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Manuel F. M. Costa
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Introduction

Celebrating Optics and Photonics and its outstanding positive impact in the world and in our everyday life (largely resulting from the current remarkable success and fast sustainable development in optics and photonics research, the Portuguese Society for Optics and Photonics (Portugal), SPOF - Sociedade Portuguesa para a Investigação e Desenvolvimento em Óptica e Fotónica (Portugal)) successfully organised its third triennial international conference on Applications of Optics and Photonics, 8 – 12 May, at the Universidade do Algarve in the lovely city of Faro, Portugal.

The conference was organized to foster the establishment of the widest range of cooperation projects and relationships with colleagues and institutions from all around the world, while increasing the external visibility of Portugal's optics and photonics research.

The success of the conference came from the enthusiastic commitment of the Portuguese optics and photonics community and of our friends from all over the world, and the endorsement and active support of the most important international scientific optics societies:

ICO, SPIE, EOS, RIAO; several national societies, committees, and boards: SEDOPTICA- Sociedad Española de Óptica (Spain), AMO (Mexico), STO (Tunisia), OPSS (Singapore), CVO (Venezuela), RCO (Colombia), SOFE (Ecuador), and SPF (Portugal);

other photonics and optics industries and companies, as well as the following projects and initiatives: Laserlab-Europe, iBROW, INNOVA, Laser World of Photonics, OPA, OQEJ-Springer, adLASER, MTBrandão, Lda.;

and the contribution from the authors and presenters of the 191 effectively presented talks in all domains of optics and photonics.

Of the over two hundred works presented, the conference included 32 invited lectures from world leading scientists and 7 plenary lectures, which gave an excellent overview of the state of the art in optics and photonics research across the world pointing out perspectives of future developments.

Well over one third of all 149 effective participants of the conference were students. This percentage increased to nearly 50% among the Portuguese participants, illustrating the vitality and growth potential of research in optics and photonics in Portugal, and all over the world in general. The support of the International Commission for Optics helped the participation of seventeen students. EOS and SPIE awarded prizes, respectively, to the best student poster presentations and best student research works presented at the conference.

At the closing ceremony of the conference the 2014 and 2015 SPOF's Best PhD Thesis in Portugal in Optic and Photonics were awarded to Hugo Martins ("Distributed and Remote Fiber Sensing Assisted by Raman Effect") and to Marta Ferreira ("Fiber Sensing Based on New Structures and Post-Processing Enhancement"), both former students of the Universidade do Porto (Portugal).

Reflecting the development of the scientific and technological research in optics and photonics in Portugal over last decades and the success of the previous editions of SPOF's conference; it was decided to change the periodicity of our AOP conferences to biannual. Therefore our next conference will be held 3 – 7 June, 2019, in Lisbon, Portugal.

We are looking forward to another exciting and most enjoyable conference!

Manuel F. M. Costa

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Validation of a semi-automatic protocol for the assessment of the tear meniscus central area based on open-source software

Hugo Pena-Verdeal
Carlos Garcia-Resua
Eva Yebra-Pimentel
Maria J. Giraldez

SPIE.

Clinical performance of an objective methodology to categorize tear film lipid layer patterns

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ABSTRACT

Purpose: To validate the performance of a new objective application designated iDEAS (Dry Eye Assessment System) to categorize different zones of lipid layer patterns (LLPs) in one image. ***Material and methods:*** Using the Tearscope-plus and a digital camera attached to a slit-lamp, 50 images were captured and analyzed by 4 experienced optometrists. In each image the observers outlined tear film zones that they clearly identified as a specific LLP. Further, the categorization made by the 4 optometrists (called observer 1, 2, 3 and 4) was compared with the automatic system included in iDEAS (5th observer). ***Results:*** In general, observer 3 classified worse than all observers (observers 1, 2, 4 and automatic application, Wilcoxon test, $p<0.05$). The automatic system behaved similar to the remaining three observers (observer 1, 2 and 4) showing differences only for Open meshwork LLP when comparing with observer 4 (Wilcoxon test, $p=0.02$). For the remaining two observers (observer 1 and 2) there was not found statistical differences (Wilcoxon test, $p>0.05$). Furthermore, we obtained a set of photographs per LLP category for which all optometrists showed agreement by using the new tool. After examining them, we detected the more characteristic features for each LLP to enhance the description of the patterns implemented by Guillon. ***Conclusions***: The automatic application included in the iDEAS framework is able to provide zones similar to the annotations made by experienced optometrists. Thus, the manual process done by experts can be automated with the benefits of being unaffected by subjective factors.

