Comparison of Multimodal Imaging for the Characterization of Geographic Atrophy

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Purpose: The purpose of this study was to compare the performances of infrared (IR), fundus autofluorescence (FAF), and multicolor (MC) imaging in the characterization of geographic atrophy, with a focus on the possibility to detect incomplete retinal pigmented and outer retinal atrophy (iRORA) on en face imaging.

Methods: The ground truth was established by two graders evaluating atrophy on spectral-domain optical coherence tomography (SD-OCT) images. A score for visibility of foveal sparing and margins of atrophy was attributed. Measurement of the atrophic area and the fovea-to-margin distance were performed. Accuracy of detection of foveal sparing was evaluated through comparison with B-scan images ground truth, with/without the inclusion of patients with foveal iRORA.

Results: Seventy patients were included in this study. Foveal sparing and atrophy’s margins subjective visibility were significantly higher rated on MC images compared to IR and FAF (P < 0.005 and P < 0.001). Agreement with OCT B-scan assessed foveal sparing revealed a significantly higher area under receiver operating characteristic curves (AUROC) for MC images at the analysis performed both with (0.876) and without (0.853) inclusion of patients with foveal iRORA (P < 0.001 and P = 0.006). Quantitative measurements revealed lower atrophy extension (P = 0.026) and fovea-to-margin distance (P = 0.019) with MC imaging.

Conclusions: MC imaging performed better at foveal sparing assessment, especially in the setting of foveal iRORA. MC also resulted in higher visibility of atrophy’s margins, lower atrophy extension measurements, and lower distance from the fovea to atrophy’s margins compared to both FAF and IR.

Translational Relevance: MC rated significantly higher in foveal sparing and atrophy detection, higher visibility of atrophy’s margins, lower atrophy extension measurements, and lower distance from the fovea to atrophy’s margins, compared to FAF and IR.

Introduction

Age-related macular degeneration (AMD) is the most common cause of legal blindness among elderly individuals in developed countries.¹ Geographic atrophy (GA), the advanced form of dry AMD, accounts for 35% to 40% of cases of severe vision loss.² GA typically develops in parafoveal patches that expand and coalesce over time, with gradual involvement of central vision and eventual involvement of the fovea.³ Atrophic involvement of the fovea represents the hallmark of the end-stage disease and represents the ultimate cause of critical vision loss in these patients. Due to this fact, intense research on foveal sparing detection and foveal sparing status preservation has been held in the last decades for a better understanding of the phenomenon and description of the prodromes of foveal involvement. Foveal atrophy is characterized by retinal pigment epithelium...
(RPE) cell death and attenuation of the choriocapillaris, Bruch’s membrane, and photoreceptor layer in the foveal region. Recently, the expert Consensus Definition for Atrophy Associated with Age-Related Macular Degeneration on optical coherence tomography (OCT) identified OCT imaging as the gold standard for atrophy diagnosis and redefined the definition of atrophy. The Classification of Atrophy Meeting (CAM) group introduced the distinction between complete retinal pigmented and outer retinal atrophy (cRORA) and incomplete retinal pigmented and outer retinal atrophy (iRORA). The cRORA is defined as a zone of homogeneous choroidal hypertransmission and absence of the RPE band measuring ≥250 μm or more with overlying outer retinal thinning and loss of photoreceptors (PRs). By contrast, iRORA is defined by the presence of incomplete or discontinuous window effect, irregularities of the RPE and Bruch’s membrane, and damage to the PRs. However, only cRORA is comprised in the definition of atrophy, whereas iRORA is considered as a sign of incipient involvement (“nascent atrophy”) with retained visual function. Even though the CAM group designated OCT as the reference method to define cRORA and iRORA, an important role was recognized in ancillary en face methods (color imaging, fundus autofluorescence, infrared reflectance, and multicolor imaging) in case of questionable or borderline features. In particular, the role of these techniques in the detection and characterization of iRORA is yet to be established. Among the most frequently used techniques for clinical evaluation of GA, it is to be mentioned fundus autofluorescence (FAF), infrared imaging (IR), and multicolor infrared imaging (MC). FAF technology combined with the use of confocal scanning laser ophthalmoscopy (cSLO) allows visualization of the topographic distribution of lipofuscin over large retinal areas in vivo, thus providing a metabolic map of changes at the level of the retinal pigment epithelium. By blue- or green-light fundus FAF, map of changes at the level of the retinal pigment large retinal areas in vivo, thus providing a metabolic quantification over the past decades. Nevertheless, FAF bears some constitutional drawbacks in atrophy evaluation, such as high susceptibility to dioptric media opacity and difficulty in the detection of foveal involvement. FAF is characterized by a dip of the signal in the fovea explained not only by the absorption of emitted light by the foveal luteal pigment (lutein and zeaxanthin in the neurosensory retina) but also by the higher density in melanin granule and a lower density in lipofuscin granules in central RPE cells. Parallely, MC imaging is gaining increasing relevance among the en face techniques used for atrophy evaluation. MC composite images are generated by the superimposition of three simultaneously acquired reflective images, using three laser wavelengths combined with cSLO: blue reflectance (486 nm), green reflectance (518 nm), and IR reflectance (815 nm). MC imaging provides high contrast resolution that allows good detection of the margins of different types of lesions, including subretinal fibrosis and reticular pseudodrusen. In addition, MC imaging has been reported to allow effective detailed evaluation of the macular status in other retinal diseases, such as retinitis pigmentosa, hydroxychloroquine retinal toxicity, acute retinal pigmented epithelitis, and Best disease. The aim of the study is, therefore, to compare the performances of IR, FAF, and MC images in the characterization of atrophy from GA, with a focus on the possibility to detect iRORA on en face imaging.

