Blue-Light versus Green-Light Autofluorescence: Lesion Size of Areas of Geographic Atrophy

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PURPOSE. Blue-light fundus autofluorescence (FAF) imaging is currently widely used for assessing dry age-related macular degeneration (ARMD). However, at this wavelength, the fovea appears as circular zone of marked hypofluorescence, due to the absorption of macular pigment (MP). This dark spot could be misinterpreted as an atrophic area and could lead to difficulties in identifying small, central changes. The purpose of the study was to analyze differences in image quality, FAF patterns, and lesion size, when using conventional blue-light (λ1 = 488 nm) and green-light (λ2 = 514 nm) FAF.

METHODS. Patients older than 50 years with central areas of geographic atrophy (GA) secondary to ARMD were enrolled. Images were recorded with a modified confocal scanning laser ophthalmoscope (cSLO). Image quality and patterns were analyzed. The quantification of the GA was performed with customized image-analysis software.

RESULTS. In total, 95 eyes were included. The borders of the central atrophic patches and the boundaries of the preserved foveal island were better identified in 514-nm images. In both excitation wavelengths the signal-to-noise ratio was sufficient for the identification of the FAF pattern. Significant differences were observed in the size of the GA areas detected in the 488- and 514-nm wavelength images (4.29 ± 3.76 mm² vs. 3.80 ± 3.68 mm²; P < 0.001).

CONCLUSIONS. The green-light FAF images (514 nm) are superior for the accurate analysis of small, central, pathologic changes, and for the determination of the central GA lesion size. Using only blue-light FAF could lead to an overinterpretation of the size of atrophic patches and the center involvement, because it suggests the presence of atrophy in the fovea. This is based on the clinicalTrials.gov number, NCT00494325. (Invest Ophthalmol Vis Sci. 2011;52:9497–9502) DOI:10.1167/iovs.11-8346

Age-related macular degeneration (ARMD) is related to changes in the retinal pigment epithelium (RPE), Bruch’s membrane, and choroid that occur with aging.1–3 The aging of the RPE is characterized by a massive accumulation of lipofuscin (LF), which contains several fluorophores. LF has been shown to contain toxic compounds including the dominant fluorophore pyridinium-bis-retinoid (A2-E), which is derived from two molecules of vitamin A aldehyde and one molecule of ethanolamine.4 A2E has been shown to inhibit lysosomal digestion of proteins and to act as a photosensitizer in blue-light–generating free radicals within the RPE cell. Based on conventional fundus photography, GA has been defined as a sharply demarcated area of apparent absence of RPE, which is larger than 175 μm, with visible choroidal vessels and no neovascular ARMD.5–7 This definition is based on histopathologic studies that have characterized clinically visible GA as areas with RPE and outer neurosensory layer cell death with occasionally visible choriocapillaris.5,6 With the advent of confocal scanning laser ophthalmoscopy (cSLO), fundus autofluorescence (FAF) can be visualized in vivo.8–10

FAF is a relatively novel imaging method that allows topographic LF mapping in vivo.8–10 In most studies blue-light FAF images, using an excitation wavelength of 488 nm, have been analyzed. It is therefore possible to use the noninvasive FAF imaging to monitor disease-associated changes in vivo. This technique is important in the characterization of the atrophic late-stage manifestation of dry ARMD; a disease characterized by the development of atrophic patches in the RPE monolayer. Because of the absence of RPE lipofuscin, areas of GA exhibit a notable reduced FAF signal. These atrophic areas can be detected clearly and can be quantified precisely in FAF images.

Imaging of FAF with a cSLO is usually performed with an excitation wavelength of 488 nm. At this wavelength, MP absorbs significantly, resulting in a circular zone of approximately 0.5 mm of marked hypofluorescence (visible as a darker spot) overlying the fovea and the surrounding area.11–14 This reduction in FAF intensity in the fovea could lead to difficulties in identifying small, central changes and may influence the identification of foveal involvement in central atrophic changes.

