Macular pigment evaluation using dual-wavelength fundus auto-fluorescence imaging

Patrik Rajs[1,2], Ivana Liehneova[1], Zbynek Stranak[2]

Introduction. Macular pigment plays an important role in the reduction of oxidative stress and in preventing retinal diseases. Quick and easy measurements of the macular pigment are essential in both clinical and research settings. Dual wavelength fundus auto-fluorescence seems to be the optimal method. This study aims to investigate changes in fundus autofluorescence in patients taking daily lutein oral supplements and develop image processing methods for follow-up evaluations of the images.

Methods. New devices allow us to examine fundus autofluorescence using both blue and green excitation wavelengths. This allows detection of the amount of macular pigment by subtracting these two images because the yellow pigment particles absorb blue wavelengths. We determined daily dose of 25 mg of lutein and 3 mg of zeaxanthin. Patients were followed up for 15 months at 3-month intervals.

Results. During our 15-month study, we observed a positive trend in pixel lightness values, suggesting an increase in macular pigments in the foveal area. In all patients taking daily lutein supplements, the foveal index significantly increased after six months, with a median change of 0.081. We did not observe a significant change after the first three months (0.006) and only a small change between the 6th and 12th-month visits (0.012).

Conclusion. With appropriate patients and procedures for capturing autofluorescence images, this is a valuable technique for macular pigment evaluation in follow-up examinations using software image post-processing and analysis with commonly available hardware. To put this into everyday practice, developing tools to automate the assessment is necessary.

Key words: retina, macula, lutein, macular pigment, auto-fluorescence

Received: October 14, 2023; Revised: November 30, 2023; Accepted: December 7, 2023; Available online: January 9, 2024
https://doi.org/10.5507/bp.2023.051

[1]Department of Ophthalmology, UJEP Masaryk Hospital, Krajska zdravotni, a.s., Usti nad Labem, Czech Republic
[2]Department of Ophthalmology, University Hospital Kralovske Vinohrady and Third Faculty of Medicine, Charles University in Prague, Prague, Czech Republic
Corresponding author: Patrik Rajs, e-mail: patrik.rajs@icloud.com

INTRODUCTION

Macular pigment (MP) is composed of three very similar molecules: lutein, zeaxanthin, and meso-zeaxanthin. These are natural lipophilic pigments of purely plant origin. In the macula, we can observe a familiar horizontal distribution of individual isomers, with concentration peaks of all three molecules in the foveola, where the highest accumulation of zeaxanthin is found, along with a foveal to parafoveal ring of lutein. In terms of vertical distribution, the macular pigment is found in both the outer and inner plexiform layers[1]. The outer plexiform layer is composed of synapses between photoreceptors and bipolar cells, while the inner plexiform layer consists of bipolar axons and ganglion cell dendrites.

This study aimed to investigate changes in fundus auto-fluorescence (FAF) in patients daily using oral lutein supplements and to design the image processing needed for follow-up evaluations of the captured images. To date, there are currently no routinely available methods for evaluating the amount of macular pigment in the retina; however, new devices allow us to examine the fundus using both blue (FAF-B) and green (FAF-G) excitation wavelengths. Specifically, FAF-B images are captured using excitation wavelengths between 435–500 nm and detection between 532–650 nm, while FAF-G images are captured using excitation wavelengths between 500–585 nm and detection between 630–750 nm. These images can be used to indirectly detect the amount of macular pigment. Yellow pigment particles absorb blue light at shorter FAF-B wavelengths; therefore, the macula appears darker than in FAF-G images (macular pigment shadow).

According to studies, it is the complex of all three molecules that has the greatest antioxidant capacity[2]. The MP layer also acts as an optical filter. By physical nature, light in the blue part of the spectrum is more likely to scatter, which can lead to image quality degradation. Specific yellow filters can correct these undesirable effects and, according to some studies, leads to improved contrast sensitivity[3].

Macular pigment plays an important role in reducing oxidative stress, and in the development of retinal diseases, especially age-related macular degeneration (AMD).

