Macular Pigment Density and Quantitative Fundus Autofluorescence in Young Healthy Subjects

Chiara M. Eandi, Marco Nassisi, Carlo Lavia, Camilla Alovisi, and Ugo de Sanctis

Department of Surgical Sciences, Eye Clinic, University of Torino, Torino, Italy

Correspondence: Chiara M. Eandi, Department of Surgical Sciences, Eye Clinic, University of Torino, Torino, Italy, Via Juvarra 19, 10122 Torino, Italy;
chiara.eandi@unito.it,
ceandi@gmail.com.

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Purpose. To measure macular pigment (MP) and find possible correlation between heterochromatic flicker photometry (HFP) and quantitative autofluorescence (qAF) in young healthy subjects.

Methods. We enrolled 80 eyes of 40 young healthy subjects. Macular pigment optical density (MPOD) was automatically calculated with a macular pigment screener (MPS; $MPOD_{HFP}$). We calculated qAF comparing gray levels (GL) of qAF images with GL of internal reference of a confocal scanning laser ophthalmoscopy. A raster of concentric rings was used to automatically calculate foveal qAF (qAFF) values (0°–1.2°); inner ring (1.3°–4.3°; qAF_3); middle ring (4.5°–7°; qAF_6); and outer ring (7.2°–9.7°; qAF_8). The test-retest coefficient of repeatability was calculated with Bland-Altman method. The between-eyes coefficient of agreement and correlation between the two techniques were calculated. Finally, an estimation of MPOD from qAF was performed (MPOD-AF), to find possible direct correlations with $MPOD_{HFP}$ obtained with the MPS II.

Results. Paired data sets of repeated measurements were not statistically different for MPS II ($P = 0.66$); log qAF_F ($P = 0.95$); log qAF_3 ($P = 0.48$); log qAF_6 ($P = 0.4$); and log qAF_8 ($P = 0.56$). Stepwise regression analysis showed negative correlation between MPS II and log qAF_F values ($R^2 = 0.35$) with Spearman coefficient (ρ) of -0.60 ($P < 0.01$) and log qAF_3 ($R^2 = 0.18$; $\rho = -0.38$.; $P < 0.01$). No correlation was found between MPS II and log qAF_6 ($\rho = 0.01$, $P = 0.93$), neither with log qAF_8 ($\rho = -0.05$, $P = 0.66$).

Conclusions. In young healthy subjects, a negative correlation between qAF values and $MPOD_{HFP}$ was found in the central degrees. However, qAF and HFP do not seem to be interchangeable: they represent two opposite ways of estimating MP.

Keywords: retina, autofluorescence, macula, pigment

Macular pigment is mainly composed of carotenoid pigments (lutein, zeaxanthin, and meso-zeaxanthin). Macular pigment has a spatial distribution corresponding approximately to the area of the foveal avascular zone and characteristically decreases at approximately 5° to 8° eccentricity.[1–3] Macular pigment is maximally located at the level of the inner plexiform layer and Henle's fiber layer of the human macula and has peculiar short wavelength (blue) light filtering and antioxidant properties.[4–6] Besides its effect in normal subjects (reduction of glare disability and chromatic aberration), macular pigment seems to protect against AMD.[7,8] Therefore, the detection and quantification of MP has become important.[9] Several techniques are available for measuring MP, both in vivo and ex vivo.[4,5,10] In vivo modalities include physical (e.g., dual-wavelength fundus autofluorescence, fundus reflectometry, and Raman spectroscopy) and psychophysical (e.g., HFP, customized [c]HFP, color matching, and motion photometry) techniques. In particular, HFP is a psychophysical technique used to MPOD by presenting a light stimulus of two alternating wavelengths (short: absorbed, and long: not absorbed). The aim of the HFP is to achieve a perception of no or minimal flicker, that occurs when the luminance of the two wavelengths appears equal to the observer.[11,12]

Fundus autofluorescence (FAF) is a physical method used to detect MP based on the properties of lipofuscin that is normally present at the level of the human RPE cells and photoreceptors outer segments.[13] Retinal pigment epithelium lipofuscin is excited in vivo between 400 and 590 nm and emits autofluorescence at 520 to 800 nm.[14] Since MP absorbs light of 400 to 550 nm and it is located anteriorly to the RPE, macular autofluorescence is attenuated by MP itself if the excitation wavelength falls between 400 and 550 nm.

