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Quantitative Assessment of Retinal Lesions in Fluorescein Angiography by Using SITE-App Algorithm

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ABSTRACT

SITE-App algorithm variant 2007 was first reported at ARVO Meeting, Fort Lauderdale, 2008, as an audio-visual poster. Results of measurements on fluorescein angiography and red free images realized by using SITE-App software were presented as posters at ARVO Meeting, Fort Lauderdale, 2009 and 2010, SITE-App variant 2021 was published on the website https://www.eyestorywithnarcisa.com, SITE-App variant 2022 is finished and soon to be released to publication.

Purpose

To design an algorithm for quantitative assessment of retinal lesions size in fluorescein angiography (FA).

Methods: Black-white fundus photos were recorded during FA from patients with age related macular degeneration, before and after Bevacizumab intravitreal injections. Area of "white lesions" was quantified in white pixels inside standardized retinal areas of interest, by using SITE-App algorithm. Method reproducibility was assessed by comparing two independent readers measurements. Method sensitivity was evaluated by comparing SITE-App results with the ones of other published quantitative methods: greatest linear dimension in millimeters - GLD[mm] or pixels - GLD[pix], mouse-delimited lesions area in MPS units - A[MPS], mm2 - A[mm2], total pixels - A[tpix] or white pixels A[wpix]. Correlation between methods was analyzed by calculation of correlation factor.

Results: SITE-App is reproducible because differences between two independent readers measurements were not statistically significant ($p>0.05$). The reproducibility was found very good for SITE-App ($p=0.49$) and A[wpix] ($p=0.22$). The reproducibility was lower, but still good for A[MPS] and A[mm2] ($p=0.13$). A[tpix] ($p=0.01$), GLD[pix] ($p=0.0003$) and GLD[mm] ($p=0.0005$) were found not reproducible. The highest sensitivity was observed for SITE-App ($p1=0.002$, $p2=0.021$) and A[wpix] ($p1=0.007$, $p2=0.02$), that also showed high correlation ($r1=0,96$, $r2=0,94$). A[MPS] and A[mm2] offered very dispersed and unsure results ($R1=1.54\pm26.1$, $R2=3.51\pm17.3$). No statistical significance of data correction for incidence angle variations of images capture was found.

Conclusion: Quantification of retinal lesions in white (or black) pixels inside of interest standardized areas (SITE-App algorithm) is reproducible, sensitive, easy, fast and low cost, very useful for longitudinal clinical studies.

Introduction

Clinical evaluation of retinal lesions represents a fundamental step in positive diagnosis and management of all retinal disorders. Tridimensional analysis starts in retinal plane with biomicroscopy, ophthalmoscopy and angiography, building a "retinal lesions map" [1-7]. New techniques of optical coherence tomography (OCTs) highly define the retinal lesions morphopathology in axial plane, realizing a "microscopic view" of retinal and pathologic tissues, layer by layer [4,8-16]. While the qualitative ocular fundus examination helps establishing the diagnosis, the monitorization of the disease evolution under different treatments requires a quantification that is essential in numerous retinal disorders with high potential of blindness like age related macular degeneration (AMD) or proliferative diabetic retinopathy (PDR) [3,17,18]. The quantitative assessment of retinal lesions, accurately realized by OCTs in axial plane [4,5,8-16,19-22]. must be completed in the retinal plane by the quantitative interpretation of fluorescein angiography (FA) and red free (RF) images. The use of contrasting dyes unveils numerous details that otherwise remain obscure in native examinations. Numerous publications reported different methods for retinal lesions size quantification on black-white photos recorded during FA, RF examination, scanning laser ophthalmoscopy, etc. Some authors measured in millimeters or pixels the retinal lesions greatest linear dimension GLD – GLD[mm] or GLD[pix] [5,14,19,22-25]. The retinal lesions mouse-driven delimited area (mdrdA) was quantified in total number of pixels – A[tpix] [7,26-30] in MPS or DA units after defining the optic nerve head (ONH) area as a standard MPS or DA unit – A[MPS] or A[DA] [5,14,20,22,23,25,31-39]. Other methods expressed the retinal lesions area in mm^2 A[mm^2] after the approximation of ONH diameter as 1.5mm or 1.8mm, consequently, the ONH area being 1.77mm^2 or 2.54mm^2. [19,25,34,36,40-43].

All these methods offer inaccurate data due to:

- Subjective nature of the human interpretation of images when establishing the retinal lesions boundaries [22,25,29].
- Ignoring the refractive power and real dimensions of the examined eye when considering the approximations [25,29,44,45].
- Omitting the variation of image recording angle that modifies the retinal lesions shape and size [27,29,44,45].