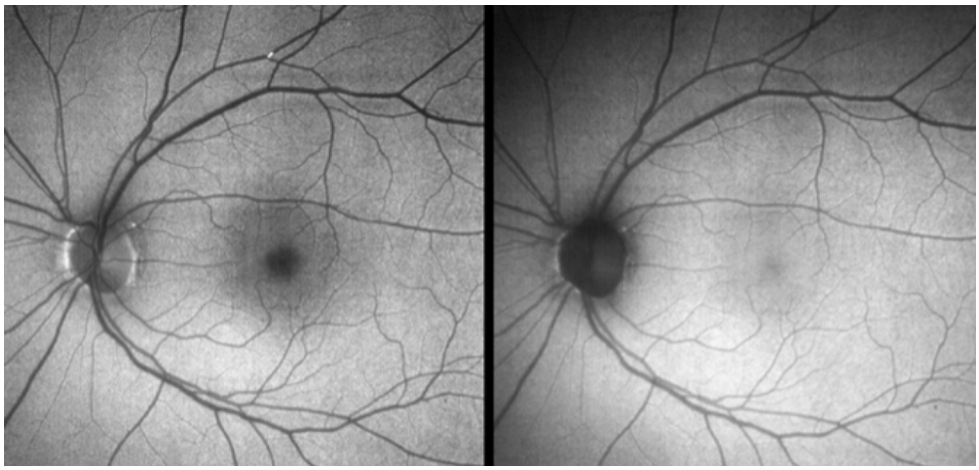

Fig. 1. FAF-B image (left), FAF-G image (right).

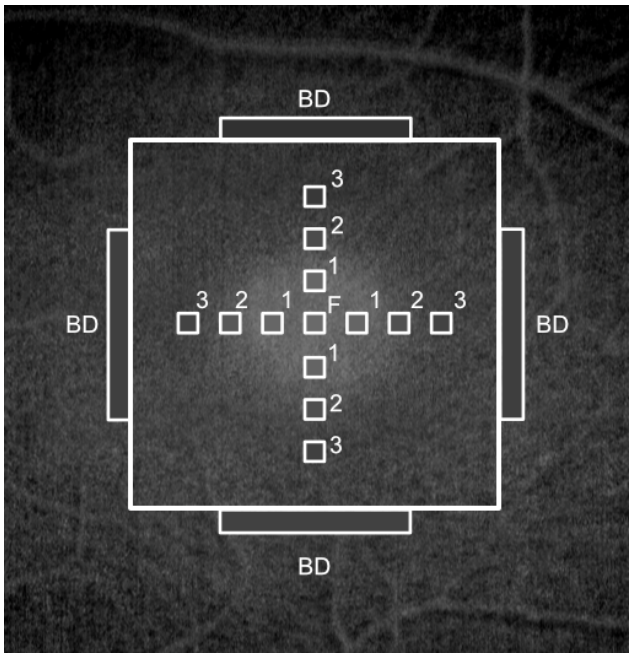

Fig. 2. Assessment Grid used for DFAF images.

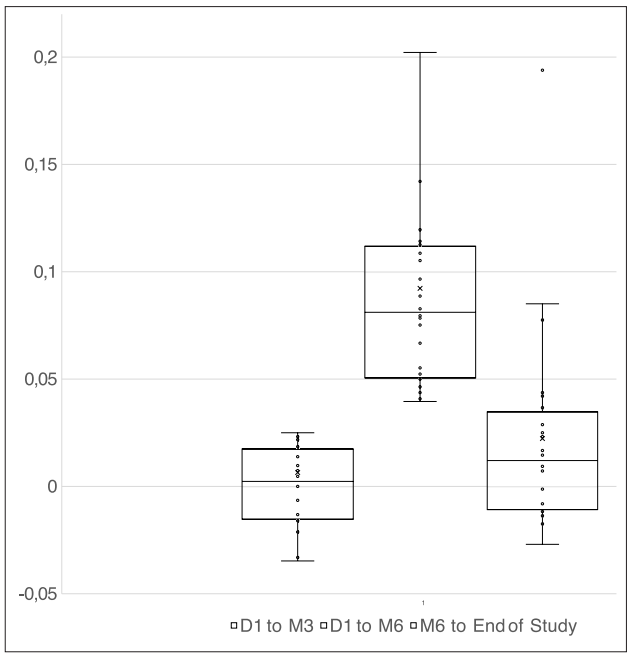

Fig. 3. Foveal Index Change in Time Intervals. D1, day 1; M, month.