Although there is not yet a technique considered as the "gold standard" for measuring MP, a concordance between MP density measured by HFP and that measured with the two-wavelength autofluorescence has already been proven both in healthy and in AMD eyes.[15,16] Even single-wavelength FAF has been previously employed to detect MP and even if it resulted less reliable when compared with the double-wavelength technique especially in peripheral areas, it showed high repeatability and a good agreement between the techniques was found within the fovea center.[17]

Quantitative autofluorescence (qAF), recently introduced, seems a more standardized and repeatable technique compared to FAF and has been studied in the evaluation of autofluorescence in healthy and pathologic eyes.[18–20]

The aim of the study was to assess the MPOD in a healthy group of young adults using an HFP technique ($MPOD_{HFP}$) and to find a possible correlation with quantitative autofluorescence measured in the same area.

Table 1. Demographic Data

Subjects enrolled, *n*	40
Subjects who completed all tests, *n*	40
Sex, male/female	18/22
Age, y, mean ± SD (range)	25.87 ± 3.93 (20–34)
Race (%)	Caucasian (100)
BCVA, Snellen equivalent, mean (range)	20/20 (20/20–20/20)

Methods

Subjects

We enrolled 80 eyes of 40 healthy subjects in the study from the population of staff and students of the University Eye Clinic of Torino, Italy, between October and December 2015. Mean (±SD) age of the study population was 25.87 (±3.93) years (range, 20–34 years). They were all Caucasian. All subjects successfully completed the MP and qAF tests. Demographic data are provided in Table 1. All subjects were in good general and ocular health, had clear media with no lens opacities, normal retinal status, good fixation, and a refractive error from −3.0 to + 2.0 diopters. Best corrected visual acuity (BCVA) was 20/20 (Snellen equivalent) in all subjects. Exclusion criteria were familiarity for AMD, smoking, and any kind of therapy including nutritional supplements.

The tenets of the Declaration of Helsinki were followed, institutional review board approval was granted, and informed consent was obtained from all subjects. The retinal light exposures (recommended maximum power: 280 μW; 30° × 30° field; 488 nm) are below the limits recommended by the American National Standard Institute (ANSI) standards for durations up to 8 hours.[21,22]

Procedures

On the same day, the subjects underwent BCVA exam and $MPOD_{HFP}$ measurement using a macular pigment screener (MPS II/MPS 9000; Elektron Technology, Cambridge, UK). An Early Treatment Diabetic Retinopathy Study chart was used to measure BCVA, expressed as Snellen equivalent. Pupil dilatation was performed after BCVA and MPS II examinations with topical 1% tropicamide solution and 2.5% phenylephrine. After 30 minutes, in order to obtain adequate mydriasis of at least 7 mm, subjects underwent qAF.

Macular Pigment Measurement

Measurements of $MPOD_{HFP}$ were all performed by the same trained investigator (CL). We used the HFP technology of the MPS II to measure $MPOD_{HFP}$ as described in details elsewhere.[23,24] Briefly, a light stimulus of two alternating wavelengths (blue and green, 465 nm and 530 nm, respectively) was presented and the subject reported the appearance of flicker as the temporal frequency of blue-green flickering lights was reduced.[8,25] In other words, the subject registered his first perception of flicker throughout different blue-green ratios.

Data were taken analyzing the central (0°) and peripheral (8°) regions. The target consists of a 1° circular aperture in an integrating sphere. This is surrounded by a uniformly illuminated white area subtending approximately 30°. The peripheral measurement is achieved by fixating on a larger 1.75° red spot located at 8° horizontal eccentricity (Figs. 1A, 1B). We then automatically calculated $MPOD_{HFP}$ using the difference between central (0°) and peripheral (8°) values. The software of the MPS II uses a new algorithm that automatically analyzes the results and gives three possible outcomes (accept, caution, and reject). In order to collect reliable data, we decided not to approve "caution" and "reject" results. Every test was repeated twice (with 30-minute intervals between measurements) to verify the repeatability of the instrument. When unacceptable results (i.e., "caution" or "reject") were observed, the test was interrupted and the subject was asked to repeat it the following day. For example, the MPS II reports unacceptable results in case of too few data points or shallow graph, noisy data or flicker too high, as stated in the user manual. Every subject was able to perform the test and included for statistical analysis.