Keywords: Lipid layer pattern; Tearscope-plus; Tear film; interference phenomena; image categorizaton.

1. INTRODUCTION

The lipid layer of the tear film plays a major role in limiting evaporation during the inter-blink period and also affects tear film stability. Tear film quality and lipid layer thickness can be assessed by non-invasively imaging the superficial lipid layer by interferometry and these two variables are correlated.[1] Guillon proposed five main grades of lipid layer thickness interference patterns for observations made using the Tearscope-plus (figure 1): open meshwork (OM), closed meshwork (CM), wave or flow (W), amorphous (AM) and colour fringe (CO). This author also described abnormal lipid layer patterns (LLPs)[2].

Although this method has proven useful to evaluate the quality and structure of the tear film,[1] it is affected by the subjective interpretation of the observer. Thicker lipid layers ($\geq$ 90 nm) are readily observed since they produce color and wave patterns. However, thin lipid layers ($\leq$ 60 nm) are difficult to visualize, since color fringes and other distinct morphological features are not present and their interpretation is more subjective.[3] Training also affects the interpretation of the LLPs and there is a learning curve for Tearscope tear interference pattern grading.[4]

In an effort to find a more objective way to classify the LLPs provided by the Tearscope, a new software application has been recently developed. Using this new software, several authors have reported good agreement among experienced observers[5] when interpreting LLPs and the method is currently the focus of a funded research project by the present authors.

Therefore, the purpose of this study, based on the objectives of the DEWS[6], was to validate the performance of an objective software application (iDEAS) to categorize different zones of LLPs in one image. The validation was done by comparing the zones of LLP categorization between all observers (4 experienced observers and the automatic system) in order to find out whether the five observers categorized similarly

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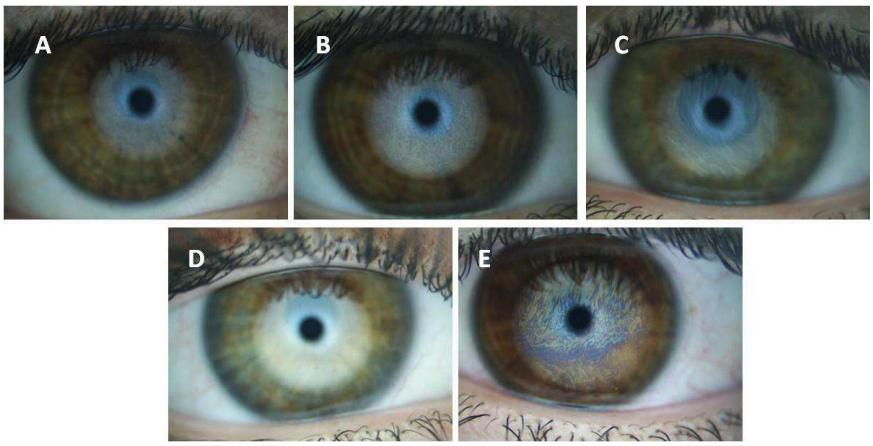

Figure 1. Lipid layer patterns as seen by Tearscope plus. A) Open meshwork, B) Closed meshwork, C) Wave, D) Amorphous and E) Color fringe.

2. MATERIAL AND METHODS

2.1 Subjects and procedure

Subjects were recruited among patients visiting the Optometry Clinic of the Optometry Faculty (Universidad de Santiago de Compostela, Spain) for an eye examination. Subjects were excluded if they had ocular infection or ocular allergy, were contact lens users, toke a prescription eye medication, or were pregnant or breastfeeding. They were asked not to wear eye makeup prior to the clinical protocol. All subjects were given written information about the study before they signed a written statement of consent to participate. The study protocol adhered to the tenets of the Declaration of Helsinki and was revised and approved by the Ethics Committee of the University of Santiago de Compostela (Spain).