The purpose of this prospective cross-sectional study was to analyze differences in image quality, FAF pattern, and lesion size measurements of areas with GA at the central posterior pole by using conventional blue-light autofluorescence (λ1 = 488 nm) and green-light autofluorescence (λ2 = 514 nm).

MATERIAL AND METHODS

Patients from the outpatient department (Department of Ophthalmology, Inselspital, University Bern, Switzerland) were screened between May 2007 and September 2011 for study participation. Patients older than 50 years with clear optical media and areas of unifocal or multifocal GA secondary to dry ARMD were enrolled. If both eyes qualified equally for the study, one eye was randomly chosen. Before any study procedures, informed written consent was obtained from every patient with an explanation of the nature and possible risks of the study. The research complied with the Declaration of Helsinki and was approved by the Institutional Review Board.

Exclusion criteria were any confluent papillary atrophy in contact with central atrophy, signs of wet ARMD, any history of retinal surgery (including laser treatment) or diabetic retinopathy, vascular occlusion, and hereditary retinal dystrophy. We performed a comprehensive ocular examination with best corrected visual acuity (BCVA) using...
Early Treatment Diabetic Retinopathy Study (ETDRS) charts, dilated binocular ophthalmoscopy, and color fundus photography (FF 450 plus; Carl Zeiss Meditec, Jena, Germany). All patients underwent fluorescein angiography (Heidelberg Retina Angiograph, HRA2; Heidelberg Engineering, Heidelberg, Germany) to confirm the diagnosis of dry ARMD and to exclude neovascularization.

FAF images were recorded according to a standardized protocol14,15 with a modified cSLO (HRamp; Heidelberg Engineering). This instrument has been optimized for FAF imaging at excitation wavelengths of \( \lambda_1 = 488 \text{ nm} \) and \( \lambda_2 = 514 \text{ nm} \). The patients were positioned in front of the cSLO and instructed to look straight ahead and hold steady. After alignment and focusing using 20° reflectance images at 488-nm wavelengths, the retina was bleached for at least 30 seconds. Then 20° FAF images were acquired at 488- and 514-nm wavelengths. Nine FAF images were recorded for each wavelength. The images obtained for each wavelength were averaged using the system software to reduce speckle noise. This method improves the signal-to-noise ratio by a factor of 3. The averaged images were then normalized and graded as follows:

1. Good quality: All areas of GA were clearly visible, and all boundaries of the areas were delineable. The signal-to-noise ratio was sufficient for the identification of the FAF pattern in the junctional zone around the GA and for pattern grading of the posterior pole.
2. Intermediate quality: All areas of GA were visible, and all boundaries of all GA areas were discernible. However the signal-to-noise ratio was too low for identification of the FAF pattern in the junctional zone, but was still sufficient for pattern grading of the posterior pole.
3. Inferior quality: Neither all borders of the GA area nor FAF abnormalities were entirely readable.

In all images of good or intermediate quality, the FAF patterns and the total size of the GA area were analyzed. We quantified the total size of the area with commercial software (Region Finder, ver. 1.0.16; Heidelberg Engineering). The software includes algorithms for semi-automated segmentation of atrophic areas and for automated identification of interfering vascular structures.17 Starting from a user-defined seed point placed in a dark area of the image, a so-called region-growing algorithm identifies the border of this dark area and calculates a mean gray value of the pixels. The FAF intensity of every picture element (pixel) is given in gray value. The dramatic decrease in the FAF signal in the GA areas compared to the signal in the nonatrophic retinal areas is used by the software for the segmentation of the GA areas. After the definition of the center of a region by the operator (reader), the region-growing algorithm tends to grow toward the borders of the region, taking into account all pixels with a signal intensity below a certain threshold. This threshold is defined by a parameter referred to as growth power. The higher the growth power, the larger the enclosed area. The proper adjustment of this parameter allows for the precise measurement of the area with GA. For scaling, the individual scaling factor that is registered by the software (HEYEX; Heidelberg Eye Explorer; Heidelberg Engineering) during acquisition is used. Given the digital image resolution of \( 768 \times 768 \) pixels of a 30° × 30° frame, one pixel roughly corresponds to 11 \( \mu \text{m} \).