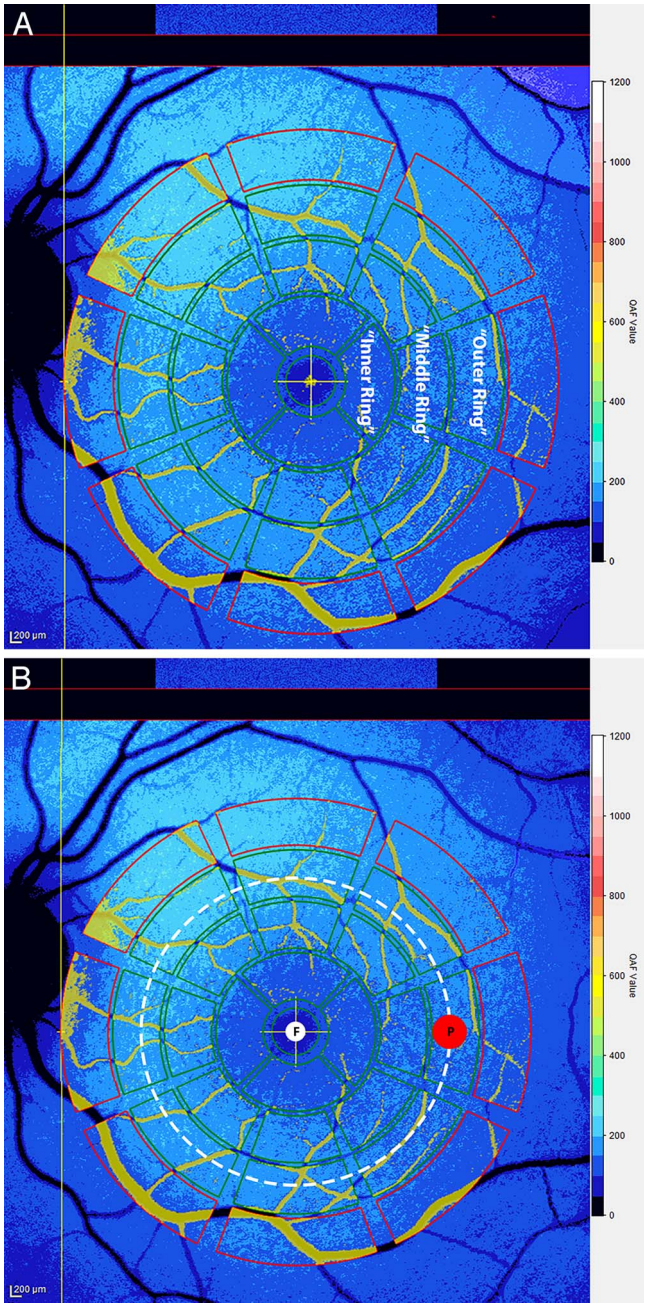

Figure 1. Quantitative fundus autofluorescence image with the scan pattern centered on the fovea. (**A**) Only green segments were included in our analysis: qAF_F includes the central area; qAF_3 includes the inner ring; qAF_6 includes the middle ring; qAF_8 includes the outer ring. (**B**) Comparison of the areas analyzed by two devices: green segments corresponding to an area of 8.5° radius were analyzed by qAF, while the white dashed circular line delimits an area of 8° radius analyzed by the MPS II. The *white* and *red circles* are the MPS II targets: *F* is the 1° central target; *P* is the 1.75° peripheral target.

Quantitative Fundus Autofluorescence

An experienced operator (MN) acquired all qAF images using a confocal scanning laser ophthalmoscopy (cSLO) device (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany). Quantitative AF incorporates a fluorescence reference internal to the imaging device so that the reference is part of the FAF image. Analysis consisted of comparing the gray levels (GL) of the FAF image with the GL of the internal reference, accounting thereby for changes in laser power and detector sensitivity. Furthermore, the Heidelberg acquisition software includes corrections for magnification and optical media density from normative data on lens transmission spectra.

Here there is a summary of qAF images acquisition protocol as detailed description is reported elsewhere.[18] Thirty minutes after pupil dilation, subjects were examined with room lights turned off and underwent a 20- to 30-second bleaching period to reduce photopigment absorption.[18] The fundus image was focused to reach maximum FAF signal intensity, and the detector sensitivity was increased until the cSLO image was as bright as possible, avoiding nonlinear effects at high GL (indicated by red pixels). The Heidelberg software limits the sensitivity setting to a value of 90 to avoid being in the nonlinear detector realm.

Three images (each of 12 frames, in video format) were recorded with room lights turned off in the high speed mode (8.9 frames/second) within a $30^\circ \times 30^\circ$ field (768×768 pixels). Images were obtained within a session (~3–6 seconds apart) using the same positioning in the chin/head rest, alignment of the camera, focus, and sensitivity. After image acquisition, the quality of subject fixation was clinically evaluated by the mean of a target positioned at 40 cm, together with the resolution of each image. All patients presented with good fixation.