Materials and Methods

Study Population and Design

This retrospective study analyzed patients referred to the Department of Ophthalmology at Centre Hospitalier Intercommunal de Creteil, France, between March 2012 and January 2022. The study was performed in accordance with the Declaration of Helsinki and current French legislation and with the approval of our local ethics committee. All selected patients had been previously diagnosed with AMD and subsequent chorioretinal atrophy resulting from GA. Moreover, the availability of B-scan and en face images derived from spectral-domain optical coherence tomography (SD-OCT) was necessary for enrollment. In particular, a B-scan section of the fovea and en face images acquired with the IR, FAF, and MC techniques were collected for evaluation. Exclusion criteria included atrophy from other causes, active or past macular neovascularization (MNV), presence of subretinal fibrosis, presence of macular edema or subretinal fluid, presence of subretinal hyperreflective material (SHRM), previous laser treatment at the posterior pole, low-quality images due to the opacity of dioptric means, or significant lens reflex. Anamnestic details such as time from AMD diagnosis and oral supplementation treatment were noted.

Image Analysis and Procedures

Only atrophy located at the posterior pole was considered for qualitative and quantitative evaluation by two expert graders (authors A.M. and I.D.R.).
The presence of atrophy was detected with multimodal imaging from SD-OCT (Spectralis, Heidelberg Engineering, Heidelberg, Germany) in accordance with the Consensus definition of 2018. In detail, OCT B-scan acquisitions were used as the ground truth for atrophy assessment and distinction between iRORA and cRORA. IR imaging (820 nm wavelength), blue light autofluorescence (FAF, 488 nm excitation wavelength), and composite MC 30 × 30 degrees field-of-view en face images of the posterior pole were evaluated in a blinded fashion for the presence of atrophy both qualitatively (subjective evaluation of easiness of detection) and quantitatively (post hoc comparison with ground truth OCT B-scan images). Random re-evaluation of the images was blindly presented to each grader to assess intra-reader agreement. 

At IR imaging, atrophy appears as well-demarcated, bright zones of hyper-reflectance spanning at least 300 μm in diameter, which may be unifocal or multifocal. FAF enhances areas of atrophy as dark hypoautofluorescent patchy confluent regions with or without hyperautofluorescent margins. Last, MC imaging visualizes atrophy as sharply demarcated areas of partial or complete depigmentation of RPE typically associated with improved visualization of the choroidal vasculature.