The tear film lipid layer was examined using a Tearscope-plus® (Keeler, Windsor, UK). This instrument, designed by Guillon, is the instrument most commonly used in clinical practice for the rapid assessment of lipid layer thickness and has been described in detail elsewhere.[1] Briefly, the Tearscope projects a cylindrical source of cool white fluorescent light onto the lipid layer. Thus, any observed phenomena are unique to the specific light source of the Tearscope.

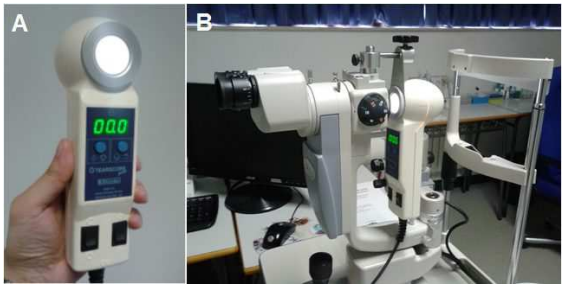

Figure 2. Tearscope plus. This device enables specular reflection oven the entire cornea through a cylindrical source of white fluorescent light onto the lipid layer. a) Tearscope plus hand-held instrument. b) Tearscope plus attached to a slit lamp, which enables high magnification.

Tearscope was attached to a Topcon SL-D4 slit lamp with a R900 Goldmann tonometer support (figure 2). The slit-lamp magnification was set at x16 and illumination was that provided by the Tearscope (the slit lamp source is not switched on). Tearscope offers two sets of illumination; in all the measurements we chose the brightest one.

To capture LLPs videos a Topcon DV-3 digital camera was used and attached to the slit lamp. All videos were stored in a computer via Topcon IMAGEnet i-base software at a spatial resolution of 1024 x 768 pixels in the RGB color space.

All images were then uploaded to the database included in the iDEAS software for image classification. Around 2000 images were uploaded in the iDEAS database at the time of the study. Then, a set of images that better fulfilled the quality requirements (free of blur, lipid layer well spread after a complete blink and well centered) were selected. This yielded 50 images that were used for our study.

2.2. iDEAS

iDEAS (Dry Eye Assessment System) is a web application designed to join several services in the field of optical image processing.[7] For the reader's interest, a wide description of the iDEAS framework is indicated elsewhere, where the technical features are indicated in detail.[8] Here it was described the functionalities that were used in this study.

Regarding the image tools, it has two main functionalities. On the one hand, by clicking the classify icon on an image, the application classifies the tear film image and assigns a specific LLP type to the whole image. This automatic classification tool has been proposed elsewhere,[9, 10] and was properly validated by optometrists by comparing LLP classifications of 105 images with those provided by 3 observers.[5]

On the other hand, by clicking the annotation icon on an image, the web application allows users to manually trace different regions associated each one with a specific LLP. The observer only has to click and drag a pointer on the image to delineate a region. This determines that an image of several discrete LLP zones can be subjectively categorized in one image. The importance of this functionality lies in the fact that the lipid layer of the tear film may show a multiple LLP (representing various lipid thicknesses), which indicates, as Guillon reported this is a sign of a meibomian gland abnormality[1] and suggests that the regularity of the LLP is a useful clinical sign.

In this sense, a new automatic tool is being developed in order to identify several LLP zones in a single patient image. First attempt to achieve this goal was presented earlier,[11] based on the techniques for color texture analysis used previously[10] and decision voting system. However, the main problem of this first approach is that it uses the unreal background category, which represents the areas of the images in which there is no LLP. Thus, the method proposed here consists of a weighted voting system which takes into account the class-membership probabilities provided by a soft classifier, and uses a minimum threshold to distinguish the background from the Guillon categories without using any unreal category. Preliminary results demonstrate the feasibility of this new tool, which will enhance the applications of the Tearscope and offer the clinician and researcher a not too sophisticate device to assess quality aspects of the tear film.

The research methodology proposed to create tear film maps based on the lipid interference patterns consists of five stages (Figure 3).

Figure 3. Scheme of the research methodology to create tear films maps.