At the end of the examination image quality was verified according to the qAF analysis software manual, (Spectralis Quantitative Autofluorescence Analysis Software User Manual, Software, version 6.0; Heidelberg Engineering) and the first two exams, if acceptable, were used to test instrument repeatability. Every frame inside each video was aligned and averaged with the system software and saved to create the images for analysis.

To determine qAF, images were exported from a commercial software program (Heidelberg Eye Explorer; Heidelberg Engineering) to a custom-made image analysis program (Heidelberg Engineering), which was developed according to the software created by Francois Delori (IGOR; WaveMetrics, Lake Oswego, OR, USA).[18] The software allowed us to draw a raster that consists of four concentric rings of segments around the foveal area (Fig. 1A). The pattern was centered on the fovea and its peripheral margin was tangential to the temporal edge of the optic disc: the distance between them was defined as foveal distance (FD). For each segment, qAF was automatically computed from the mean GL in that segment and the GL of the internal reference.[18] The software accounted for the presence of vessels in the segments.

The average FD, used to define the measurement areas, was 12.3° (visual field degrees). The central circle (fovea) had a radius of 1.2°. The radii of the centerlines for the inner, middle, and outer rings were respectively 2.8°, 5.7°, and 8.5°. The mean difference in radius between the area analyzed by the MP II and the area analyzed by qAF_6 (middle ring) is 0.4° (Fig. 1B).

Four measurements were obtained from each image (Fig. 1A): qAF of the central foveal area (qAF_F); mean qAF between the four segments of the inner ring (qAF_3); mean qAF between the eight segments of the middle ring (qAF_6) and mean qAF between the eight segments of the outer ring (qAF_8). The more external ring was not included in our correlation analysis.

Data Analysis and Statistics

All data are presented as mean ± standard deviation. The Shapiro-Wilk test was used to verify the normal distribution of data.[26] Differences between data sets were evaluated with paired *t*-tests. A value of $P < 0.05$ was considered significant. Statistical analyses were conducted using commercial software (SPSS 19.0; SPSS, Inc., Chicago, IL, USA).

Repeatability. Test–retest repeatability and between-instrument agreement between measurements were analyzed using the Bland-Altman method.[27] The coefficient of repeatability (CR) was calculated as:

$$CR_{MPOD} = \pm 1.96 \times \sigma_{MPOD_2 - MPOD_1} \quad (1)$$

$$CR_{qAF_F} = \pm 1.96 \times \sigma_{\log(qAF_2) - \log(qAF_1)}, \quad (2)$$

where $MPOD_{HFP1}$ and $MPOD_{HFP2}$, and qAF_1 and qAF_2 are respectively the two measurements made for each eye; σ is the standard deviation. As previously described,[19] we used log(qAF) instead of qAF in all analysis. The same formula was used to calculate repeatability of qAF_3, qAF_6, and qAF_8.

Agreement Between Eyes. Between-eyes coefficient of agreement (CA) was calculated as:

$$CA_{MPOD_{HFP}} = \pm 1.96 \sigma_{\overline{MPOD_{HFP_{OD}}} - \overline{MPOD_{HFP_{OS}}}} \quad (3)$$

$$CA_{qAF_F} = \pm 1.96 \sigma_{\overline{\log(qAF_{OD})} - \overline{\log(qAF_{OS})}}, \quad (4)$$

where $\overline{MPOD_{HFP}}$ and $\overline{\log(qAF)}$ are the mean values of $MPOD_{HFP1}$ and $MPOD_{HFP2}$, $\log(qAF_1)$ and $\log(qAF_2)$, respectively. The same formula was used to calculate agreement between eyes of qAF_3, qAF_6, and qAF_8.

Correlation Between qAF and MPOD. With an expected correlation coefficient of 0.5 and a significance level of 0.05, we estimated a sample size of 38 eyes to reach a statistical analysis power of 0.9.[28] Regression analysis and Spearman coefficient were used to calculate the correlation between each log: qAF_B qAF_3, and qAF_6 with $MPOD_{HFP}$.

We also estimated MPOD values from qAF_B as previously described [17], using the formula:

$$MPOD_{AF} = \frac{1}{K_{(488)}} \times \log\left(\frac{qAF_8}{qAF_F}\right), \quad (5)$$

where $K_{(488)}$ (0.781) is the known extinction coefficient of the MP at 488 nm, relative to the extinction coefficient at 460 nm.[29] The same formula was used to estimate MPOD from qAF_3 and qAF_6. We then correlated all $\overline{MPOD_{AF}}$ with $\overline{MPOD_{HFP}}$.