For qualitative analysis, the ability to detect the margins of the atrophy and the presence of foveal sparing were evaluated as ordinal variables by attributing a score to each image as follows: 0 = not visible, 1 = barely visible, 2 = mostly visible, and 3 = fully visible. Foveal sparing was defined as the absence of atrophic involvement of the fovea as described above for the three techniques. Differences in subjective grading were assessed with McNemar Bowker test for multiple correlated proportions. Concerning the quantitative evaluation of foveal atrophy, OCT B-scan images were considered as the ground truth and foveal iRORA on en face imaging was defined by the presence of mottled or soft atrophic changes within a non-atrophic foveal region: with IR it appeared as fainter reflectiveness with internal irregularities, at FAF it appeared as dishomogeneous autofluorescence within a middle-shaded fovea, and at MC imaging it appeared as a zone of initial speckled depigmentation. Patients with foveal iRORA were included in the foveal sparing patients’ group. Accuracy of the detection of foveal sparing with the three different en face methods was evaluated two times: one with the inclusion of the patients with iRORA in the foveal sparing group and one with the exclusion of the patients with iRORA from the study population. For each of the six performances, an area under receiver operating characteristic curve (AUROC) curve was drawn. In the quantitative analysis, each grader individually contoured the area of atrophy from the en face images using Spectralis built-in tool with automatic area calculation, as previously described. Areas resulting from each patch of atrophy were summed. The minimal distance from the supposed location of the fovea to the closest margin of the atrophy was measured by each grader using the built-in caliper of the Spectralis device with automatic computation of the distance. Quantitative comparisons for both the area of the extension of the atrophy (expressed in mm²) and the distance of the margin of the atrophy to the fovea (expressed in μm) as measured by the graders (subjective measurement) were performed.

Outcomes

The main outcome of the study was the comparison of subjective and objective foveal sparing detectability among the three techniques. Outcome measure for subjective detectability was a difference in qualitative score attribution from the graders in foveal sparing visibility at en face images with each of the techniques. Outcome measure for objective detectability was the comparison of AUROC curves for foveal sparing detection as verified with B-scan ground truth images. Secondary outcomes were the comparison of subjective visibility of the margins of the atrophy and the comparison of subjective measurements of atrophy’s extension and the fovea-to-margin distance (distance from the fovea to the closest margin of the atrophy).

Statistical Analysis

Statistical analysis was performed using SPSS software (IBM SPSS Statistics 26.0). Qualitative variables were expressed as the mean number of cases over total and percentage. Fleiss kappa analysis was performed to assess inter-reader and intra-reader agreement for qualitative variables. McNemar Bowker test was used to assess the statistical significance of the differences in outcome among the different imaging methods. The receiver operating characteristics (ROC) curves were elaborated to visualize the diagnostic accuracy of each method for foveal sparing and the area under the curves (AUC) was calculated. The resulting AUROC’s were compared using the DeLong method. Quantitative parameters were reported as mean and standard deviation (SD). Inter-reader and intra-reader agreement for quantitative parameters were assessed with intraclass correlation coefficient (ICC). Bland Altman analysis was applied for qualitative evaluation of agreement in quantitative measures among the different imaging techniques. ANOVA for repeated measures was used to compare
results from the different methods. A $P$ value of 0.05 was considered statistically significant.

**Results**

**Patient Demographics and Clinical Characteristics**

Seventy (70) patients with a mean age of 76.4 ± 8.7 years were included. Thirty-seven patients (53%) were men. The mean time lapse from diagnosis of AMD was 125.7 ± 36.8 months. Fifty-four patients (77.1%) had been under oral supplementation treatment with lutein/zeaxanthin, vitamin C, vitamin E, zinc, and beta carotene for at least 5 years. Complete foveal sparing was detected on OCT B-scan examination in 29 eyes (41.4%), whereas foveal iRORA was present in 14 patients (20.0%).