Two independent, trained readers (MN, VW) assessed the image quality and quantified the total GA size in FAF images at excitation wavelengths of \( \lambda_1 = 488 \text{ nm} \) and \( \lambda_2 = 514 \text{ nm} \). The readers analyzed the images in separate sessions at least 1 day apart. In each session, the images were presented in a randomized fashion to minimize any memory effect. For statistical analysis of lesion size the measurements between the two independent graders were averaged. Inter-FAF mode variability was analyzed using Bland-Altman plots. The interobserver agreement for both FAF modes (e.g., \( \lambda_1 = 488 \text{ nm} \) and \( \lambda_2 = 514 \text{ nm} \)) was analyzed with the Bland-Altman plots and the Lin's concordance correlation coefficient (statistical analyses: Prism ver. 5.00; GraphPad Software, San Diego CA) for all statistical analyses. \( P \leq 0.05 \) was accepted as statistically significant, given the exploratory nature of these analyses. Demographic characteristics of the patients were summarized using descriptive statistics. Descriptive statistics are expressed as the mean ± SD and percentages.

**RESULTS**

In total, 95 eyes with GA areas secondary to dry ARMD involving the central posterior pole of the eye were included in this unmasked, prospective, cross-sectional study. Baseline demographics of all 95 patients are described in Table 1.

<table>
<thead>
<tr>
<th>Mean age ± SD, y (range)</th>
<th>78.8 ± 7.4 (62.9–90.9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, n (%)</td>
<td>Male 34 (36)</td>
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<tr>
<td></td>
<td>Female 61 (64)</td>
</tr>
<tr>
<td>Mean letter score ± SD (range)</td>
<td>55 ± 20 letters (0–81)</td>
</tr>
<tr>
<td>LogMAR</td>
<td>−0.6</td>
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<tr>
<td>Spherical equivalent ± SD, D (range)</td>
<td>+0.5 ± 0.27 (+5.0 to −12.0)</td>
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<tr>
<td>Lens status, n (%)</td>
<td>Phakic 47 (49)</td>
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<tr>
<td></td>
<td>Pseudophakic 48 (51)</td>
</tr>
<tr>
<td>Type of IOL, n (%)</td>
<td>Clear 29 (60)</td>
</tr>
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<td></td>
<td>Blue-blocking 19 (40)</td>
</tr>
</tbody>
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\( n = 95 \).

![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933457/) Example of a good-quality FAF image (right eye; patient O-Y; female; age, 78 years; BCVA. 63 letters; correction, +1.25 and +0.5 D/96°). All areas of GA are clearly visible, and all boundaries are delineable. The signal-to-noise ratio is sufficient for the identification of the FAF patterns in the junctional zone around the GA and for the detailed grading of the posterior pole. Left: FAF image with an excitation wavelength of 488 nm (total GA size, 1.621 mm²); middle: FAF image with an excitation wavelength of 514 nm (total GA size, 1.554 mm²); and right: color fundus photograph.
FAR imaging with the modified cSLO was possible in all 95 eyes using the 488- and 514-nm excitation wavelengths. In all eyes, we found a reduced FAF signal in the GA site when using both wavelengths.

Assessment of the Image Quality
In both, blue (λ1 = 488 nm) and green (λ2 = 514 nm) FAF images, the signal-to-noise ratio was sufficient for the quality assessment. We did not observe differences in the quality assessment between both excitation wavelengths and the two independent readers. In general, the 488-nm images had a slightly better contrast outside the macular region. Borders of the GA patches involving the macula or boundaries of any existing preserved foveal island were easier to identify in the 514-nm than in the 488-nm FAF images.