However, because of the lack of other much more accurate variants, the previously mentioned methods are still largely used in longitudinal clinical trials requiring retinal lesions quantification [14,20,23,31,36,38,39,46,47].

We designed an easy, fast and low-cost algorithm (SITE-App) for quantitative assessment of retinal lesions on black-white FA and RF fundus photos [48,49,50]. By standardizing the area of interest inside of which the measurements are performed, by replacing the human subject with the computer in images processing and interpreting, as well as by expressing the results in relative units, SITE-App aims to lower the errors, improving the measurements reproducibility and sensitivity.

Methods

Patients Selection and Imagistic Procedure

The research used fundus photos that belong to the study investigating the effect of Avastin in different proliferative retinopathies (Clinical Trials.gov: NCT00564148). The study was conducted in accordance with Declaration of Helsinki guidelines and was approved by Local Ethics Committee in Iasi, Romania. Written informed consent was obtained from all individuals included in the study, after the explanation of the nature and possible consequences of all procedures. A VISUCAMlite Digital Camera (Carl Zeiss Meditec AG, Jena, Germany) suitable for photographing, displaying and storing the data, operating with Microsoft Windows (Microsoft Corporation, Redmond, Wash., US) was used to capture FA images from eyes with AMD and PDR, before and after ivitB - intravitreal injections with 1.25 mg Bevacizumab (Avastin®, Genentech, San Francisco, CA, USA). In order to compare the area of neovascularization and leakage that are variable with angiographic times, pairs of photos before/after treatment, 45° angular field, focused on ETDRS fields 1 or 2 were recorded in the same angiographic phase (early < 1 min, mid 1-3 min or late 5-10 min), in dark room, through mydriatic pupil exceeding 5 mm [51]. Aiming to establish a fixed referential regarding the pictures brightness and contrast, for each pair of images, the capture was realized by using the same fundus illumination and magnification, after eliminating possible visible reflections and shadows.

Site-App Algorithm Steps

SITE-App algorithm was developed to analyze quantitatively, in retinal plane, the retinal lesions size, by interpreting the black-white fundus photos recorded during FA and RF examinations. After images processing in Adobe Photoshop, the retinal lesions area is measured in pixels, inside of interest standardized retinal areas, by using SITE-App software.

Adobe Photoshop Processing

The aim of Adobe Photoshop processing ((Figure 1), Movie 1.A) is to regularize the grey scale tones and to improve the contrast between the retinal lesions and surrounding background. Rough black-white fundus photos with 720x576 pixels resolution are exported in JPEG (X.jpg) graphic file format from fundus camera to a computer with the operating system Microsoft Windows and having installed the Adobe Photoshop program (CS2 Version 9.0; Adobe Systems Incorporated, San Jose, CA, US). Imported image (Figure

1A) is opened in Adobe Photoshop. Photos "pure black" edges are excluded with the magic wand, by pressing "ctrl+shift+i". The remaining ocular fundus image is adjusted from Menu (Image), by automatic regularization (Auto) of grey scale Levels.