**Detection of Foveal Sparing Using MC, FAF, and IR**

Regarding the visualization of foveal sparing, the qualitative assessment revealed a significant difference among the three methods in graders’ score attribution ($P = 0.005$, McNemar Bowker test; **Table 1**). Regarding IR imaging, most of the images were classified as “barely visible” or “mostly visible” (24.4% and 34.3%, respectively), with a significantly higher rate of “not visible” score compared to the other techniques (20.0%, $P = 0.013$). For IR imaging, there was a moderate inter-reader and intra-reader agreement in foveal sparing detection (respectively, $k = 0.55$, confidence interval $[CI] = 0.52–0.57$ and $k = 0.58$, $CI = 0.56–0.60$). Foveal sparing evaluated with FAF imaging was considered as “barely visible” in 21.4% of the eyes and “mostly visible” in 32.8%, whereas it was judged as “not visible” only in 10% of the eyes. The time for image evaluation by the two graders using the Spectralis averaged 2 minutes and 9 seconds for IR, 2 minutes and 5 seconds for FAF, and 2 minutes and 17 seconds for MC (per image). Evaluation of FAF images was characterized by a good inter-reader ($k = 0.68$, $CI = 0.66–0.69$) and intra-reader ($k = 0.70$, $CI = 0.67–0.72$) agreement. MC imaging evaluation revealed a significantly higher percentage of “fully visible” eyes (47.1%) compared with the other two ($P = 0.026$). Moreover, only 2.8% of the images were labeled as “not visible” ($P = 0.037$). MC imaging-based assessment of foveal

| Table 1. Comparison of Qualitative Outcome Measures Using the Three Imaging Techniques |
|---------------------------------------------------------------|
| **Visualization of margins**                                |
| IR ($n = 70$) | FAF ($n = 70$) | MC ($n = 70$) | $P$ Value |
| Not visible (score 0) | 5 (7.1%) | 1 (1.4%) | 1 (1.4%) | $<0.001$ |
| Barely visible (score 1) | 26 (37.1%) | 10 (14.3%) | 8 (11.4%) |
| Mostly visible (score 2) | 24 (34.3%) | 39 (55.7%) | 26 (37.1%) |
| Fully visible (score 3) | 15 (21.4%) | 20 (28.6%) | 35 (50.0%) |
| Inter-reader agreement ($k$) | 0.58 (0.56–0.59) | 0.74 (0.73–0.75) | 0.80 (0.79–0.81) |
| Intra-reader agreement ($k$) | 0.57 (0.55–0.59) | 0.75 (0.73–0.77) | 0.81 (0.79–0.82) |
| **Visualization of foveal sparing**                        |
| IR ($n = 70$) | FAF ($n = 70$) | MC ($n = 70$) | $P$ Value |
| Not visible (score 0) | 14 (20.0%) | 7 (10%) | 2 (2.8%) |
| Barely visible (score 1) | 17 (24.4%) | 15 (21.4%) | 7 (10.0%) |
| Mostly visible (score 2) | 24 (34.3%) | 23 (32.8%) | 28 (40.0%) |
| Fully visible (score 3) | 15 (21.3%) | 25 (35.7%) | 33 (47.1%) |
| Inter-reader agreement ($k$) | 0.55 (0.52–0.57) | 0.68 (0.66–0.69) | 0.81 (0.79–0.82) |
| Intra-reader agreement ($k$) | 0.58 (0.56–0.60) | 0.70 (0.67–0.72) | 0.83 (0.81–0.84) |
| AUROC without iRORA (56 patients) | 0.701 (0.685–0.728) | 0.807 (0.788–0.829) | 0.853 (0.834–0.865) | 0.006 |
| AUROC with iRORA (70 patients) | 0.674 (0.654–0.698) | 0.759 (0.742–0.768) | 0.876 (0.867–0.881) | $<0.001$ |

The table displays the average number of eyes with percentage for each score category as well as data concerning inter-reader and intra-reader agreement for the three imaging methods (Fleiss $k$). The "$P$ value" column displays the value of alpha error resulting from McNemar Bowker test, representing the significance of the differences in scores among the three methods.

AUROC, area under the receiver operating characteristics curve; FAF, blue autofluorescence imaging; cRORA, complete retinal pigmented epithelium and outer retinal atrophy; iRORA, incomplete retinal pigmented epithelium and outer retinal atrophy; IR, infrared imaging; MC, multicolor composite imaging; $n$, number of eyes.
Figure 1. Example of foveal sparing as visible at OCT B-scan (upper image), IR en face (lower left image), MC en face (lower middle image), and FAF en face (lower right image). Of note, the contours of the macular area spared from atrophy (central darker gray in IR images and central darker red in MC images) are sharper and more delineated in MC image than in IR image. In the blue FAF image, a diffused mottled autofluorescence is present and foveal sparing is not recognizable. FAF, fundus autofluorescence; MC, multicolor; N-IR, near infra-red; OCT, optical coherence tomography.

sparking displayed a good inter-reader ($k = 0.81, CI = 0.79–0.82$) and a very good intra-reader agreement ($k = 0.83, CI = 0.81–0.84$). Examples of foveal sparing, foveal iRORA, and foveal cRORA as visible at OCT B-scan, IR, FAF, and MC en face imaging can be found, respectively, in Figures 1–3.