Of the 95 eyes, we found good image quality in 58 (61%) and intermediate quality in 26 (27%). Representative examples for the image quality assessment are shown below in Figures 1 and 2 (good quality) and Figure 3 (intermediate quality). Inferior quality was found in 11 (12%) eyes. The reasons for reduced image quality included slow eye movements during image acquisition, which were not corrected by the averaging algorithm of the software (9/11 eyes), and vitreous floaters (2/11 eyes).

Neither the lens status (phakic versus pseudophakic; P > 0.05, χ² test) nor the type of the intraocular lens (IOL; clear versus blue-blocking IOL; P > 0.05, χ² test) had any influence on the FAF image quality. The refraction or the spherical equivalent also did not influence the image quality. For the analyses of the lesion size and the FAF patterns, we excluded all 11 (12%) of the 95 eyes because of inferior image quality.

Assessment of the Total GA Size
In all the FAF image sets, the total size of GA was delineated and quantified. However, in 15 (18%) of the 84 eyes, the total size of the atrophic patches was too large to visualize all peripheral borders in the FAF images (Fig. 4). Therefore, the size of the GA was assessable only in image sets from 69 eyes. We found significant differences in the size of the GA areas detected in the 488- and 514-nm wavelength images (4.29 ± 3.76 mm² vs. 3.80 ± 3.68 mm²; P < 0.001). The Bland-Altman plot for the differences between the FAF modes is presented in Figure 5. The total size of GA showed no difference (P > 0.05) between both readers for a given wavelength. The correlation between both observers was good with a Lin concordance correlation coefficient of 0.965 for the 488-nm images and of

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**FIGURE 2.** Example of a good-quality FAF image (right eye, patient WL; female; age, 92 years; BCVA, 38 letters; correction, −1.00 and +0.50 D/112°). Central boundaries of the atrophic areas were more clearly visible if the dark spot produced by the MP was absent. The absence of the spot resulted in a significantly smaller size of the atrophic patches if FAF with an excitation wavelength of 514 nm was used. *Left:* FAF image with an excitation wavelength of 488 nm (total GA size, 4.789 mm²); *middle:* FAF image with an excitation wavelength of 514 nm (total GA size, 3.917 mm²); *right:* color fundus photograph.

**FIGURE 3.** Example of an intermediate-quality FAF image (right eye; patient AA; female; age, 90 years; BCVA, 44 letters, correction, −1.00 and +0.75 D/172°). All areas of GA are visible and all boundaries of all areas are discernible. The signal-to-noise ratio was too low for sufficient identification of all FAF patterns in the junctional zone. *Left:* FAF image with an excitation wavelength of 488 nm (total GA size, 2.643 mm²); *middle:* FAF image with an excitation wavelength of 514 nm (total GA size, 2.169 mm²); *right:* color fundus photograph. The foveal island is clearly visible, especially in the 514-nm FAF image (middle) because the “dark shadow” produced by the MP does not cover the central region. If only FAF images with an excitation wavelength of 488 nm (left) were used, it could lead to a false interpretation of the amount of central region involved.

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0.930 for the 514-nm images. Bland-Altman plots for inter-reader variability are presented in Figure 6.

DISCUSSION

Most of the epidemiologic and natural history studies that have addressed the enlargement of GA areas over time have used color fundus photographs or fundus-camera–based FAF images. Although this technique can be used to detect the presence of GA, graders at reading centers have reported difficulty reproducibly measuring atrophic areas because of intersubject variability in fundus pigmentation, media opacities, and the presence of drusen and small satellites of atrophy.

The purpose of this study was to analyze differences in image quality, FAF patterns and total GA size measurements using conventional blue-light autofluorescence (488 nm) and green-light autofluorescence (514 nm). Imaging of FAF distribution using conventional blue-light autofluorescence is a relatively well established method to assess the LF distribution in a clinical setting. For image acquisition, we used a modified cSLO optimized for macular pigment measurements (Jahn C, et al. IOVS 2004; 45:ARVO E-Abstract 2968). This instrument allows FAF imaging with two excitation wavelengths (λe = 488 nm and λe = 514 nm) (Jahn C, et al. IOVS 2004; 45:ARVO E-Abstract 2968).