Results

Using the Shapiro-Wilk test, all data resulted normally distributed ($P > 0.05$).

Repeatability and Agreement Between Eyes

Main results are summarized in Table 2. Mean $\overline{MPOD_{HFP}}$ was 0.47 ± 0.1 optical density units (DU). Mean $\overline{\log(qAF)}$ was 1.66 ± 0.17 for log qAF_B 1.92 ± 0.12 for log qAF_3, 2.32 ± 0.13 for log qAF_6, and 2.31 ± 0.12 for log qAF_8. We found CR was ±0.17 for the MPS II and ±0.031, ±0.013, ±0.018, and ±0.013 for log qAF_F, log qAF_3, log qAF_6, and log qAF_8, respectively (Fig. 2).

Paired data sets of repeated measurements were not statistically different for the MPS II ($P = 0.66$); log qAF_F ($P =$

Table 2. Statistical Analysis Showing the Results of Test-Retest Repeatability, CR, and COA Between the Two Techniques of MP Measurement (HFP and qAF)

Technique*	Mean ± SD	CR	*P* Value (X_1) vs. (X_2)	*P* Value $\bar{X}_{OD}$ vs. $\bar{X}_{OS}$	CA
MPS II	0.47 ± 0.1	±0.17	0.66	0.93	±0.51
log qAF_F	1.66 ± 0.17	±0.031	0.95	0.95	±0.11
log qAF_3	1.92 ± 0.12	±0.013	0.48	0.52	±0.16
log qAF_6	2.32 ± 0.13	±0.018	0.4	0.75	±0.17
log qAF_8	2.31 ± 0.12	±0.013	0.56	0.35	±0.15

X_1 and X_2, first and second measurement respectively; $\bar{X}_{OD}$ and $\bar{X}_{OS}$, mean values in the right and left eye, respectively.
* The values of MPS II are expressed in optical DUs. Log qAF values are expressed in log qAF units.

0.95); log qAF_3 ($P = 0.48$); log qAF_6 ($P = 0.4$); and log qAF_8 ($P = 0.56$).

Paired data sets of measurements for the two eyes were not statistically different for the MPS II ($P = 0.93$); log qAF_F ($P = 0.95$); log qAF_3 ($P = 0.52$); log qAF_6 ($P = 0.75$); and log qAF_8 ($P = 0.35$), with CA of ±0.51 ($MPOD_{HFP}$), ±0.11 log qAF_F units, ±0.16 log qAF_3 units, ±0.17 log qAF_6 units, and ±0.15 log qAF_8 units.

Correlation Between qAF and MPOD

A stepwise regression analysis showed a negative correlation between $MPOD_{HFP}$ and log qAF_F (coefficient of determination, $R^2 = 0.35$) and log qAF_3 ($R^2 = 0.18$) with a Spearman coefficient (ρ) of –0.60 ($P < 0.01$) and $P = –0.38$ ($P < 0.01$), respectively. No correlation was found neither between $MPOD_{HFP}$ and log qAF_6 ($\rho = 0.01$, $P = 0.93$) nor between $MPOD_{HFP}$ and log qAF_8 ($\rho = –0.05$, $P = 0.66$; Fig. 3). Mean $MPOD_{AF}$ was 0.82 ± 0.25 from qAF_F, 0.5 ± 0.21 from qAF_3 and –0.01 ± 0.04 from qAF_6. It showed a positive correlation with $MPOD_{AF}$ values in the foveal area ($R^2 = 0.23$) with $\rho = 0.49$ ($P < 0.0001$) and in the inner ring ($R^2 = 0.12$) with $\rho = 0.32$ ($P < 0.01$) but not in the middle ring ($R^2 = 0.01$) with $\rho = –0.13$ ($P = 0.22$; Fig. 4)

Discussion

Macular carotenoids (lutein, zeaxanthin, and meso-zeaxanthin) are gaining an increasing interest among scientists and clinicians, since their correlation with retinal pathologies such as age-related macular degeneration has been widely studied and evaluated.[30–33] However, in order to verify their potential contribution to the natural history of macular disease, a valid, reliable, and simple measurement technique of these pigments would be needed. Several MP-measuring devices are available and psychophysical techniques claim to be the most accurate and reproducible.