Concerning the quantitative assessment of foveal sparing, the AUROC for the detection of foveal sparing (with/without the exclusion of iRORA eyes) for each method is shown in Table 1 (see also Fig. 4). AUROC from the analysis performed after the exclusion of foveal iRORA eyes (14 eyes excluded) revealed a significant difference between the curves ($P = 0.006$) (see Fig. 4). In detail, MC reported an AUROC of 0.853 (CI = 0.834–0.865), which was significantly higher than the 0.807 (CI = 0.788–0.829) of FAF ($P = 0.023$). AUROC for IR was 0.701 (CI = 0.685–0.728), which was significantly lower than both MC ($P = 0.011$) and FAF ($P = 0.038$). AUROC from the analysis performed on the total study population (including patients with foveal iRORA), also revealed statistically significant differences among the three methods ($P < 0.001$). MC showed a significantly higher AUROC compared to both FAF ($P = 0.003$) and IR ($P = 0.012$) imaging, with a visibly higher spread between MC and FAF curves compared to the analysis performed with
the exclusion of iRORA eyes. The analysis of the total population revealed an AUROC of 0.876 (CI = 0.867–0.881) for MC imaging and an AUROC of 0.759 (CI = 0.742–0.768) for FAF imaging.

Detection of Geographic Atrophy Margins Using MC, FAF, and IR

The analysis of graders’ qualitative rating of visibility of the atrophy’s margins revealed statistically significant differences among the three techniques ($P < 0.001$, McNemar Bowker test; see Table 1). On IR imaging, the margins of atrophy were judged as “not visible” in 7.1%, “barely visible” in 37.1%, “mostly visible” in 34.3%, and “fully visible” in 21.4% of cases with a moderate inter- and intra-reader agreement (respectively, $k = 0.58$, CI = 0.56–0.59 and $k = 0.57$, CI = 0.55–0.59). Analysis of FAF images led to a significantly lower rate of “barely visible” and “not visible” margins (respectively, 14.3%, $P < 0.001$ and 1.4%, $P = 0.039$) and a significantly higher rate of “mostly visible” margins (55.7%, $P = 0.003$) compared with IR imaging. Inter- and intra-reader agreement in FAF images interpretation were both high (respectively, $k = 0.74$, CI = 0.73–0.75 and $k = 0.75$, CI = 0.73–0.77). Analysis
of MC images resulted in a significantly higher rate of “fully visible” margins (50.0%) compared to both IR ($P < 0.001$) and FAF ($P = 0.002$), a significantly lower rate of “mostly visible” margins compared to FAF images ($P = 0.004$) and a significantly lower rate of “not visible” margins compared to IR images ($P = 0.039$), with a very good inter-reader ($k = 0.80$, CI $= 0.79–0.81$) and intra-reader ($k = 0.81$, CI $= 0.79–0.82$) agreement in images interpretation. A detailed description of the results of qualitative analysis is displayed in Table 1.

Quantification of Geographic Atrophy Extension on MC, FAF, and IR

Comparison of quantitative measurements of atrophy extension revealed statistically significant differences among the three techniques ($P = 0.026$). Mean atrophy extension was significantly lower with MC imaging ($16.9 \pm 5.3 \text{ mm}^2$) compared with both IR and FAF imaging (respectively, $P = 0.042$ and $P = 0.029$), whereas differences between IR and FAF trended toward statistical significance ($P = 0.057$). ICC
Figure 4. ROC curves showing the accuracy of foveal sparing detection using N-IR, FAF, and MC images as compared to the ground truth OCT B-scan images. The analysis was performed both excluding patients with iRORA (left image, 56 eyes) and including them (right image, 70 eyes). BAF, blue autofluorescence; FAF, fundus autofluorescence; iRORA, incomplete retinal pigmented and outer retinal atrophy; N-IR, near-infrared imaging; MC, multicolor imaging; OCT, optical coherence tomography.