The input data is a tear film image acquired with the Tearscope-plus, and the output data is a labeled image based on the interference patterns defined by Guillon. Firstly, the region of interest, in which the further analysis will take place, is located. Next, each local window inside it is analyzed in terms of color and texture features, and its class-membership probabilities are calculated by using a soft classifier. Following, the segmentation is performed using the weight voting system in such a way that every pixel of every window receives a vote associated to each class c:

$$v_c = w_1 \cdot p_c + w_2 \cdot p_c / d$$

where p_c is the probability of belonging to the class c, d is the distance from the pixel to the center of the window, and w_1 and w_2 weight the probability and the distance, respectively. As windows are overlapped, each pixel belongs to several categories and so the votes received from each category are added up. Thus, the pixel is assigned to the most voted category only if its total number of votes is greater than a threshold. Finally, the tear film map obtained is post-processed in order to eliminate the small regions which usually correspond to false positives or noisy areas.

2.2.1. Characterization of the lipid layer patterns

Both image tools available in the iDEAS web system are based on the characterization of the lipid layer patterns by means on color and texture information. Broadly speaking, color and interference patterns are the two discriminant features of the Guillon categories for lipid layer assessment. On the one hand, some categories show distinctive color characteristics which motivate the color analysis step. On the other hand, the interference phenomena can be characterized as a texture pattern, since thicker lipid layers show defined patterns while thinner layers are more homogeneous.

For color analysis, the use of the Lab color space is considered according to previous research.[12] The CIE 1976 L*a*b color space[13] (Lab) is a chromatic color space which describes all the colors that the human eye can perceive. It was defined by the International Commission on Illumination, abbreviated as CIE from its French title Commission Internationale de l'Eclairage. Lab is a 3D model where its three coordinates represent: the luminance of the color L, its position between magenta and green a, and its position between yellow and blue b. Its use is recommended by CIE in images with natural illumination. In addition, this color space is perceptually uniform, a very important characteristic since experts' perception is trying to be imitated. The use of the Lab color space entails converting the three channels of the Tearscope image in RGB into the three components of Lab, which will be subsequently analyzed in terms of texture.

Regarding texture analysis, the co-occurrence features technique is applied due to its effectiveness to characterize the LLPs.[12] Co-occurrence features[14] allow to define a texture descriptor based on the computation of the conditional joint probabilities of all pair wise combinations of gray levels, given an interpixel distance and an orientation. The method generates a set of gray level co-occurrence matrices (GLCM), and extracts several statistical measures from their elements. The Chebyshev distance is here considered and, in general, the number of matrices for a distance d is 4d. From each GLCM, a set of 14 statistical measures proposed by Haralick et al.[14] are computed, which represent features such as contrast or homogeneity. Finally, the mean and the range of these 14 statistics are calculated across matrices and a set of 28 features composes the texture descriptor for a particular distance.

2.3. Pattern classifications. Agreement among 4 experienced observer's methodology

Four observers experienced in LLP grading were asked to categorize the LLPs found in 50 images selected as described above (section 2.1). In each image, the observer marked the LLP zones of interest using the iDEAS tool (Figure 4a). The iDEAS program was then used to extract the zones for which there was agreement among all 4 observers (Figure 4b). These agreed areas were catalogued as "correct images" to be included in the database created to guide LLP categorization.

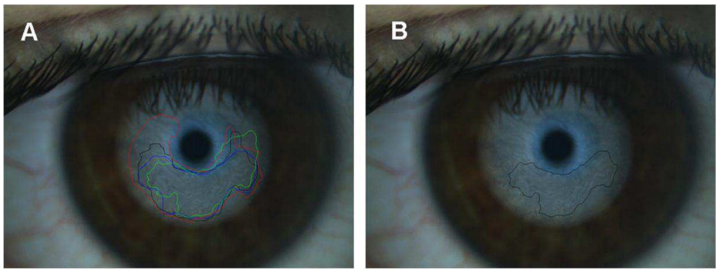

Figure 4. Image interpreted by 4 observers using the iDEAS. A) Overlapping zones marked by the observers; each color indicates the zone categorized by one observer. B) Zone for which there was agreement among the 4 observers.

2.4. Data and statistical analysis

In order to validate this new application, the system was compared with the annotations made by four experienced observers. This validation process consists in dealing with the system as it was another observer, and so a comparison between five hypothetical observers was performed. In this manner, the areas marked by a reference observer were compared with the areas annotated by the other four observers. That is, the number of pixels of the reference observer which match with other observers are added up and graphically represented. Therefore, if the reference observer is compared with the rest of observers, there are a number of pixels in which the reference observer agrees with 0, 1, 2, 3 or 4 observers. Thus, the agreement with 4 observers means a total agreement between the five hypothetical observers considered, whilst the agreement with 0 observers implies that those pixels were marked only by the reference observer.