In the present study, we evaluated the concordance of two MP-measuring devices (MPS II and qAF) in young healthy subjects. Repeatability was also calculated for both instruments, and new coefficient of repeatability was investigated in more eccentric parafoveal areas (qAF_3, qAF_6, and qAF_8). In particular, the coefficient of repeatability for $MPOD_{HFP}$ was 0.17. This was similar to what previously reported for $MPOD_{HFP}$ measured with MPS II/MPS9000 ranging between 0.19 and 0.33.[34]

Although recently introduced, qAF has already demonstrated good reproducibility both in healthy and pathologic eyes.[19,20] In our study, we observed good reproducibility in all analyzed areas of the scan pattern. The within-session repeatability for the qAF in the foveal area (qAF_F) was 7.4%, comparable to that reported by Delori et al.[18] (6.9%). In the more peripheral areas (qAF_3, qAF_6, and qAF_8) the within-session repeatability was 3%, 4.2%, and 3% respectively. Since it has not been investigated before, no comparison was possible for the peripheral areas.

In this study, we investigated the potential correlation between MP measurements obtained with the MPS II technology and quantitative measurements of the autofluorescence (qAF). Interestingly, a negative and statistically significant correlation between $MPOD_{HFP}$ and log (qAF) has been found in the central area up to approximately 4.3° (–0.60 and –0.38, for the qAF_F and qAF_3 respectively; $P < 0.01$), while in the more eccentric region (qAF_6) no correlation was found. This negative correlation mainly relies on the high concentration of macular pigment in the foveal area, absorbing the FAF blue-light masking retinal fluorophores, while the lack of correlation found between qAF_6 and $MPOD_{HFP}$ is probably due to the decrease in macular pigment concentration from the foveal region to more peripheral areas. The investigated area, which is a function of the radius of the chosen reference, critically improves its dimensions compared to qAF_3, making of the more peripheral segments the most consistent part to determine the mean qAF. Although several factors can influence retinal pigment distribution (which in the 40% of healthy eyes does not follow a Gaussian distribution),[3] our results suggest that qAF values can be able to reflect its concentration. In particular, the lower are the values of qAF, the higher is the concentration of MP. A direct estimate of MPOD from qAF values (Equation 5), in order to directly compare the values with those from the MPS II, was also performed. In the qAF_F area, $MPOD_{AF}$ was 0.82 DU, compared to 0.47 DU obtained with the HFP, This discrepancy can be partially explained by the different sampling apertures of the instruments (1° and 2.4° diameter for HFP and qAF, respectively) and by the intrinsic limit of the single-wavelength AF, that provides AF values from MP and even the RPE, resulting in an overestimation of the measurements. Delori et al.[18] estimated that the fluorescence in the fovea was on average 61% ± 9% of its value at 7° temporal. By applying a correction formula to consider the gap between foveal and peripheral AF values due to lipofuscin autofluorescence, $MPOD_{AF}$ values were reduced to 0.54 DU, more similar to those obtained with HFP technique. Although the formula for direct MPOD estimation is suitable for double-wavelength systems, a positive correlation between qAF_F and MPS II MPOD values was found, somehow enforcing our findings and suggesting its possible employment in the clinical practice.

Several studies compared physical such as dual-wavelength autofluorescence and psychophysical (heterochromatic flicker photometry) techniques for the measurement of MP.[13,15,34] In particular, MP values measured with the dual-wavelength autofluorescence OCT device (Heidelberg Engineering) were comparable to MP values obtained using the densitometer in subjects free of retinal diseases.[34] However, these findings were not confirmed in subjects with retinal changes secondary to early AMD.[15] The correlation between these two techniques varies also according to the area where the measurements were taken, constantly decreasing moving away from the

