Improvement and evaluation of tearscopy potentials

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Abstract

New potentials of improved tearscopy technique based on photoregistration of colour interference phenomenon are presented. A new softwear "Lacrima" was developed in order to objectivate the results and to get more detailed data, it allows to reveal data on lipid layer thickness (LLT), estimate relative area of examined zone (in %) with certain LLT as well as regularity of lipids distribution. Digital images of interference picture of precorneal tear film of 13 healthy objects (26 eyes) and 20 patients with exogenous dry eye syndrome were analyzed. In eyes with dry eye syndrome compared to normal eyes increase of uncertainty zone and zone with minimal LLT were revealed for 13,2 and 12,8% respectively. Relative area of zones with increased LLT decreased for 16,7 and 56,3 % respectively. Computer analysis of interference picture of precorneal tear film using softwear "Lacrima" allows to extend capabilities of tearscopy and get additional data on lipid layer status.

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The conjunctival cavity constantly contains about 6—7 mcL of lachrymal fluid. Blinking motions cause tear to spread over the surface of the anterior segment of the eyeball in the form of thin tear film.

The tear film that covers the cornea surface, is called precorneal tear film. Its complex biochemical composition and structure support important physiological functions. Of these functions, primary are protection, oxygen and nutrients transportation to epithelial layers of cornea and conjunctiva as well as removal of residual metabolites and dead epithelial cells.

While lubricating the eyeball surface the tear film forms the anterior surface of the primary refractive medium in optical system [1, 2]. In order to exercise these functions and provide the conditions for the normal functioning of the corneal and conjunctival epithelium, the tear film should have certain composition, structure and sufficient stability. The tear film is nonhomogenous; its aqueous layer (98% of optical section) contains dissolved mucoproteins, their concentration increases closer to the epithelial membrane. The mucin layer is formed when water-mucin gel with the highest mucoproteins concentration contacts the surface of epithelial cells.

The external layer is a thin bilaminar lipid film. The polar lipid layer (phospholipids, sphingomyelins etc.) is located at the water-lipid boundary. The air-lipid boundary is formed by non-polar lipids (cholesterols, triglycerids, free fatty acids). The interaction of all the components of precorneal tear film occurs at the action of ionic compounds and Van der Waals forces [3].

The stability and functionality of the structure is determined by the interaction of all its components. Certain diagnostic methods enable indirect assessing of the condition of water layer.

Impressive cytology method allows estimating density and the differentiation degree of epithelium goblet cells. Their secretion forms the mucin layer of tear film [10—15].

Schirmer's test and the height of lachrymal meniscus are indirect indications of water layer condition [1, 2, 4, 5, 6, 7].

The condition of meibomian glands orifices in intermarginal area together with meibography data may provide the information about the possibility of tear film lipid layer formation [16—18].

However, the results of these allow only to indirectly determine the condition of tear film layers . Visualization and direct examination is possible only for the lipid layer. Its visualization can be done using tearscopy method that is based on the photoregistration of colour interference phenomenon [8, 9, 19, 20—25].

The interference occurs as the result of interaction of light beams being reflected by two surfaces. Its prerequisite is the difference in coefficients of refraction, i.e. the presence of optical border.

When the light passes through the tear film, the beam goes through the border between air and lipid layer, their refraction coefficients being different. This difference is the cause of the partial refraction of light beams.

The boundary between lipid and water layers is the second optical border. Their refractory indices also differ, which causes the effect of partial refraction of light beams **(Fig. 1)**.