Lutein is also one of the most concentrated carotenoids in the brain, and many studies have found a connection between cognitive function and lutein supplementation, with potential benefits for patients with Alzheimer's disease[4-6]. The amount of MP in the central retinal area correlates with the amount of lutein in the brain; measuring MP in the retina is also relevant to other fields of medicine.

METHODS

Subjects and Procedures

Twenty patients were examined every three months for 12 to 15 months (End of Study Visit). We always performed a visual acuity test (ETDRS chart) and captured true color images and FAF (-B, G) images (Fig. 1) of both eyes using a Zeiss Clarus 700 fundus camera.

For our study, we designed an assessment grid (Fig. 2) to monitor pixel lightness values in concentrically spaced regions of the central retinal area in patients using a dietary lutein supplement. We generally followed the AREDS2 study recommendation of administering daily dose of at least 10 mg of lutein and 2 mg of zeaxanthin[7]. We selected a slightly higher daily dose for our study, i.e., 25 mg of lutein and 3 mg of zeaxanthin. No further dietary restrictions were recommended. Patients were followed up for 15 months at 3-month intervals. The values measured in the grid of each image were converted into a graph to obtain a macular pigment density profile. The changing shape of the curve is striking from the individual graphs, based on the broadening of the MP shadow with a corresponding increase in the brightness of the pixels in the DFAF images. It is not possible to focus on the absolute values of the brightness of the pixels since we would run into limitations in the replicability of the exposure. For continuous tracking, it is necessary to introduce a relative expression, i.e., a value that reflects the change in the shape of the descending profile. This relative value,

which we refer to as the "foveal index" in our paper, is calculated as the ratio of the central square of the assessment grid within the foveola to the average of the nearest four squares within the fovea. The images were analyzed in 8bit grayscale color mode, where the tones of the grayscale image range from 0 (black) to 255 (white) and all 254 shades of gray in between. The original FAF images were exported as TIFF files (3916×3916 px, 96 pixels/inch), blinded, and masked to the reviewer during all analysis steps. Adobe Photoshop software (Adobe, San Jose, CA) was used for image alignment, subtraction, and pixel lightness evaluation.

FAF image analysis procedure

1. Quality and artifacts assessment
2. Subtraction of high-quality FAF Blue and FAF Green images
3. Analysis of subtracted images – for every image we used the same assessment grid consisting of 18 fields (Fig. 2, various areas in specific locations including the foveola, fovea, and parafovea).
   a. Getting average individual lightness of all 18 fields in the assessment grid (central area square 300×300 pixels, 13 squares 16×16 pixels, and four border rectangles 18×155 pixels)
   b. Exporting the lightness value of every field
4. Evaluation of the measured values and construction of MP curves
5. Calculating the "foveal index" – relative value as described above.

To prove the functionality of our MP assessment technique, we used four eyes from patients diagnosed with macular telangiectasia type 2 (MacTel Type 2) as a control group. As described in other studies[8,9], we also confirmed higher MP levels in the peripheral regions of the macula compared to the central region. The MP curve shape was reversed compared to patients without MacTel Type 2 disease, with the lowest point in the center of the assessment grid and highest points along the periphery.

Table 1. Foveal Index Changes.

	D1 – M6	M6 – End of Study	D1 – M3
Median	0.081	0.012	0.006
Average	0.092	0.022	0.008

D1, day 1; M, month.

During the study, dietary supplementation in the control group slightly enhanced the existing ring of higher MP concentrations but did not restore normal distributions, confirming the results of Zeimer et al.[10]. Analyzing the foveal index of the control group, the values were significantly higher, as expected from the curve shape, representing central depression. However, there was no measurable change during the monitored time since the amount of MP was initially very low in the foveal area.