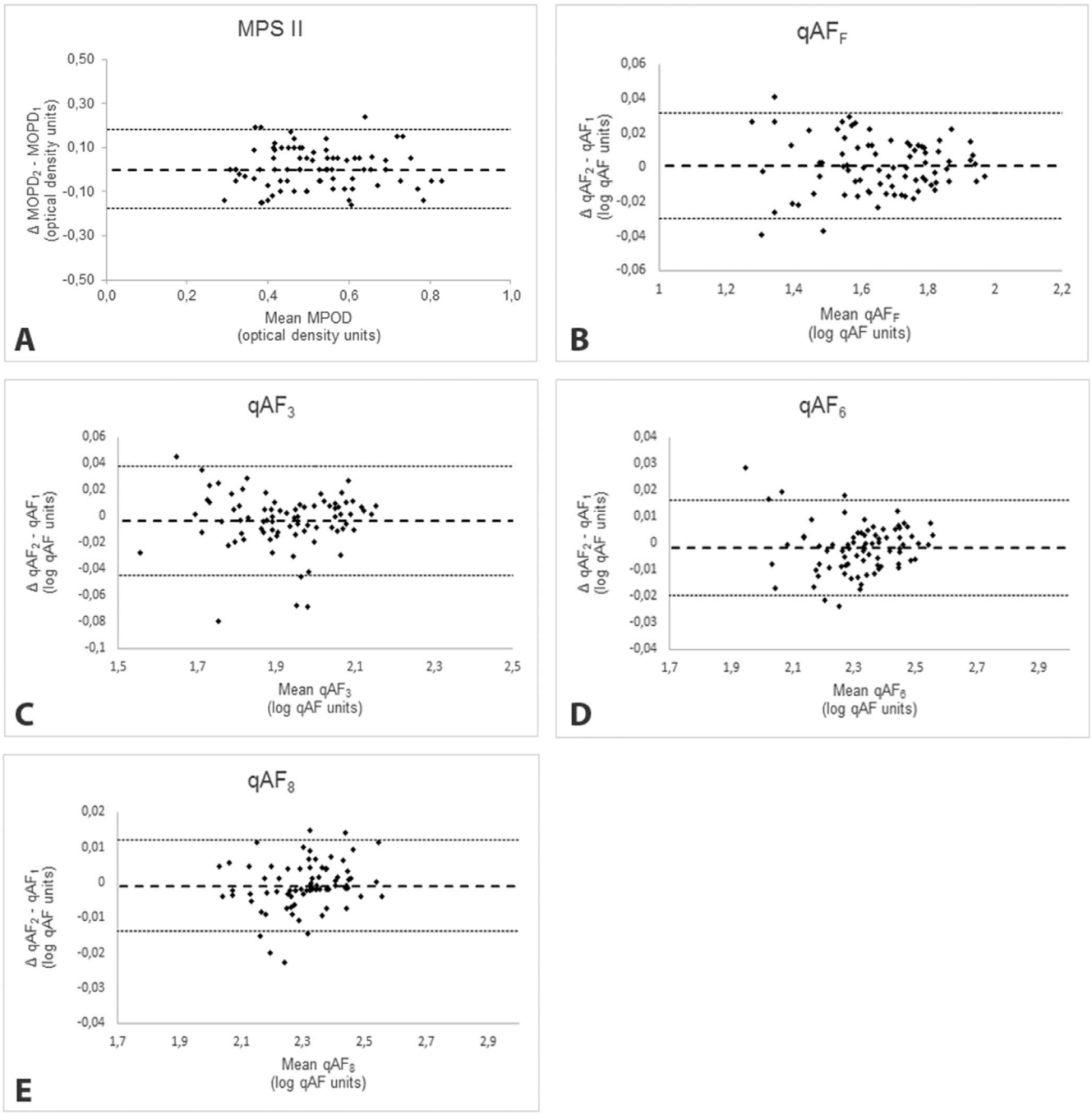

Figure 2. Bland-Altman plots showing the repeatability between two measurements. (**A**) Mean macular pigment optical density measured with the MPS II and reported in optical density units. The plot shows the variability between two tests ($MPOD_{HFP1}$ and $MPOD_{HFP2}$). (**B**) Mean qAF measured in the foveal area (qAF_F; approximately 0°–1.2°) and reported in log qAF units. The plot shows the variability between two tests (qAF_1 and qAF_2) (**C**) Mean qAF measured in the inner ring (qAF_3; approximately 1.3°–4.3°) and reported in log qAF units. The plot shows the variability between two tests (qAF_1 and qAF_2). (**D**) Mean qAF measured in the middle ring (qAF_6; approximately 4.5°–7°) and reported in log qAF units. The plot shows the variability between two tests (qAF_1 and qAF_2). (**E**) Mean qAF measured in the outer ring (qAF_8; approximately 7.2°–9.7°) and reported in log qAF units. The plot shows the variability between two tests (qAF_1 and qAF_2).

fovea.[15] In fact, although these technologies are designed to measure MP, each device works on different methodological approach, with different advantages and limitations. Even if at present qAF is mainly used for experimental research, its diffusion is encouraged and it will probably become a useful clinical technique in the evaluation and management of retinal diseases. Compared to FAF, it offers quantification of measurements and enables the ophthalmologist to evaluate predefined areas. However, unlike traditional validated MPOD-detecting techniques, it does not offer a measurement of MP but an indirect and probably less reliable estimate (e.g., nonhomogeneous MP-lipofuscin ratio). Moreover, compared to double-wavelength FAF, qAF is less reliable in detecting defined variations in macular pigment distribution, such as the ring-like pattern observed by some authors in the foveal area.[35–37] Nevertheless, due to its lesser dependence on patients' skills,