The colour of interference pattern in each point depends on the wavelength of the light beam that passes through the lipid layer and is reflected off its inner surface. The thickness of the lipid layer can be calculated on the basis of the interference colour for every point of the

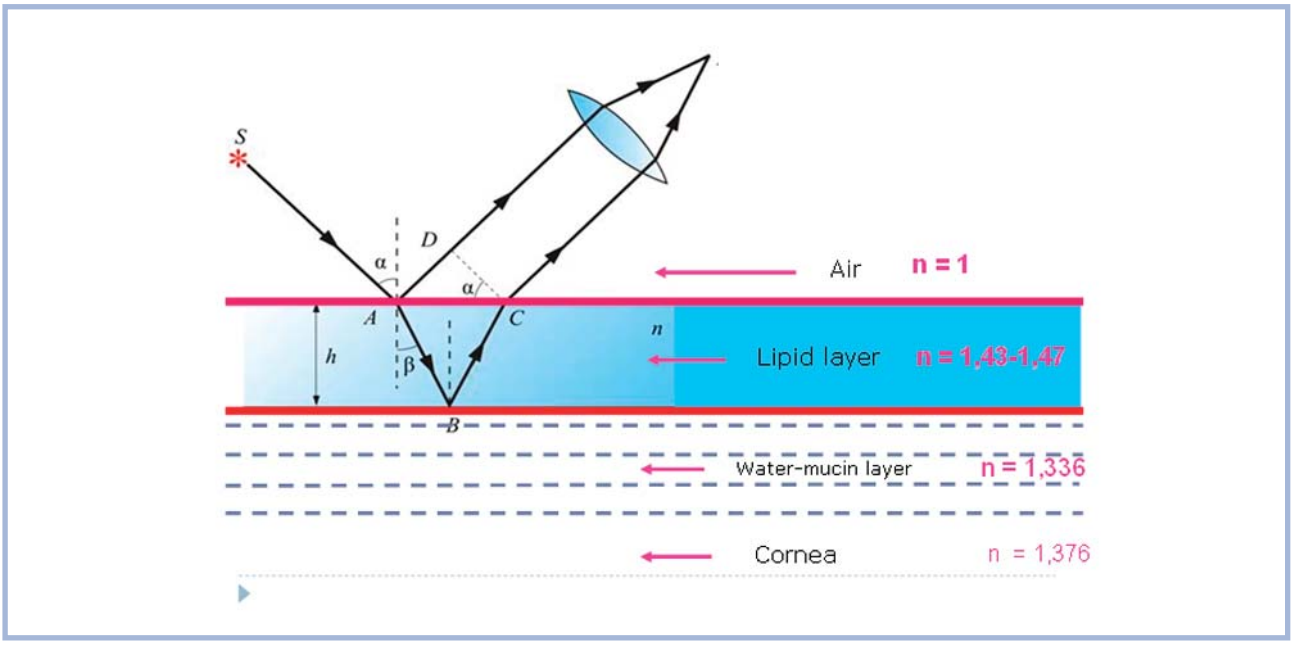

Fig. 1. **Interference scheme (n — coefficient of refraction).**

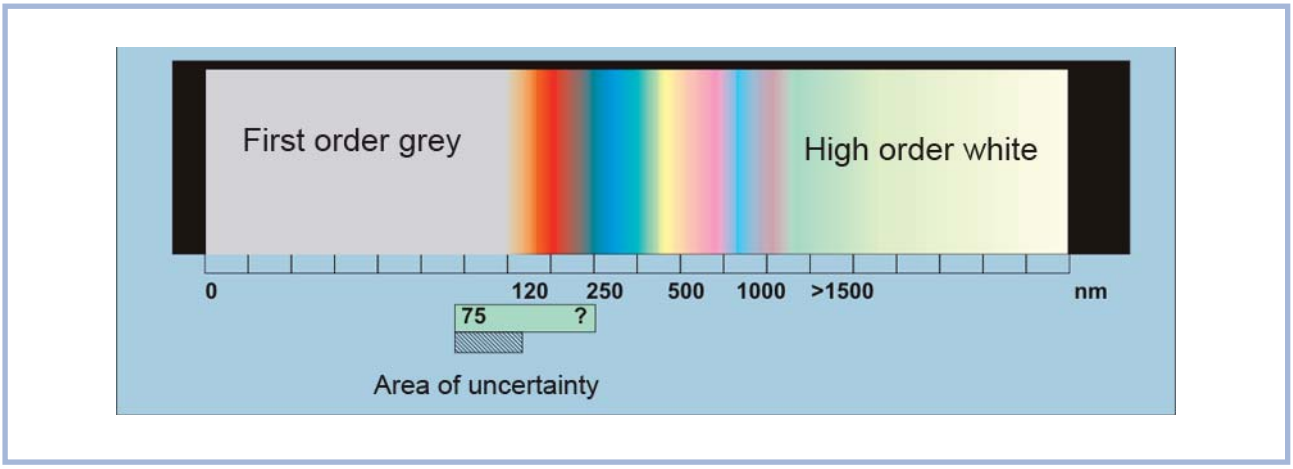

Fig. 2. **Nomographic chart of tear film thickness in accordance with interference colours for refraction coefficient of n = 1.46.**

area under examination. The appearance of iridescent spots of oil on water surface is explained by the occurrence of light interference in thin films [8, 25, 26].

The lipid layer interference pattern was first investigated by J.E. Donald in 1968 [21]. His work raised the interest of many scientists. In order to acquire images of interference pattern, special devices (tearscopes) were created). They are based on specific illuminators. The thickness of precorneal tear film layer was evaluated by interference colors. The images acquired were categorized according to features of the color pattern. Attempts were made to analyze the interrelations between various types of interference images and clinical features, particularly those of dry eye syndrome (DES), contact lenses tolerability and systemic diseases [3, 8, 9, 19, 20, 22, 26, 27].

Calculating the lipid layer thickness for each point of the area under examination is commonly performed using Michael-Levy chart. From the nomographic chart that indicates the interrelation between interference colours and film thickness we have picked out the color scale for lipid refraction coefficient **(Fig. 2)**.

However, this method of determining the thickness of lipid layer using nomographic chart, is only approximate and to a certain extent subjective, it doesn't provide full information about the layer structure and whether