Table 2  Comparison of Quantitative Outcome Measures Using the Three Imaging Techniques

|                         | IR                  | FAF                | MC                  | P Value    |
|-------------------------|---------------------|--------------------|---------------------|------------|
| **Average extension of the atrophy (mm²)** | 17.8 ± 4.7          | 18.5 ± 6.2         | 16.9 ± 5.3          | 0.026      |
| Inter-reader agreement (ICC) | 0.501 (0.478–0.522) | 0.643 (0.627–0.659) | 0.728 (0.713–0.741) |            |
| Intra-reader agreement (ICC) | 0.516 (0.497–0.531) | 0.668 (0.645–0.682) | 0.742 (0.727–0.758) |            |
| **Margins’ distance from the fovea (µm)** | 362 ± 53            | 410 ± 73           | 327 ± 29            | 0.019      |
| Inter-reader agreement (ICC) | 0.636 (0.618–0.647) | 0.537 (0.522–0.557) | 0.702 (0.691–0.717) |            |
| Intra-reader agreement (ICC) | 0.641 (0.624–0.663) | 0.529 (0.509–0.544) | 0.708 (0.691–0.721) |            |

The table displays mean and standard deviation (SD) as well as data concerning inter-reader and intra-reader agreement (ICC) for each of the three methods. The “P value” column displays the value of alpha error resulting from repeated measures ANOVA, representing the significance of the differences among the three methods.

FAF, blue autofluorescence; ICC, intraclass correlation coefficient; IR, infrared imaging; MC, multicolor composite imaging.

showed moderate inter- and intra-reader agreement in IR atrophy contouring, whereas FAF and MC both showed a good agreement. The mean distance from the fovea to the closest margin of the atrophy was 16.9 ± 5.3 µm with MC, 17.8 ± 4.7 µm with IR, and 18.5 ± 6.2 µm with FAF images (P = 0.019). ICC showed moderate to good inter and intra-reader agreement for all the three techniques, with higher values for MC and lower values for FAF (Table 2).

Discussion

In this study, we showed that visualization of foveal sparing was significantly more accurate with MC imaging compared to the other techniques both on a subjective point of view (visibility score) and on an objective point of view (AUROC with reference to OCT B-scan ground truth). Moreover, performing the analysis of foveal sparing visualization after the exclusion of patients with foveal iRORA led to a reduction in accuracy spread between the techniques, suggesting that MC might be particularly proficient in identifying areas that are not completely involved by the atrophic process and thus corresponding to iRORA (higher specificity). These differences reflect the fact that each technique offers a different insight into a particular aspect of retinal physiopathology by reflecting the health of a particular component of the retinal tissue while exploiting its ability to return detailed images when hit by visible or invisible light. N-IR light (>800 nm wavelength), penetrates retinal layers and is mostly reflected by melanin and retinal pigment, giving back precious information on the status of the outer retinal and allowing detection of subretinal changes that may be otherwise obscured or ambiguous using more traditional methods, such as retinal photography. The hyper-reflective appearance of geographic atrophy in IR images relates to the absence of RPE blockage (compared to neighboring regions) and reflection of light from the sclera. The appearance of a hyper-reflective region thus requires
the absence of masking anatomically intact RPE layer, which is a feature that does not necessarily imply metabolic activity from the RPE part. In this context, MC imaging provides a particularly qualitative image in terms of depth perception, spatial and layer-by-layer resolution due to the detailed reconstruction provided by differential reflectivity of IR light with variable wavelength. By contrast, FAF signal is generated by short-wavelength excitation of RPE lipofuscin, a complex mixture of fluorophores that are byproducts of the visual cycle and that accumulate in the RPE after phagocytosis. As a consequence, a silent area at FAF reflects functional inactivity of the RPE in that region and might be a parallel phenomenon to that of drusen collapse and reabsorption documented in early retinal atrophy. Indeed, drusen fading and imaging correlates have been linked to the risk of progression of the atrophy in the short run and are believed to represent a first step in the process of atrophy formation. In this perspective, it is not surprising that our analysis detected a significantly higher foveal involvement and higher average extension of the atrophic area detected with FAF compared with the one from the other two methods, a finding that is also consistent with previous studies in the literature. We hypothesize that this is attributable to the presence of nascent atrophy zones at the borders of the atrophic lesions as well as foveal iRORA lesions that are metabolically silent, and are therefore recognized as atrophic by FAF imaging, while still retaining partial anatomical and functional integrity as detected by IR imaging modalities, such as IR and MC imaging. This is consistent to the example shown in Figure 1, which demonstrates a poor characterization of the atrophic area, appearing as uniformly silent with minimal internal variation and no visible foveal sparing in blue FAF imaging. Moreover, the contours of the atrophic area are visibly larger using blue FAF than those of the corresponding area on IR and MC images, involving marginal zones that are still not involved in the atrophic process according to other en face methods. Initial signs of degeneration are visible in these zones with OCT B-scan. Consistently with the rest of the findings, linear distance from the closest margin of the atrophy to the fovea found to be significantly higher with FAF technique compared with the IR techniques. Moreover, visualization of margins of the atrophy was reported to be optimal with MC imaging, followed by FAF and IR imaging. De Rosa et al. similarly reported optimal performances in subretinal fibrosis margins detection in patients with AMD analyzed with MC imaging. Nevertheless, it should be acknowledged that MC imaging is but one of the available technologies allowing to capture high resolution, high contrast, (pseudo)color images of the retina, and that the analysis of atrophy visualization in GA with these other instruments (e.g. the Pomerantzef camera, the Panoret-1000, the RetCam, Staurenghi lens and Optos fundus camera; Optos PLC, Dunfermline, Scotland) might as well provide interesting complementary results. Hence, it is unclear if the performance would be similar if a different device were used and further research is needed to answer this question.