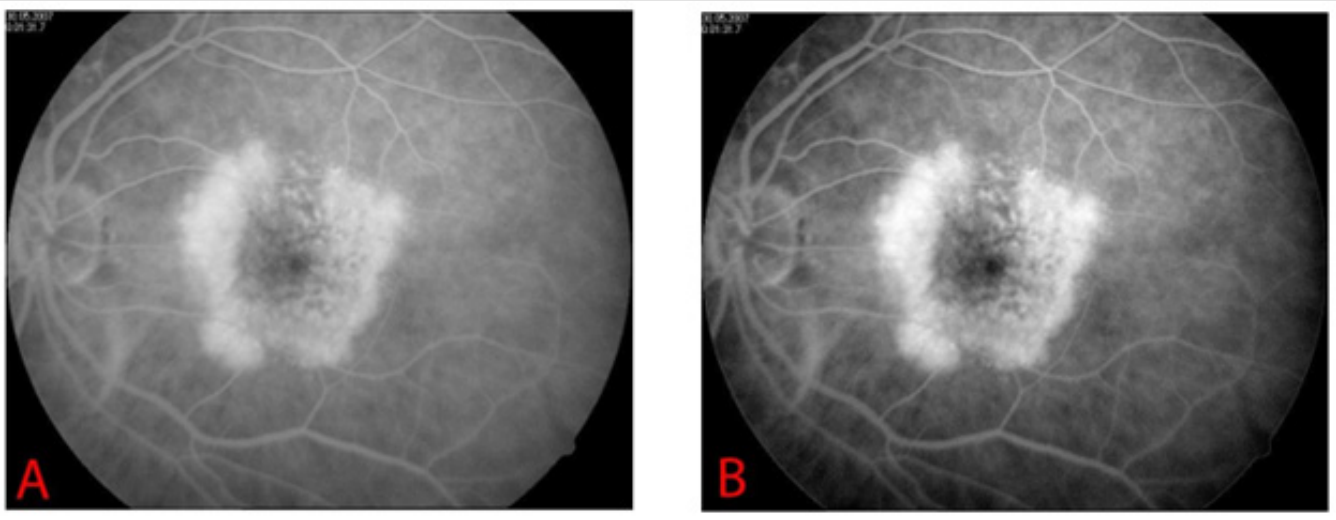

Figure 1: Adobe Photoshop processing of black-white images imported from the fundus camera.
Details visibility of imported rough image (A) is improved in the final processed image (B). Press Figure 1.A for Movie 1.A.

Interest Retinal Area Standardization

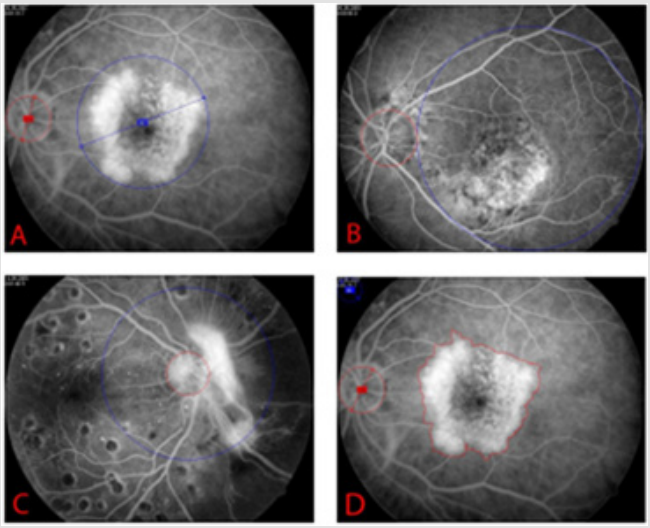

Figure 2: Establishment of the interest retinal area inside of which the measurements are performed in different quantitative methods of interpretation of FA images. Standardization of interest area as blue circle in SITE-App algorithm (A,B,C). Red circle surrounding the ONH chosen as referential (B,C). Area of interest as blue circle surrounding the single macular lesion – circumscribed circle method (A). Press Figure 2.A for Movie 2.A. Evaluation of macular lesions with tangent circles method in ETDRS field 2 (B). Press Figure 2.B for Movie 2.B. Evaluation of retinal lesions around the ONH by concentric circles method in ETDRS field 1 (C). Press Figure 2.C for Movie 2.C. Mouse-driven delimited area - mdrdA used for measurements in white pixels - A[wpix], total pixels – A[tpix], MPS units – A[MPS] or mm2 – A[mm2], after the approximation of ONH diameter as 1,5 mm and ONH area to be 1 MPS = 1.77 mm2 (D). Press Figure 2.D for Movie 2.D.

All computerized programs designed to measure in pixels a previously delimited area are impaired by errors at the level of "angles and inflections" (Figure 2D). In order to decrease these errors, SITE-App algorithm standardized the interest areas as circles – so called "blue circles" (Figures 2A-2C). For single lesions, SITE-App algorithm uses the smallest blue circle circumscribing the analyzed retinal lesions – "circumscribed circle method" (Figure 2A). In series of images recorded from the same eye, before/after treatment, the blue circle diameter is modified in order to surround the analyzed lesions in their new circumstances. For multiple retinal lesions, the blue circle is reported to "red circle", defined as the smallest circle circumscribing the ONH (Figures 2B & 2C). In series of photos followed over time, blue circle diameter is fixed, obtained by multiplication of red circle diameter (e.g. 4 x ONH diameter) while its position is chosen according to the analyzed lesions. "Tangent circles method" was designed for macular lesions and uses photos focused on ETDRS field 2 (Figure 2B). "Concentric circles method" was planned for lesions around the ONH, in ETDRS field 1 (Figure 2C).

SITE-Application Software

SITE-App software was developed for PC compatible computers with Microsoft Windows operating system [48,49,50]. SITE-App software final version can be used to measure in white, black or total pixels (wpix, bpix, tpix), MPS units, in mm or mm^2 (after approximating the ONH diameter as 1.5 mm and ONH area as 1MPS = 1.77 mm^2). The measurements may focus on distances or interest areas, inside of standardized circular or mouse-driven delimited areas - mdrdA (Figure 2D). Measurements steps are demonstrated in videos attached to (Figures 2A-2D) and (Figure 3C).