lipids are evenly distributed over the water layer surface. Objectification of the study results and acquisition of more detailed information was achieved with the help of a unique self-developed PC software program "Lacrima". The program analyzes the digital image of interference pattern and shows information about lipid layer thickness; it allows to estimate relative surface area (as %) with its certain thickness and evaluate uniformity of lipid distribution (distribution coefficient is established in the program algorithm).

Material and methods

Digital images of the precorneal tear film interference pattern were acquired by means of an originally designed illuminator adapted to photo slit lamp and providing a uniform light reflex from the cornea surface.

Received images were analyzed using a PC software program "Lacrima".

In order to evaluate the informational content of this software, is was used to analyze digital images of precorneal tear film interference pattern in 13 normal patients (26 eyes) with no signs of Dry Eye Syndrome, and 20 patients with exogenous Dry Eye Syndrome. Of these patients, 15 had DES diagnosed during their first visit for contact lenses selection, 5 had been using contact lenses for a period of 1 to 15 years.

Computer images analysis is presented in diagrams (Fig. 5,6). The diagrams show relative surface area (as %) occupied by lipid layer of a certain thickness within the area under examination. The first bar of the diagram (n/d) corresponds to the area of uncertainty where the tear film is very thin, hence the evaluation of its thickness by this method proved to be impossible. The other bars of the diagrams are organized in ascending order by thickness of the precorneal tear film.

All the patients went through standard examination including a test of tear production (Schirmer's test) and a test for assessing precorneal tear film stability (Norn's test).

Results

The total level of tear production in DES patients was by 34.9% less than in the normal group. Norn's test results were by 55.9 % less, the difference being statistically-valid ($P < 0.05$) **(Table 1, Fig. 3)**.