RESULTS

Subjects

Out of a total of 20 patients (40 eyes), we had to exclude three patients for non-compliance, three for poor image quality due to cataract progression, and two patients diagnosed with another macular disease. Twenty-four eyes with only mild dry AMD changes or without any macular pathology were included. There was no significant change in the best corrected visual acuity (BCVA) for any of the 24 eyes during the study. The average BCVA, measured in ETDRS letters, was 82.708 (ranging from a minimum of 70 letters to a maximum of 85 letters, with a median of 84 letters). The maximal change in visual acuity observed between the start of the study (screening) and the end was no more than two letters. A history of oral carotenoid supplementation was considered an exclusion criterion. The participants were between the ages of 62 and 80 yrs., with an average age of 69.67 (median age of 69 yrs.). Cataracts were also considered an exclusion criterion, but artephakic patients were allowed. There were 18 eyes with IOLs (phacoemulsification and PC IOL implantation) and six eyes with crystalline, natural lenses without significant opacification. Our patients did not suffer from any serious comorbidities, and they were mostly treated for high blood pressure and hyperlipidemia; all were well compensated.

Foveal Index Changes

We confirmed a positive trend in pixel lightness values, suggesting increased MP concentration (Fig. 3). In all patients taking daily lutein supplements, the foveal index significantly increased after six months (the median index change was 0.081). We did not see a significant change after the first three months (the median index change was 0.006) and observed only a small change between the 6th and 12th-month visits (0.012)

Table 2. Results of the estimation.

Variable	Estimate	Std Error	Prob>|t|	Lower 95%	Upper 95%
Intercept	0.8770173	0.022904	<.0001*	0.8275504	0.9264842
After 3 months	0.0023612	0.011593	0.8392	−0.020758	0.0254806
After 6 months	0.0897063	0.011202	<.0001*	0.067365	0.1120475
After 9 months	0.0982058	0.018572	<.0001*	0.0611728	0.1352389
End of study	0.1133708	0.010718	<.0001*	0.0919949	0.1347467

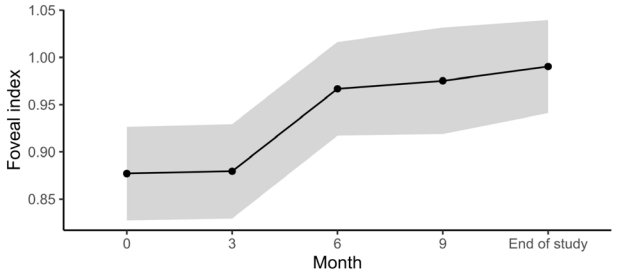

Fig. 4. Plot of the Expected Foveal Index after supplementation over the course of the study.

Empirical analysis

This section presents the empirical findings from our study. The analysis used the mixed effect model with a fixed effect of months, which were treated as categorial variables. The specifications are as follows:

$$Foveal_{ime} = \alpha + \beta_1 Month3_{ie} + \beta_3 Month6_{ie} + \beta_2 Month9_{ie} + \beta_4 EndofStudy_{ie} + \gamma_i + \delta_{e|i} + \varepsilon_{iem}$$

Where $Foveal_{ime}$ is the dependent measurement variable on patient *i,* in month *m*, and eye *e.* α is the intercept. β_1, ..., β_4 are coefficients that are of interest since they measure the change in the foveal index (after supplementation) at 3 (β_1), 6 (β_2), 9 (β_3) months during the supplementation and at the end (β_4) of the study. The end of the study was 12 to 15 months after the start of oral supplementation. γ_i is the random patient effect and $\delta_{e|i}$ is an eye within a random patient effect. These random effects are included in the regression to capture the correlation of observed values within a patient and within the eye of a patient, respectively. The model is estimated using a restricted maximum likelihood estimation.

Table 2 presents the results of the estimations. The estimated coefficients show supplementation effects compared to the supplementation time (time 0). The average value of the foveal index before supplementation is represented by the intercept. The findings show no statistically significant difference between foveal index values at the beginning of the study and the values after three months of daily usage. However, there was a statistically significant effect after six months. The effect persists until the end of the study, with an average increase in the value of the foveal index ranging from 0.089 to 0.113. By inspecting the confidence intervals, we see that supplementation effect does not significantly increase after the sixth month.

In Fig. 4, we plot the expected foveal indices 3, 6, and 9 months after the start of treatment and at the end of the study. The figure shows a pattern that is also visible in Table 2. There is a statistically significant increase in the foveal index six months after the treatment, after which the value remains stable.