- Lesions relative area expressed in wpix (SITE-App method) inside of concentric ((Figure 2), Movie 2.A) or tangent ((Figure 2), Movie 2.B) standardized blue circles
- Lesions greatest linear dimension in mm or pix ((Figure 3), Movie 3.C)
- mdrdA expressed in wpix, tpix, MPS units and mm^2 ((Figure 2D), Movie 2.D).

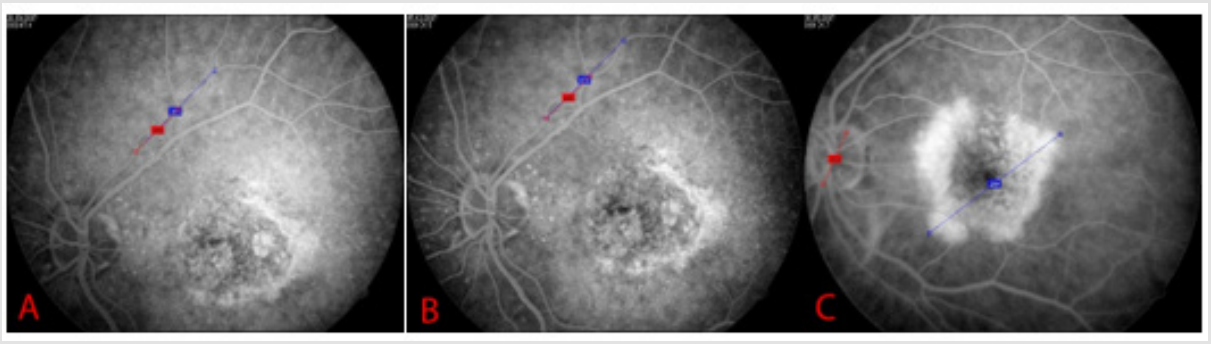

Figure 3: Measurement of distances by using SITE-App software. Measurements of fixed segments on two successive fundus photos recorded from the same eye, before (A) and after (B) treatment, used for definition of correction factor Fx. The distance between the 2nd and 3rd branch of the temporal superior retinal vein (red segment) varies from 108 pix (image A) to 109 pix (image B). The distance between the 2nd and 4th branch of the temporal superior retinal vein (blue segment) varies from 203 pix (image A) to 199 pix (image B). Measurement of retinal lesions greatest linear dimension GLD (blue segment) in pixels or mm, after approximation of ONH diameter (red segment) as being 1,5 mm (C). Press Figure 3.C for Movie 3.C.

Correction Factor Fx Definition

When central fixation is lost, fundus photos capture under exactly the same angle is difficult [2,27,29,44,45]. Assuming that incidence angle variation introduces important errors in data series recorded over time from the same eye, some authors defined a correction factor [29]. Aiming to decrease these errors, SITE-App algorithm was designed to perform measurements on photos focused on ETDRS fields 1 or 2. In order to analyze if a small variation in incidence angle still introduces statistical significant errors, we defined the correction factor Fx as the ratio between the areas (πr^2) of the same circle captured in two successive photos A and B (Figures 3A & 3B): $Fx = rA^2 / rB^2$. Any fixed segment can be used for Fx definition: ONH diameter, distances between 2 successive branches of temporal retinal veins, etc (Figures 3A & 3B). In order to report lesions from image B to the same angle through which image A was recorded, area S resulted from image B measurements must be therefore multiplied with Fx ($SB = S \times rA^2 / r B^2$).

Statistical Methods

We assessed different methods reproducibility by comparing 2 independent readers measurements and by calculating the p value

with TTEST function in Microsoft Office Excel for one tail distribution (Tails = 1) and paired type (Type 1) (Wilcoxon-Mann-Whitney test or rank-sum test). Average m and standard deviation σ were calculated and the confidence interval limits were established for a probability of 95% (m±2σ). Differences between measurements were therefore considered statistically significant for $p<0.05$. One analyzed method was considered reproducible if 2 readers measurements did not differ statistically significant.

Methods sensitivity was assessed by calculating the percent % of each parameter (lesions area or GLD) modified as the treatment effect, in 2 independent readers results. The ability of a method to reveal a higher % of modification in the parameters values was interpreted as higher sensitivity. Pearson correlation coefficient r was calculated in order to assess the correlation between different methods. Values of r close to 1 revealed high correlation between methods, values close to 0 - low correlation, positive values of r - direct correlation, while negative values - inverse correlation.

Results

Case Reports

Case 1: Is a 74-year-old man with exudative AMD (eAMD). Fundus photos were recorded in the same angiographic time of the same FA session: 2.11 min (Figure 4A), 2.15 min (Figure 4B). The illumination variation during images capture severely influenced the number of wpix and bpix counted in the same standardized circular area tangent to ONH, having 4 times the ONH diameter: 151990 wpix and 11 bpix in image A, 25465 wpix and 126536 bpix in image B.