Good or intermediate image quality is needed, no matter what excitation wavelength is used. In FAF images of inferior quality, neither the FAF patterns nor all borders of GA are readable. A high-contrast difference between atrophic and nonatrophic areas is necessary for an exact measurement of the GA area size. This high-contrast difference between the atrophic and nonatrophic areas is also needed for a clear delineation of GA boundaries.

In most eyes with clear optical media (87%), good or intermediate FAF imaging quality was observed with both excitation wavelengths. Overall, image quality and distribution of FAF patterns did not differ between the two wavelengths.

Both wavelengths could be used for a fast and noninvasive detection of disease associated changes in patients with GA. Areas with GA show decreased FAF signal due to the lack of RPE, which contains LF as a dominant fluorophore. The areas adjacent to the GA show a broad spectrum of autofluorescence pattern. These patterns were well established using blue-light...
autofluorescence images and seem to offer a certain predictive value for disease progression (Bindewald A, et al. IOVS 2004; 45:ARVO E-Abstract 2960).26

In this study, areas of GA showed a marked reduced autofluorescence signal with both wavelengths. Adjacent to the GA areas, we were able to detect the previously described FAF patterns with both wavelengths. The relative distribution of FAF patterns in the present study was similar to those in other patient cohorts previously tested.24

Quantification of the atrophic patches using customized image-analysis software has been described.17 FAF with both excitation wavelengths (488 and 514 nm) allowed the delineation of the borders of the GA. Significant differences were found when the size of the atrophic patches was compared between both excitation wavelengths. We observed significant differences in the size of the GA areas detected in the 488- and 514-nm wavelength images (4.29 ± 3.76 mm² vs. 3.80 ± 3.68 mm²; P < 0.001). The interobserver agreement was good, and there was no statistically significant difference between the readers (P > 0.05).

Central pathologies were more easily distinguishable and more clearly visible, if the longer excitation wavelength (514 nm) was used. Preserved foveal islands were more clearly distinguished in FAF images with the 514-nm excitation wavelength. The dominant cause of misinterpretation at the 488-nm wavelength was the dark spot overlaying the fovea due to MP absorption. The spot could lead to a false or an overinterpretation of the GA size and the center involvement, because it suggests the presence of atrophy in the fovea, which could explain the differences in the size measurements between both wavelengths. The differences in the size measurements between both FAF modes are not important, if only the progression rate or the enlargement of GA areas is studied in a longitudinal fashion. But in our opinion, it is important to give the correct baseline characteristic for the center involvement and for a correlation with functional values such as BCVA in epidemiologic or natural history studies.5,18,19

In current clinical studies, only blue-light FAF is used to determine the size of atrophic lesions. However, an accurate measurement with green-light FAF is important in future clinical trials for the evaluation of central GA progression. As a result, the use of green-light FAF images could give us a better opportunity in estimating an approximate time of visual loss. Depending on which wavelength is used, a detailed description is needed for a precise interpretation of focally increased or decreased FAF signals. Therefore, future studies are necessary to evaluate the clinical value of this new imaging method to understand green-light autofluorescence better.

Although lipofuscin is the dominant fluorophore in the ocular fundus, there are many other fluorophores present anterior and posterior to the RPE cell monolayer, including elastin and collagen.27 Fluorophores such as melanin could contribute to the detected FAF above 695 nm if near-infrared (IR) wavelengths were used28 and could complicate the interpretation of the FAF images obtained with wavelengths longer than 600 nm instead of the conventional blue light.

To conclude, the green-light FAF images obtained with a 514-nm excitation wavelength are superior for an accurate determination of small, central, pathologic changes, and of the central atrophic lesion boundaries.

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References