This comparison between experts was performed in such a way that each observer is used as the reference observer once, and so a total of five comparisons are obtained.

As an example, figure 5 illustrates the annotations made by the 4 observers over 5 representative images, and their respective output images provided by the automatic system. As can be seen, there are areas of the images in which the observers agree with the LLP, whereas there are other areas in which there is no agreement. The same situation can be appreciated if the output images are compared with the observers' annotations.

The size of ***Perfect categorization (PC)*** (those pixels in agreement between reference observer and the all remaining 4 observers) was not considered, because all the five observers show the same value. Therefore, the analysis of the comparisons was performed considering the ***Erroneous categorization (EC)***, that is, those pixels that reference observed agreed with 0 observers or were marked only by the reference observer, where the lower the size of the EC area, the better the performance of LLP categorization.

Kolmogorov-Smirnov test was done for each variable and it was found that they did not follow a normal distribution ($p < 0.05$), so non parametric statistics were used. In order to perform multiple comparisons a Kruskal-Wallis test was used. Finally, to perform pair wise comparisons, Friedman test was used for independent variables whereas a Wilcoxon test was used for dependent variables.

Figure 5. Annotations made by the 4 observers over 5 representative images, and their respective output images provided by the automatic system.

3. RESULTS

Table 1 shows the descriptive statistics of the size of the EC area (amount of pixels) for each observer irrespective of the LLP.

Table 1. Descriptive statistics of size areas quantified in pixels of area for each observer irrespective of lipid layer pattern. Median and interquartile range (IQ) are indicated. "*Erroneous categorization*" means those pixels that reference observer agreed with 0 observers or were marked only by the reference observer. *Observer 5 refers to the automatic system.

Amount of pixels EC. Median (IQ)				
Observer 1	**Observer 2**	**Observer 3**	**Observer 4**	**Observer 5***
2416 (3773)	1829 (3617)	6354 (8282)	1509 (2841)	3360 (5015)

In order to assess whether there were statistical differences between observers' categorization, a Friedman test was used, and there was found a difference between them ($p < 0.001$). Wilcoxon test was used to check pair differences between observers (table 2) and it was found that observer 3 showed a size of EC area significantly higher than the remaining 4 observers ($p \leq 0.006$ for all comparisons, table 2), indicating that observer 3 categorized worse than the remaining observers. There was also a statistical difference between observer 4 and new application categorization ($p < 0.044$)

Table 2. Wilcoxon test outcomes. Pair wise comparisons between the amount of EC pixels categorized by two observers. In each comparison median of differences and level of statistical significance (*p*) are indicated. *EC*: "*Erroneous categorization*", means those pixels that reference observer agreed with 0 observers or were marked only by the reference observer. *Observer 5 refers to the automatic system

Pair wise comparisons. Lipid layer pattern categorization					
	Observer 1	**Observer 2**	**Observer 3**	**Observer 4**	**Observer 5***
Observer 1					
Observer 2	P = 0.26				
Observer 3	**P = 0.01**	**P = 0.006**			
Observer 4	P = 0.09	P = 0.50	**P < 0.001**		
Observer 5*	P = 0.55	P = 0.51	**P = 0.003**	**P = 0.04**	

Finally, it was compared the LLP categorization between observers for each LLP. This was done in order to find out whether the differences between the observers categorization were dependent on the LLP type. Table 3 shows the descriptive statistics of the size of the EC area (amount of pixels) categorized by each observer for each LLP. Friedman test showed that there were statistically significant differences between observers only for open meshwork LLP ($p = 0.02$), but that was not true for the remaining LLPs; closed meshwork ($p = 0.19$), wave ($p = 0.39$), amorphous ($p = 0.41$) and color fringe ($p = 0.19$). In this case pair wise comparisons (Wilcoxon test) was only done for OM pattern, because this was the only LLP that showed differences between observers. Table 4 shows the Wilcoxon test for open meshwork LLP and it was found that there were significant differences only when comparing the size of EC area between observer 1 and observer 4 ($p = 0.01$) and between observer 4 and the automatic system ($p = 0.02$).

