pattern.[15] A report that both evaporation and meibomian gland dropout increase with age is consistent with an expected correlation between evaporation and lipid thickness.[16]

If tear thinning between blinks is largely due to evaporation,[8,10,11] then tear thinning rate should be inversely related to mean lipid thickness. This has been demonstrated by

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"spectral interferometry"; for example, five subjects with lipid thickness less than 24 nm all had "rapid" thinning rates of over 4 μm/min, whereas 31 of 33 subjects with lipid thickness over 30.5 nm had "slow" thinning rates of less than 4 μm/min.[17] However, the correlation between thinning rate and lipid thickness was not perfect, with a Spearman correlation of R = -0.385. Possible reasons for this lack of perfect correlation include contributions of nonevaporative mechanisms, such as tangential flow of tears (along the corneal surface), measurement errors in lipid thickness and thinning rate, the effect of variability of precorneal air currents on evaporation rate, the effect of lipid layer composition and structure on evaporation, and nonuniformity of lipid layer thickness within the 33 μm diameter measurement spot. With regard to this last reason, it was found that fits to the observed reflection spectra could be improved by assuming that the lipid thickness varied within the measurement spot, but it was not possible to determine the exact characteristics of this thickness variation.[17]

To appreciate the possible contribution of thickness variation to evaporation rate, consider the rabbit tear film discussed above,[14] but after the lipid layer over half the cornea has been removed and added to the other half to make a double thickness. The total evaporation over the exposed half will be half that of an eye totally denuded of lipid, ie, 15/2 = 7.5 times greater than that for a uniform lipid layer having the same total precorneal lipid. Even ignoring evaporation from the other half of the corneal surface, evaporation would be greatly increased by this redistribution of lipid. Thus, evaporation depends not only on mean lipid thickness but also on its spatial variability.

An aim of this study was therefore to investigate the spatial variability of the lipid layer with a purpose-built microscope of the highest practical resolution. Previous purpose-built systems for imaging the lipid layer have generally covered a large area with relatively low resolution,[18-22] whereas higher resolution microscopy has used systems designed for other purposes, such as differential interference microscopy,[23-25] slit-lamp biomicroscopy,[23,26] noncontact endothelial cameras,[20,25] specular microscopy,[27] and confocal microscopy.[28,29] Limitations of such systems may include objectives of relatively low numerical aperture (thus limiting resolution), blur due to lipid layer movement during relatively long exposures, and lack of focusing and alignment aids; the current system was designed to minimize such limitations.

In addition to investigating spatial variability, this study provides details of the fine structure of the lipid layer and its changes in the interval between blinks. While this study demonstrates the characteristics of images obtained from both normal eyes and eyes with dry eye disorders, a more systematic analysis of differences between dry and normal eyes will be deferred to future publications.

Table 1. Nomenclature

Name	Example Figures
Backgrounds	
Sea	6, 8, 9, 10, 13
Irregular	5, 7, 11, 12
Thick objects	
Spots	6
Lenses	8, 9
Islands	8, 9
Snowflakes	10
Clouds	10
Droplets	5, 7b, 11, 12
Thin objects	
Lakes	8a, 9, 13
Dots	14, 15

II. THE MICROSCOPE

A. Design Features

The microscope includes the following five features. First, a long working distance (13 mm) microscope objective is used with the highest readily obtainable numerical aperture (0.6). Second, because such a high resolution objective has a limited depth of focus (about 1 μm), 2000 images are recorded with a video camera over a 20-sec period, with the expectation that some images will be in focus. Third, illumination is from a stroboscopic light source having a flash duration of less than 0.1 msec, to avoid blurring from movement of the lipid layer and eye. Fourth, it is arranged that the image is in focus when the edge of the image is sharp — in this way, it is possible to decide whether an apparently blurred image is due to lack of sharp features or to poor focus. Fifth, an aid is included to help align the cornea at normal incidence to the axis of the objective so that the whole lipid image can be in focus.

B. The Recorded Image

Figure 1 shows that the microscope records three "spots." On the left, the "image spot" records a 200