Case 2: Is a 68-year-old man with eAMD. On 3 fundus photos recorded during FA, under different incidence angles, we analyzed fixed segments and areas. ONH diameter varied from 90 pix, to 98 pix, to 96 pix, and its area in tpix from 6349 tpix, to 7521 tpix, to 7209 tpix (Figures 5A-5C). Distance between 2nd and 3rd branch of temporal superior retinal vein showed similar variations: 196 pix, to 195 pix, to 201 pix (Figures 5A-5C).

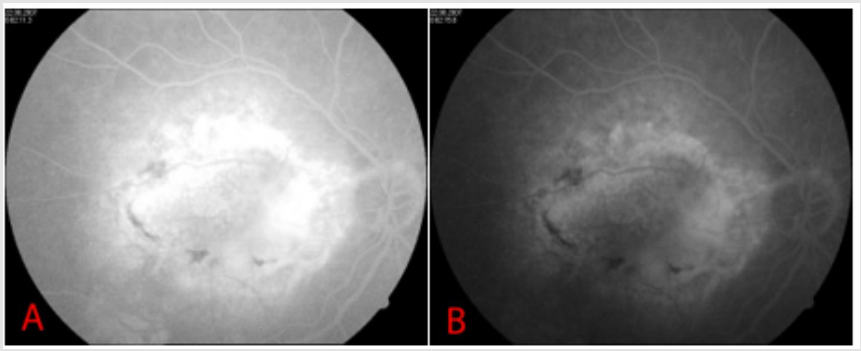

Figure 4: Images recorded from the Case 1 during the same session of fluorescein angiography, with different illuminations, in close angiographic times: 2.11 min (A), 2.15 min (B). Measurements inside of the blue circle tangent to the ONH and having 4 times the ONH diameter showed a marked variation in the number of wpix and bpix: 151990 wpix and 11 bpix in the Image A, comparative to 25465 wpix and 126536 bpix in the Image B.

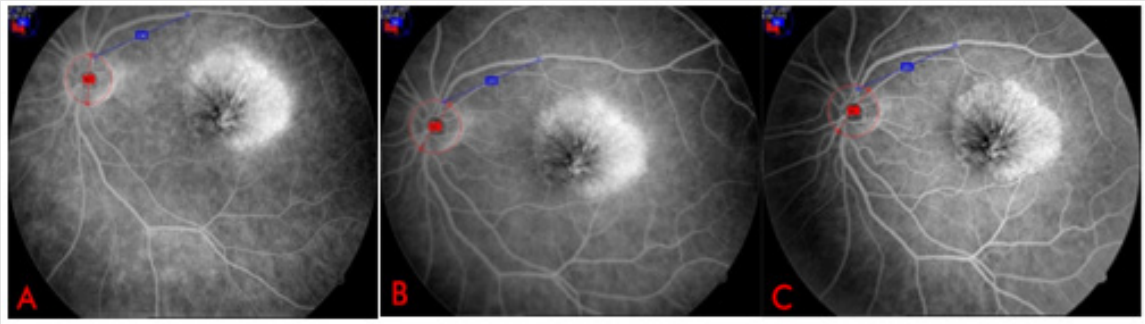

Figure 5: Different fixed distances of the ocular fundus having different sizes as they were photographed under different incidence angles. The ONH diameter varies from 90 pix (A) to 98 pix (B), to 96 pix (C), while the distance between the 2nd and 3rd branch of the superior temporal retinal vein varies from 196 pix (A), to 195 pix (B), to 201 pix (C). Consequently, the ONH area in tpix varies according to the eye rotation from 6349 tpix (A), to 7521 tpix (B), to 7209 tpix (C).

Importance of Adobe Photoshop Processing before Measurements

N = 50 angiographic images from eyes with eAMD were used to analyze the SITE-App method reproducibility and importance of Adobe Photoshop processing of rough images before measurements. Two independent readers measured in pix the ONH diameter and white lesions area A[wpix] inside of blue circle having 3 times the ONH diameter - tangent circle method (Table 1). Adobe Photoshop processing did not significantly influence the agreement between the two readers measurements of ONH diameter, as m and σ of not-processed/processed images groups had close values: 117.68±8.17/117.48±8.51 for R1 and 116.36±9.21/116.84±9.64 for R2. Two readers measurements of area in wpix did not differ statistically significant (p>0.05), showing that SITE-App algorithm is a reproducible method, no matter if images are processed (p=0.13) or not-processed (p=0.09) in Adobe Photoshop. However, the reproducibility was increased after images processing as p value was higher, suggesting an improvement in images contrast and details visualization.