Table 3. Descriptive statistics of the size of the EC area (amount of pixels) categorized by each observer for each LLP. Median and interquartile range (IQ) are indicated. *EC*: "*Erroneous categorization*", means those pixels that reference observer agreed with 0 observers or were marked only by the reference observer. *Observer 5 refers to the automatic system.

	Amount of pixels EC. Median (IQ)				
	Observer 1	**Observer 2**	**Observer 3**	**Observer 4**	**Observer 5***
Open meshwork	4234 (9489)	1820 (5602)	10144 (8660)	1328 (2236)	5503 (4284)
Closed meshwork	3350 (5778)	2795 (3650)	4780 (16017)	1417 (2446)	4130 (4402)
Wave	2819 (1690)	1720 (3271)	5684 (15277)	3369 (9278)	2592 (7920)
Amorphous	717 (2102)	770 (3432)	3308 (7747)	1314 (3921)	2174 (3387)
Color fringe	3155 (4145)	1586 (8338)	7267 (21206)	1223 (1558)	3134 (7795)

Table 4. Wilcoxon test outcomes for Open meshwork lipid layer pattern categorization. Pair wise comparisons between the amount of EC pixels categorized by two observers. In each comparison median of differences and level of statistical significance (*p*) are indicated. *EC*: "*Erroneous categorization*", means those pixels that reference observer agreed with 0 observers or were marked only by the reference observer. *Observer 5 refers to the automatic system.

Pair wise comparisons. Lipid layer pattern categorization

	Observer 1	**Observer 2**	**Observer 3**	**Observer 4**	**Observer 5***
Observer 1					
Observer 2	P = 0.21				
Observer 3	P = 0.09	P = 0.21			
Observer 4	P = 0.09	P = 0.12	**P = 0.01**		
Observer 5*	P = 0.67	P = 0.48	P = 0.12	**P = 0.02**	

4. CONCLUSION

This study showed a new automatic tool, within the framework iDEAS, to identify several LLP zones in a single patient image. In a previous research,[8] a preliminary study showed that the automatic system showed similar categorization than observers, especially for CO and OM LLPs, whereas W pattern showed less percentage of coincidences.[8] In the present study the automatic application was validated by comparing its performance against new four experienced observers.

Categorizations of LLP zones were compared between the 5 observers. In general, observer 3 classified worse than the remaining 4 observers (tables 1 and 2) and the automatic system was classified worse only when comparing with the observer 4 (observer 4 was the most conservative). However, these differences were found only for the OM pattern (tables 3 and 4), so in the remaining lipid layer categories there was a consistency between observers in the examination of the LLP areas.

From the results found here, the automatic application is able to provide zones similar to the annotations made by experienced optometrists. Thus, the manual process done by experts can be automated with the benefits of being unaffected by subjective factors. This new software clearly improves the previous automatic application that was designed to categorize the whole pattern.[5] However, there is still large room for improvement on the processing time needed to generate the output image (10 minutes on average). Consequently, our future research will involve developing an optimized version whose processing time makes it useful in any clinical routine.

As an interferential phenomena, the LLPs are related to colors appearance, and some authors only addressed the color of the pattern.[3, 15] The authors considered that the best way to address the LLPs is by doing an analysis based in both texture and color. However, the lipidic reflection does not always show a CO pattern. The observation of a colorless pattern (grey color) is because its thickness is below the minimal thickness to produce interference fringes. Korb established the lipid layer thickness that corresponded to each color[3] as well as Isenberg et al.[16] From those classifications it can be seen that thinner LLPs (from OM to W) showed a grey background, and yellow color begins to appreciate in the transition of W and A patterns. In our experience,[5] we have noted that the thicker the lipid layer, the more marked appear the details of the pattern. Thus, as the lipid layer gets thicker, the pattern produced goes through the following stages: almost no details on a dark background (OM); changing to clearly visible dark streaks on an ever-brighter gray background followed by the merging of streaks to form large zig-zags (CM); until they thin out, and give

rise to the flow wave pattern (W); the gray background then turns yellowish until waves disappear to give the amorphous pattern (AM); and finally on this yellow background, appear the first brown/blue colors (CO).

In conclusion, the automatic application included in the framework iDEAS is able to categorize LLP zones as done by experienced optometrists. This device offers a clear benefit in categorizing the heterogeneity of the LLP without being affected by subjective factors.

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