DISCUSSION

Quick and easy measurement of the amount of macular pigment is essential for both clinical and research purposes. Dual wavelength fundus auto-fluorescence (DFAF) seems to be the best currently available method. However, some factors can influence the measurements that need to be considered. For example, cataracts block the FAF signal, particularly the FAF-B, which is important to consider during an analysis. The concentration of lutein in the lens increases with age[11], which provides an additional protective layer for the retina. Despite this, the FAF signal is reconstructed after cataract surgery using IOL implants[12], which is why we included pseudophakic patients. Surprisingly, we did not observe any measurement difficulties with intraocular lenses (IOL) with yellow filters (Alcon Clareon Aspheric Hydrophobic Acrylic IOL, CNA0T0). Considering that the FAF-B excitation wavelength is between 435–500 nm and detection between 532–650 nm, we found that the increased concentration of lutein in crystalline lens that occurs with aging and the cataract progression generally blocked wavelengths between 434–650 nm (FAF-B) more significantly than IOL filters. The total transmittance of a "young" lens increases rapidly at around 390 nm and reaches 90% (ref.[13]) at 450 nm. The rate of increase is considerably slower for an "older" lens. For example, a 63-year-old lens begins transmitting at 400 nm but does not reach 90% (ref.[13]) transmittance until 540 nm. When comparing the marketed range of spectral transmittance values of IOLs made from hydrophobic acrylate/methacrylate copolymer with bonded UV-absorber and Alcon's proprietary blue light filtering chromophore, there is an increasing trend toward IOLs transmittance values of over 450 nm[13,14], where the curve of crystalline lens transmittance has already started to flatten. In this regard, FAF signal evaluations could be used to objectively grade cataract progression[15]. We also must consider light scattering caused by the lens, which is why the multifocal IOLs caused some difficulties in capturing FAF images. Of course, the condition of other optical media (cornea, vitreous) must also be mentioned. Pupil constriction also heavily influences the signal, hence the need for sufficient mydriasis. Therefore, mydriasis should be used routinely for DFAF MP measurements[16]. When comparing images of individual visits, we calculated the relative number (ratio), which we call the "foveal index," to eliminate incorrect interpretations caused by small changes in exposure between each visit. In the context of our study, it is necessary to mention the pitfalls of using Broad Line Technology with wider wavelength detection, where at higher MP concentrations, shielding can occur during green wavelength mode capturing because of overlapping, which can cause a change in the curve shape at the top, where the central value (Fig. 2, area F) is lower compared to the closest juxtafoveal values (Fig. 2, areas 1). In that case, the foveal index is higher than 1.0, and we must exclude underlying RPE pathology.

The FAF imaging devices available today can be divided into conventional fundus cameras, cSLO (confocal

scanning laser ophthalmoscope), and new systems based on Broad Line Technology (BLFI), which allow wider wave spectrums to be captured simultaneously. There are no defined standards, therefore, final images are device-specific. Differences in imaging can be observed between the capturing principles (camera chip or cSLO-generated image) and between different devices that use slightly different optical filters or differently configured software working with the signal. For scientific purposes, a modified Heidelberg Spectralis device with an MPOV module (Macular Pigment Optical Volume) that uses the DFAF principle is available.

CONCLUSION

Overall, with the appropriate patient and an eligible procedure for capturing images, this is a valuable technique for follow-up MP evaluation examinations using software image post-processing and analysis with commonly available hardware. Tools that can automate the assessment must be developed to make this technique available for everyday practice. Based on the individual steps, the use of artificial intelligence is suggested.

Author contributions: PR, IL: conceived the presented idea, data collection; PR: designed the image processing methods and performed the computations. IL, ZS: verified the methods and contributed to the interpretation of the results. All authors discussed the results and contributed to the final manuscript.

Conflict of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

Ethic approval and consent to participate: Study was approved by the Ethics committee (UJEP Masaryk Hospital, Krajska zdravotni, a.s., Usti nad Labem, Czech Republic). Every patient had to give written permission before receiving any type of medical treatment, test, or examination.