A comparative study of precorneal tear film was conducted in non-DES cases and patient groups with signs of eye-watering distortions in order to determine the in-

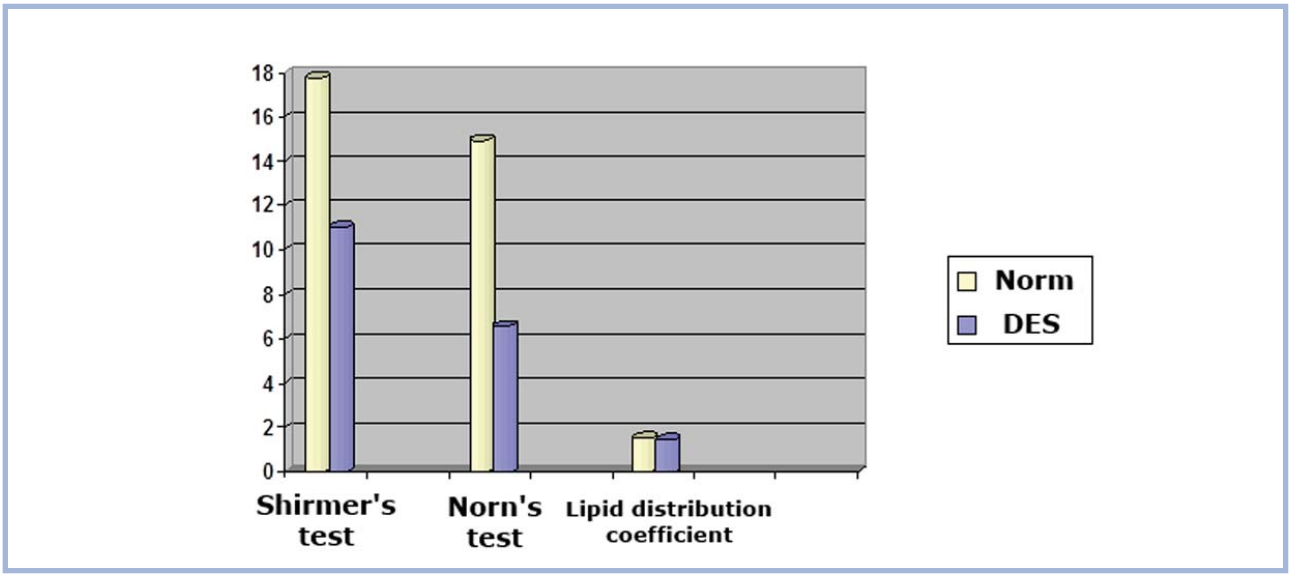

Fig. 3. **Total tear production, precorneal tear film strength and lipid distribution coefficient in norm and DES cases.**

Table 1. **Precorneal tear film condition, total level of tear production and lipid distribution coefficient in norm and DES cases (M±σ)**

Measurements	Norm	DES	Confidence level
Shirmer's test (mm)	17,80±5,9	11,05±2,9	$p<0,05$
Norn's test (sec)	14,95±4,9	6,59±3,1	$p<0,05$
Lipid distribution coefficient (K)	1,51±0,3	1,46±0,3	$p>0,05$