Table 1: Measurements on Not Processed / Processed Fundus Images.

Parameter	Not processed images	Processed images
ONH diameter [pix] – R1	117.68 ± 8.17	117.48 ± 8.51
ONH diameter [pix] – R2	116.36 ± 9.21	116.84 ± 9.64
A [wpix] – R1	p = 0.09*	p = 0.13*
A[wpix] – R2		

Note: Values presented as m± σ; [pix] = parameter expressed in pixels; A[wpix] = area of white lesions inside of standardized blue circle, expressed in white pixels; R1 = reader 1; R2 = reader 2.

*p > 0.05 is not statistically significant

Reproducibility of SITE-App Compared to Other Quantitative Methods

Macular lesions of n=48 angiographic images recorded from eAMD patients were interpreted quantitatively by using SITE-App algorithm (circumscribed circle method) and other quantitative methods reported in literature (Table 2). Best reproducibility was observed for SITE-App (p=0.49), followed by mdrdA expressed in wpix A[wpix] (p=0.22). MdrdA expressed in MPS units or mm^2 offers reproducible results (p=0.13). GLD method was found not reproducible because 2 readers measurements differed statistically significant in both variants – GLD[mm] (p=0.0005) and GLD[pix] (p=0.0003). Lesions area in tpix A[tpix] calculated after manually mouse delimited borders was also found not reproducible (p=0.01).

Table 2: Quantification of retinal lesions in different methods.

Method	Delimitation of area for measurements	Lesions area/ dimension of interest	Units	p values
SITE-App algorithm	Standardized blue circle circumscribing white macular lesions	Relative area of white macular lesions	white pixels	0.49*
GLD [mm]	–	White lesion greatest linear dimension	Mm	0.0005†
GLD [pix]	–	White lesion greatest linear dimension	Pixels	0.0003†
A[MPS]	Mouse-driven delimited area of white macular lesion	Total delimited area	MPS units	0.13*
A[mm2]	Mouse-driven delimited area of white macular lesion	Total delimited area	mm2	0.13*
A[tpix]	Mouse-driven delimited area of white macular lesion	Total delimited area	total pixels	0.01†
A[wpix]	Mouse-driven delimited area of white macular lesion	Only white lesion included in the mouse-driven delimited area	white pixels	0.22*

Note: *p>0.05 revealing not statistically significant differences.

†p<0.05 revealing statistically significant data

Sensitivity of SITE-App as Compared to Other Quantitative Methods

N=24 pairs of angiographic photos were recorded from eAMD eyes, treated with ivitB. Each pair of photos before-after treatment has been chosen to have up to 30 seconds interval between angiographic frames. By using different quantitative methods, 2 independent readers (R1, R2) quantified retinal lesions dimensions (Table 3). SITE-App was the most sensitive in surprising statistically significant differences in the status before-after treatment (p1=0.002, p2=0.021). It was followed by the method of quantification in wpix inside of mdrdA, that also revealed the treatment positive effect (p1=0.007, p2=0.02). The methods of GLD[mm] (p1=0.36, p2=0.13) and A[MPS] (p1=0.16, p2=0.25) did not reveal any statistical modification in the lesions size after ivitB. A[tpix] method offered con-

tradictory results between the 2 readers (p1=0.31, p2=0.04). The evolution of retinal lesions area/dimensions was analyzed as percents of the status before treatment - Abefore, by using the formula: [%] = (Abefore – Aafter) / Abefore. m and σ of percents for 2 independent readers were calculated, for each quantitative method (Table 3). Retinal lesions area measurements in wpix revealed statistically significant differences in the status before and after treatment, in both methods, no matter if the measurements were performed inside of standardized circumscribed circle area as in SITE-App (m1±σ1=17.03±25.81, m2±σ2=13.44±29.09) or inside of mdrdA (m1±σ1=13.7±26.3, m2±σ2=11.37±26.9). Very dispersed and unsure results offered all the other methods: GLD[mm] (m1±σ1=-0.83±17.19, m2±σ2=-1.98±8.44), A[MPS] (m1±σ1=1.54±26.1, m2±σ2=3.51±17.3) and A[tpix] (m1±σ1=-2.14±8.23, m2±σ2=-4.59±11.5).

Table 3: Sensitivity of different quantitative methods R1 = reader 1; R2 = reader 2; A before = dimension/area before treatment; A after = dimension/area after treatment; m = average; σ = standard deviation.