The perception of a less distinct margin of the lesion with FAF method might reflect the gradual fading of RPE activity at the borders of the lesion, which in turn results in a gradually shading autofluorescence whose interpretation would benefit a binarization process. Similarly, the presence of sparse RPE defects with a punctiform initial window defect in nascent atrophy at the borders of the lesion confers to IR images as a fuzzy aspect of the margins, impairing the performances of the technique in their near detection. By contrast, the superimposition of images derived from different layers and the color in MC composite images provides a good contrast sensitivity and depth perception, allowing optimal visualization of full-thickness defects in the outer retina. In fact, MC appears to be an even more reliable method for qualitative and quantitative characterization of the atrophy compared to classic IR imaging, being characterized by a very good inter-reader and intra-reader agreement. Nonetheless, our study confirmed the high intra- and inter-reader agreement in atrophy characterization with FAF reported by previous literature. Last, it is important to notice that we excluded patients with previous or active MNV due to the supposed differences in the evolution and pathogenesis of the atrophy related to MNV exudation. Moreover, the presence of MNV and associated lesions (such as subretinal fibrosis) might introduce confounding elements in imaging evaluation of the outer layers of the retina, thus providing additional arguments for exclusion of these patients from our study. By contrast, calcific drusen were present in most patients in our cohort due to high overlap with GA in patients with dry AMD. Consistent with previous literature, we noticed a high performance of MC imaging in the visualization of calcific drusen, due to the sharp demarcation of their contours and the bright yellow appearance (different from other drusen, appearing as shady greenish formations with orange halos). The presence of calcific drusen thus did not impair detectability of atrophy’s margins with MC imaging. Among the limitations of the study, we can also mention the lack of a control group with atrophy from other causes, the absence of the use of other imaging modalities for atrophy evaluation, such as OCT angiography, green FAF, and fundus...
photography, and the retrospective nature of the evaluation. Last but not least, the intrinsic subjectivity based on graders’ evaluation needs to be mentioned as a limit to reproducibility. Nonetheless, we believe the findings to be relevant for clinical purposes and we hope that future literature on the matter will further expand the subject.

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Ethics Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent to Participate: Informed consent was obtained from all individual participants included in the study.

Authors’ Contributions: Conceptualization of the study: A.M., I.D.R., and E.C. Methodology: E.C. Software: E.C. Validation: E.S. and A.M. Formal analysis: E.C. and I.D.R. Investigation: D.C., C.J.M., E.C., and I.D.R. Resources: E.S. and A.M. Data curation: E.C. and I.D.R. Writing of the manuscript and original draft preparation: E.C. and I.D.R. Writing of the manuscript and review and editing: E.S. and A.M. Supervision: E.S. and A.M. Project administration: E.S. All authors have read and agreed to the published version of the manuscript.

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