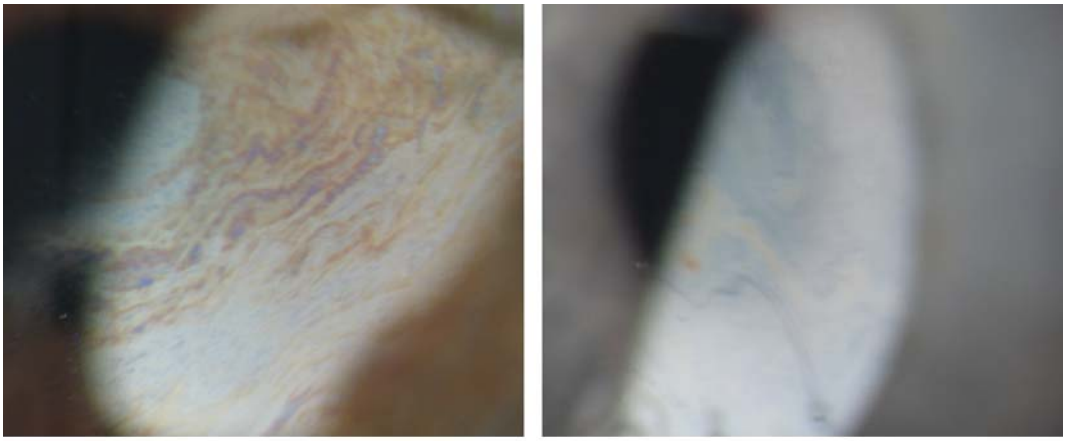

Fig. 4. **Interference pattern of precorneal tear film in norm (a) and DES (b) cases).**

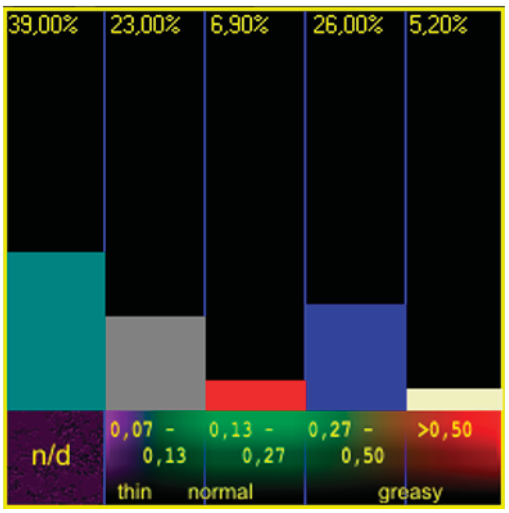

Fig. 5. **Lipid layer thickness of precorneal tear film in norm (a). Computer model of the precorneal tear film (b).**
Lipid distribution coefficient (K) =1,7.)

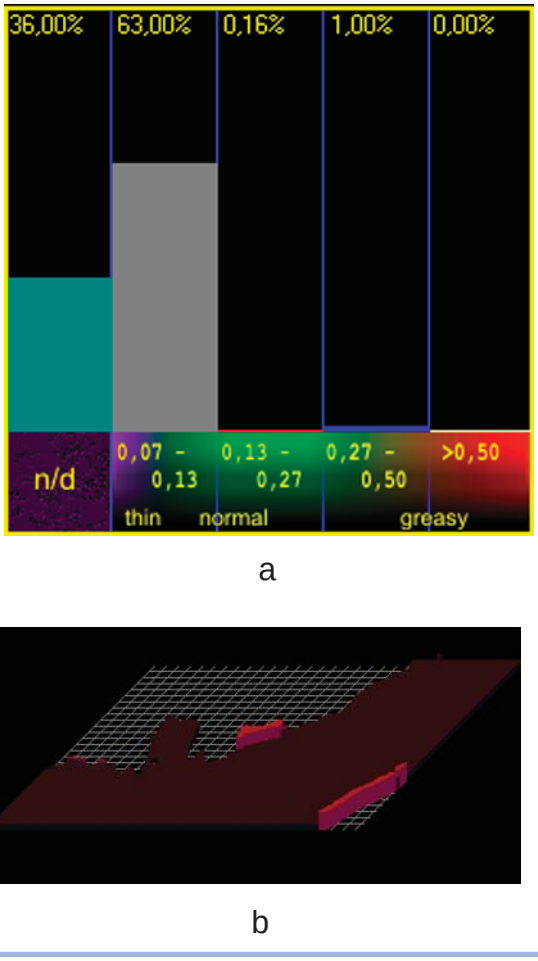

Fig. 6. **Lipid layer thickness of precorneal tear film in DES patient (a). Computer model of the precorneal tear film (b).**
Lipid distribution coefficient (K) =0,53.