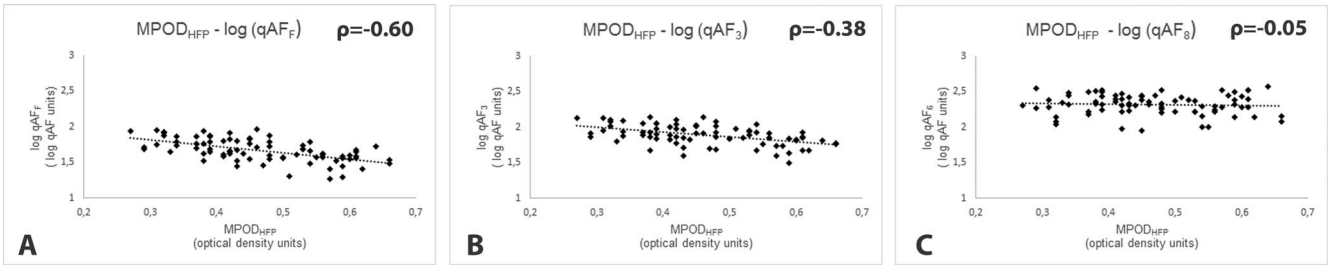

Figure 3. Scatter plots showing the correlation between $MPOD_{HFP}$ and qAF measurements in the foveal area (qAF_F; approximately 0°–1.2°; $P < 0.01$). (**A**) Inner ring (qAF_3; approximately 1.3°–4.3°; $P < 0.01$). (**B**) Outer ring (qAF_8; approximately 7.2°–9.7°; $P = 0.93$). (**C**). ρ, Spearman coefficient.

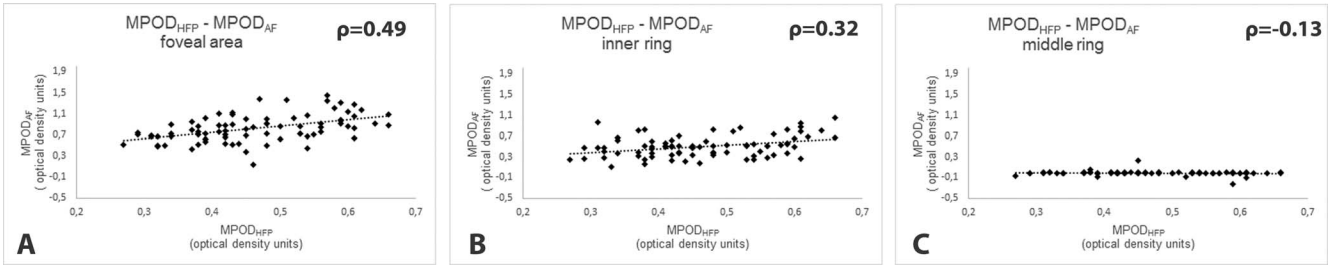

Figure 4. (**A–C**) Show the correlation between MPOD measured with MPS II ($MPOD_{HFP}$) and MPOD estimated using qAF values ($MPOD_{AF}$) from the foveal area ($P < 0.0001$), the inner ring ($P < 0.01$), and middle ring ($P = 0.22$), respectively. ρ, Spearman coefficient.

faster execution, real-time guidance for satisfactory acquisition of images, and its association with morphologic examinations (pixel-to-pixel correlation with spectral domain OCT), it can be used as an alternative indirect indicator of MP, of macular health, and possibly of susceptibility to develop macular degeneration.

This study has several limitations. The areas analyzed with qAF and MPS II are slightly different (Fig. 4). The intrinsic limit of single-wavelength FAF made it impossible to clearly localize and quantify MP patterns of distribution (e.g., ring-like), potentially affecting the correlation we reported, especially in the qAF_3 area. Moreover, a relatively small number of eyes and most importantly healthy and young subjects were enrolled; in older healthy subjects with some degree of nuclear sclerosis, the correlation we found between measurements could be different.

In conclusion, $MPOD_{AF}$ values in central areas significantly correlate with $MPOD_{HFP}$ in a group of young healthy subjects. However, qAF and HFP do not seem to be interchangeable. They represent two opposite ways of estimating MP, suggesting that patients would rather be followed with a single type of technique.

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