Methods	p - values R1 (p1) R^2 (p^2)	[%] = (Abefore – Aafter) / Abefore as m ± σ
SITE-App algorithm	p1=0.002†	m1±σ1=17.03±25.81
	p2=0.021†	m2±σ2=13.44±29.09
GLD[mm]	p1=0.36*	m1±σ1= -0.83±17.19
	p2=0.13*	m2±σ2= -1.98±8.44
A[MPS]	p1=0.16*	m1±σ1=1.54±26.1
	p2=0.25*	m2±σ2=3.51±17.3
A[tpix]	p1=0.31*	m1±σ1= -2.14±8.23
	p2=0.04*	m2±σ2= -4.59±11.5
A[wpix]	p1=0.007†	m1±σ1=13.7±26.3
	p2=0.02†	m2±σ2=11.37±26.9

Note: *p>0.05 revealing not statistically significant differences

†p<0.05 revealing statistically significant data

Correlation Between the Quantitative Methods

Table 4: Correlation between the sensitive quantitative methods.

Methods compared	Pearson correlation factor r	Interpretation / agreement between readers
- circumscribed circle method SITE-App	r1=0.96	- strong direct correlation
- mouse-driven delimited A[wpix]	r2=0.94	- good agreement between readers
- circumscribed circle method SITE-App	r1=0.48	- poor interreaders agreement
- mouse-driven delimited A[MPS]	r2=0.06	- medium direct / poor direct correlation

In the same group of 24 pairs of angiographic photos statistically interpreted for sensitivity assessment, Pearson correlation factor r calculated for 2 independent readers revealed a strong correlation (r1=0.96, r2=0.94) between the two most sensitive methods – SITE-App and A[wpix] (Table 4). Values of r calculated for SITE-App (circumscribed circle method) and A[MPS] have shown a poor agreement between readers: medium direct correlation for R1 (r1=0.48), but very poor direct correlation for R2 (r2=0.06).

Is Fx Compulsory to Be Applied?

N=24 pairs of angiographic images recorded from eAMD eyes before-after ivitB were used to calculate Fx by using different fixed segments: ONH diameter s(ONH), distance between 2nd and 3rd branch of superior temporal retinal vein s(2-3) and distance between 2nd and 4th branch of superior temporal vein s(2-4) (Table 5). No statistically significant differences were found between s(ONH) and s(2-3) (p=0.45), between s(ONH) and s(2-4) (p=0.31), or between s(2-3) and s(2-4) (p=0.21), suggesting that Fx calculation may consider any of these landmarks. For macular lesions, SITE-App uses photos focused on standardized ETDRS field 2. In order to appreciate if applying Fx is still required even if the variation of incidence capture angle is very small, series of results obtained from 2 readers were compared in the variant corrected and not-corrected (Table 5). For all 3 considered fixed landmarks, the differences between 2 readers measurements were not statistically significant (p>0.05), suggesting that data correction is not required in the context of SITE-App standardization.

Table 5: Statistical Significance of Data Correction for Incidence Angle Variation.

Readers	s(2-3)	s(ONH)	s(2-4)
R1	p1=0.43*	p1=0.48*	p1=0.22*
R2	p2=0.35*	p2=0.39*	p2=0.39*

Note: R1 = reader 1; R2 = reader 2; s (ONH) = optic nerve head diameter; s (2-3) = distance between 2nd and 3rd branch of superior temporal retinal vein; s (2-4) = distance between 2nd and 4th branch of superior temporal vein.

*p>0.05 revealing not statistically significant differences

Discussion

The development of SITE-App algorithm started from the necessity of retinal lesions quantification in retinal plane, as a component of the complete tridimensional examination in retinal disorders. The neovascularisation and leakage represent the characteristic lesions in the main retinal diseases leading to blindness, eAMD and PDR. Such lesions can be evaluated quantitatively only on black-white photos, after systemic administration of dyes, mainly fluorescein. Numerous ways for quantitative retinal lesions interpretation were imagined, in the attempt to improve the measurements repro-

ducibility, accuracy and sensitivity, decreasing as much as possible the errors incidence [1,22,25,27,29,44,45]. These objectives also represented the aims of this research, that, before designing the method, started to analyze "Where do the errors come from, and how the errors could be avoided?". The errors sources can be detected after dividing the process of quantification in its main steps:

a) Images capture and selection.

b) Delimitation of interest area for measurements.

c) Measurements procedure.

Obtaining high quality fundus photos is the first requirement, essential for any quantitative method of retinal lesions interpretation. Besides the basic recommendations very well described in literature (sharpest focus, well pupil dilation, best contrast, etc) [1,25]. some other conditions also seem to be important for obtaining accurate measurements on series of black-white photos [25,57,58]. For accurate measurements of lesions size, maintaining the same magnification of the fundus photos appears to be an essential condition. This aspect was outlined in numerous published works [15,27,29,46,52,55,60]. being also used as a preestablished condition in our research. From the best of our knowledge, the standardization of the light exposure during images capture was not yet reported in literature as an important condition to prevent errors. Some authors suggested the regularization of grey scale tones by using Adobe Photoshop processing [24,30,59].