REFERENCES

1. Snodderly DM, Brown PK, Delori FC, Auran JD. The macular pigment. I. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. Invest Ophthalmol Vis Sci 1984;25(6):660-73.
2. Li B, Ahmed F, Bernstein PS. Studies on the singlet oxygen scavenging mechanism of human macular pigment. Arch Biochem Biophys 2010;504(1):56-60.
3. Nolan JM, Power R, Stringham J, Dennison J, Stack J, Kelly D, Moran R, Akuffo KO, Corcoran L, Beatty S. Enrichment of Macular Pigment Enhances Contrast Sensitivity in Subjects Free of Retinal Disease: Central Retinal Enrichment Supplementation Trials - Report 1. Invest Ophthalmol Vis Sci 2016;57(7):3429-39. doi: 10.1167/iovs.16-19520
4. Craft NE, Haitema TB, Garnett KM, Fitch KA, Dorey CK. Carotenoid, tocopherol, and retinol concentrations in elderly human brain. J Nutr Health Aging 2004;8(3):156-62.
5. Nolan JM, Loskutova E, Howard A, Mulcahy R, Moran R, Stack J, Bolger M, Coen RF, Dennison J, Akuffo KO, Owens N, Power R, Thurnham D, Beatty S. The impact of supplemental macular carotenoids in Alzheimer's disease: a randomized clinical trial. J Alzheimers Dis 2015;44(4):1157-69. doi: 10.3233/JAD-142265
6. Lindbergh CA, Renzi-Hammond LM, Hammond BR, Terry DP, Mewborn CM, Puente AN, Miller LS. Lutein and Zeaxanthin Influence Brain Function in Older Adults: A Randomized Controlled Trial. J Int Neuropsychol Soc 2018;24(1):77-90. doi: 10.1017/S1355617717000534
7. AREDS2 Research Group; Chew EY, Clemons T, SanGiovanni JP, Danis R, Domalpally A, McBee W, Sperduto R, Ferris FL. The Age-Related Eye Disease Study 2 (AREDS2): study design and baseline characteristics (AREDS2 report number 1). Ophthalmology 2012;119(11):2282-9. doi: 10.1016/j.ophtha.2012.05.027
8. Charbel Issa P, van der Veen RL, Stijfs A, Holz FG, Scholl HP, Berendschot TT. Quantification of reduced macular pigment optical density in the central retina in macular telangiectasia type 2. Exp Eye Res 2009;89(1):25-31.
9. Srinivasan R, Teussink MM, Sloan KR, Bharat RPK, Narayanan R, Raman R. Distribution of macular pigments in macular telangiectasia type 2 and correlation with optical coherence tomography characteristics and visual acuity. BMC Ophthalmol 2022;22(1):264.
10. Zeimer MB, Krömer I, Spital G, Lommatzsch A, Pauleikhoff D. Macular telangiectasia: patterns of distribution of macular pigment and response to supplementation. Retina 2010;30(8):1282-93.
11. Roberts JE, Dennison J. The Photobiology of Lutein and Zeaxanthin in the Eye. J Ophthalmol 2015;2015:687173.
12. Reiter GS, Schwarzenbacher L, Schartmüller D, Röggla V, Leydolt C, Menapace R, Schmidt-Erfurth U, Sacu S. Influence of lens opacities and cataract severity on quantitative fundus autofluorescence as a secondary outcome of a randomized clinical trial. Sci Rep 2021;11(1):12685. doi: 10.1038/s41598-021-92309-6
13. Boettner EA, Reimer Wolter J. Transmission of the Ocular Media. Invest Ophthalmol Vis Sci 1962;1(6):776-83.
14. US DFU ClareonTM Aspheric Hydrophobic Acrylic IOL with the AutonoMeTM Automated Pre-loaded Delivery System Model: CNA0T0, Rev. 0120, Available from: https://www.accessdata.fda.gov/cdrh_docs/pdf19/P190018C.pdf
15. Eom Y, Suh YW, Kim SW, Lee YJ, Jeon HS, Lee SY, Lee IH, Song JS, Kim HM. New technology using crystalline lens autofluorescence for presbyopia and cataract grading. Graefes Arch Clin Exp Ophthalmol 2023;261(7):1923-32. doi: 10.1007/s00417-023-05999-1
16. Loskutova E, Butler JS, Hernandez Martinez G, Flitcroft I, Loughman J. Macular Pigment Optical Density Fluctuation as a Function of Pupillary Mydriasis: Methodological Considerations for Dual-Wavelength Autofluorescence. Curr Eye Res 2021;46(4):532-8.