Table 2. **Lipid layer thickness of precorneal tear film in norm and DES cases (M±σ)**

Thickness (micron)	Relative surface area under examination (%)		Confidence level
	Norm	DES	
Uncertainty area	38,0±0,06	43,0±0,08	$p<0,05$
0,07—0,13	39,0±0,11	44,0±0,09	$p<0,05$
0,13—0,27	6,0±0,04	5,0±0,04	$p<0,05$
0,27—0,5	16,0±0,11	7,0±0,07	$p<0,05$
>0,5	1,0±0,02	2,0±0,06	$p>0,05$

formative value of computer analysis of interference pattern . The results can be demonstrated by clinical examples **(Fig. 4, 5, 6)**. The differences in received images are clearly evident (Fig. 4), which is supported by computer modelling of the tear film (Fig. 5B, 6B).

Detailed analysis of lipid layer thickness performed by means of "Lacrima" software is shown in diagrams (Fig. 5A, 6A). When compared to DES cases results, higher value of relative surface with minimal lipid layer thickness (0.07-0.5 micron) was found in contrast with the results of tear film examination in patient with no signs of DES (63% and 23% respectively). High thickness areas in DES cases are minimal while areas with lipid layer of more than 0.5 micron are completely absent.

Significant decrease of the lipid distribution coefficient (0.53) was noted with Dry Eye Syndrome signs in relation to the value (1.7) acquired during the examination of tear film in a patient with no clinical signs of its distortions.

Statistical analysis of data in the two patient groups showed differences in the lipid layer structure and allowed to estimate the correlation between relative surface area and its different thickness **(Table 2; Fig. 7)**.

Compared to norm, the increase of uncertainty area and area with minimal lipid layer thickness (by 13.2% and 12.8% respectively) was noted in Dry Eye Syndrome cases. The relative surface area of high lipid layer thickness decreased by 16.7% and 56.3% respectively. The difference was statistically unreliable in areas with lipid layer

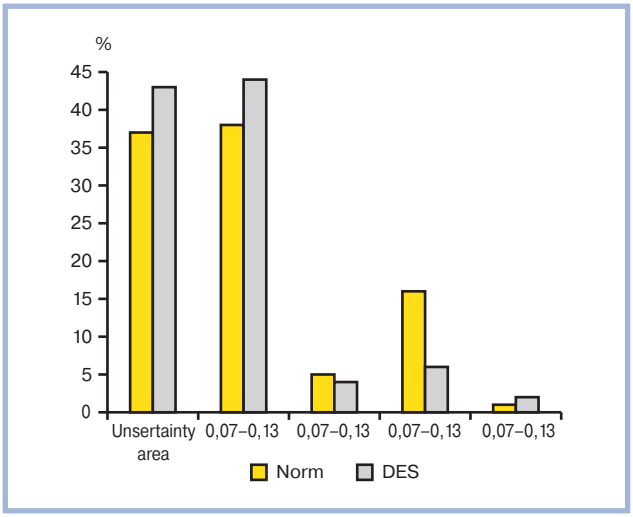

Fig. 7. **Lipid layer thickness of precorneal tear film in norm and DES cases (mean values).**

of more than 0.5 micron. However, certain extensions of such areas were seen in DES cases, which can be related to inequality of lipid distribution over the surface of water-mucin layer.

Distribution coefficient differences were statistically unreliable. Decrease tendency was noted in DES cases (mean values were 1.51 for norm cases and 1.46 for DES cases) (Fig. 3).

Conclusion

As can be seen from the above, computer analysis of precorneal lipid layer interference pattern using "Lacrima" software allows to extend the potential of tearscopy method and aquire additional information on lipid layer condition.

The method may help diagnose various forms of Dry Eye Syndrome and prescribe adequate medical treatment.

The method is fairly informative and useful for assessment of treatment efficiency.

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