While others the use of Gaussian filters superposed on the captured images [25]. Our observations illustrated by the reported case 1 revealed that variations in the illumination used during photos recording cannot be replaced by Adobe Photoshop processing and introduces high errors in the measurements results expressed in wpix or bpix. We therefore established several conditions for digital fundus photos capture: dark room (to create similar levels of ambient illumination for the whole series of photos [25] mydriasis exceeding 5 mm (to eliminate shadowing due to small pupil [25]), same illumination fixed in the 1st examination for one eye (to obtain similar reflectance of fundus background and similar contrast between the interest lesions and retinal background at the lesions boundaries [24,25,42,55]. Our results showed that the measurements performed on Adobe Photoshop processed photos do not statistically significant differ from the ones not-processed. However, we learned from our data that Adobe Photoshop processing increases the contrast at the lesions boundaries. The area of retinal lesions positive on FA photos depends on the angiographic time1. Numerous authors reported measurements on frames selected "during the same angiographic time" trying to record modifications over time focused on the same area of neovascularization and leakage [4,5,22,29] . In our study, we have chosen a smaller interval between angiographic frames selected from 2 consecutive examinations, 30 seconds, because the fulfilling of normal and pathologic blood vessels is not uniform and largely varies from one eye to another [25]. Consequently, a smaller interval between selected angiographic frames will better focus on a special stage of neovascularization extension.

The retinal lesions evaluation in the retinal plane, on fundus photos, consider the approximation of the volumetric biologic structure as a plane one. No mathematical function can be perfect in this transformation (polynomial, affine, translation, rotation, etc) [25]. so that all quantitative methods are affected by errors from this point of view. By taking into account the modifications induced by the eye and camera movements, some authors defined formulas to correct the lesions size and shape [25,29]. Our case 2 also demonstrated that different fixed landmarks of the fundus have different sizes if they are photographed under different incidence angles. In order to decrease these errors, SITE-App focuses on ETDRS field 2 when quantifies macular lesions. Statistical interpretation of our data suggested that correction factor Fx can be defined by using any fixed segments, but there is no statistical difference between the data corrected by this factor and the ones not corrected. This observation would suggest that the focus of photographs on the same standardized ETDRS field 2 sufficiently correct the modifications of incidence angle for images recording. Before performing the measurements, the interest dimension should be established. As any biologic tissue has a tridimensional growth or involution, the monitorization of a single dimension cannot characterize the evolution in the most accurate way. The best parameter for retinal lesions evaluation in retinal plane is therefore their area. By comparing the results of different quantitative methods, our data suggested that, as compared to all the other methods measuring the lesions area, the GLD method is indeed not reproducible, has the lowest sensitivity and offers contradictory results between readers when assessing the evolution of macular lesions in eAMD.

The majority of previously reported methods assessed quantitatively the neovascularization and leakage evolution inside of manually delimited area – mdrdA [20,22,27-35,37,40,42,43,59]. In all these methods, the image interpretation is affected by errors in an infinite number of points along the irregular lesions boundaries (Figure 2D). Our data revealed these errors by showing statistically significant differences between measurements of 2 independent readers. Aiming to lower the errors and to increase the measurements accuracy, SITE-App algorithm standardized the interest area inside of which the measurements are performed, and designed the circumscribed circle method for quantification of single macular lesions (Figure 2A). The errors are significantly decreased because the reader has to interpret only a few points where the circum-

scribed circle is tangent to the lesion (Figure 2A). By standardizing the interest area, SITE-App algorithm has also other advantages to the mdrdA methods:

- Allows for measurements in multiple, not-homogeneous macular lesions (tangent circle method, (Figure 2B)).
- Can also quantify lesions around the optic disc (concentric circle method, (Figure 2C)) or numerous mixed retinal lesions (e.g. neovascularization and subretinal hemorrhages; neovascularization and fibrosis, etc).

In summary

The highest quality of black-white FA and RF images is essential for an accurate quantitative analysis and can be obtained in standardized conditions established for each series of images recorded from one eye. The most accurate measurements are performed inside of standardized interest areas, by expressing the analyzed retinal lesions in wpix or bpix, according to lesions appearance on RF or FA images. SITE-App is an easy, fast and low cost algorithm that includes, in its steps, conditions for the most reproducible and sensitive measurements.

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Disclosure

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