DOI: 10.4274/tjo.galenos.2020.49107
Turk J Ophthalmol 2021;51:169-176

Review

# The Use of Fundus Autofluorescence in Dry Age-Related Macular Degeneration

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**Abstract**

Fundus autofluorescence (FAF) has been a well-known imaging method for quite some time. However, with developing technologies and novel imaging devices, FAF is being used more often to diagnose and monitor retinal diseases. The density of lipofuscin (LF) and other fluorophores in the retina have a determining role in FAF images. In dry age-related macular degeneration (AMD), hyperautofluorescence is seen in cases of increasing LF in the retina pigment epithelium, whereas hypoautofluorescence is detected in decreasing LF resulting from geographic atrophy. In recent years, studies have shown that FAF images provide prognostic information in patients with AMD. This review aims to highlight the importance of FAF imaging in dry AMD.

**Keywords:** Age-related macular degeneration, fundus autofluorescence, geographic atrophy, lipofuscin, retina

## Introduction

Fundus autofluorescence (FAF) is a noninvasive imaging method based on the principle of stimulating fluorophores with specific wavelengths and measuring the light they emit through barrier filters.

The presence of autofluorescence in the fundus was first detected in images taken immediately before performing fundus fluorescein angiography (FA) and was called pseudofluorescence.[1] The introduction of confocal laser scanning ophthalmoscopy (cSLO) systems increased the quality of FAF images, and the method became widely used in the diagnosis and follow-up of retinal diseases.

FAF images demonstrate fluorophore density in the retina. Lipofuscin (LF), found in the retinal pigment epithelium (RPE), is one of the main fluorophores in the retina. An increase in the amount of LF leads to hyperautofluorescence and a decrease results in hypoautofluorescence.

FAF imaging has been embraced as a useful imaging method for explaining the pathophysiological mechanisms of retinal diseases, evaluating the risk of progression, and monitoring treatment outcomes.

The aim of our review is to provide basic information about FAF imaging and emphasize the importance of its use in dry age-related macular degeneration (AMD).

### The Retinal Pigment Epithelium and Lipofuscin

The RPE consists of a single layer of polygonal cells separating the choroid from the neurosensory retina. It has a critical role in normal retinal functioning, being responsible for

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**Received:** 13.05.2020 **Accepted:** 26.11.2020

**Cite this article as:** Şahinoğlu Keşkek N, Şermet F. The Use of Fundus Autofluorescence in Dry Age-Related Macular Degeneration. Turk J Ophthalmol 2021;51:169-176

phagocytosis and lysosomal destruction of photoreceptor outer segments.[2] Each RPE cell phagocytoses 3 billion outer segments during its lifetime.[2]

With aging, insufficient clearance of the photoreceptor outer segments leads to accumulation of LF in the RPE. LF is a macular fluorophore that absorbs blue light at a wavelength of 470 nm and emits yellow-green light at a wavelength of 600-610 nm.[3] It is a heterogeneous molecule consisting of vitamin A and byproducts of the visual cycle. The accumulation of LF in the RPE lysosomes increases with age, with LF and melanolipofuscin occupying a quarter of the RPE cytoplasm and after the age of 70 years.[4] However, excess LF accumulation is pathological and causes apoptosis in the RPE. The amount of LF is also known to increase in retinal degenerative diseases such as AMD and macular dystrophies such as Best and Stargardt disease.[5]

Although LF is composed of many different molecules, the most important is N-retinyl-N-retinylidene ethanolamine (A2E). A2E is formed in the outer segment of the photoreceptor by the combination of two vitamin A aldehyde (all-trans retinal) molecules with a phosphatidyl ethanolamine.[6] A2E is believed to accumulate in lysosomes because lysosomal enzymes are unable to recognize and degrade it. Another theory to explain the excess accumulation of LF is that the reactive A2E molecule inhibits the metabolism of lysosomal enzymes and inactivates proteolytic enzymes.[7] The accumulation of this substance in the lysosomal compartment of RPE cells is characteristic of the aging process.

Another component of LF, all-trans retinal, is a toxic aldehyde produced in the photoreceptor outer segments as a result of light exposure. As photoreceptors do not have cis-trans isomerase function, all-trans retinal cannot be converted to 11-cis-retinal.[8] All-trans retinal accumulates in the photoreceptor and forms bisretinoids. The oxidation of bisretinoids yields LF.[9] Light-induced photoreceptor loss is known to reduce the accumulation of LF.[10]

LF has other components besides A2E and all-trans retinal.[11] These molecules include A2E precursors, fragments of oxidized A2E molecules, and protein and lipid peroxidation products.[11]

The retinoid fluorophores that form LF are composed of long conjugated bonds. These structures allow retinoid fluorophores to absorb light and then emit fluorescence. Their autofluorescent properties allow LF granules in RPE cells to be detected by fluorescence microscopy.[12]

### Fundus Autofluorescence and the Working Principle

In fluorescent microscopy, ultraviolet light is used to detect LF in *in vitro* examinations. LF excitation can occur at a broad spectrum of wavelengths ranging from 300 nm to 600 nm. Although the emission spectrum is also wide (480-800 nm), its peak is between 600 and 640 nm.[13]

The definition of autofluorescence emerged with the use of fundus FA, while the quantitative evaluation of autofluorescence was performed using fluorometry in 1989 by Kitagawa et al.[14,15]

An important factor that prevents the acquisition of clear FAF images is autofluorescence from anatomical structures anterior to the retina, such as the lens. In recent years, improvements in camera systems and new, sophisticated imaging methods have produced clearer FAF images.

### Wide-Field Retinal Imaging: Wide-Angle Fundus Cameras

Fundus cameras for retinal imaging were first introduced by Carl Zeiss in 1926.[16] While the first device enabled imaging of a 20-degree area of the fundus, the field of view has expanded with developing technology. Today, many devices used in clinical practice provide 50-degree images. Fundus cameras with a field of view greater than 50 degrees are described as "wide-field". Devices that image even larger retinal areas, called "ultra-wide-field", have recently been introduced.[17] One of these devices, the Heidelberg Spectralis (Heidelberg Engineering, Inc., Heidelberg, Germany), can provide 102-degree retinal images. In addition, performing FAF, FA, and indocyanine green angiography examinations with the same device enables evaluation of the choroidal and retinal structures extending to the equator.

Another wide-field imaging system that became commercially available in 2000 is the Optos (Optos PLC, Dunfermline, UK), which includes a cSLO system and allows the retinal periphery to be examined in a single image without pupil dilation or a contact lens. Within the Optos system, an ellipsoid mirror is used to visualize the peripheral retina.[18] This design provides a wide-field image. If the patient is very cooperative and the pupil is well dilated, images up to the ora serrata can be obtained.

In addition to the conveniences it provides, the Optos system has some disadvantages. These include the inability to visualize the entire peripheral retina, the fact that image coloring differs from the actual appearance, and the low posterior pole resolution compared to standard fundus cameras and high-resolution confocal scanning laser systems such as the Spectralis.

Both the Optos and Spectralis systems can provide simultaneous FA and indocyanine green angiography images. Although the Spectralis does not produce clear FAF images with its ultra-wide-field lens system, the 30- and 55-degree FAF images have high resolution.

### Confocal Scanning Laser Ophthalmoscopy

The cSLO was developed by Webb et al. and introduced into clinical use by von Rückmann et al.[19] In this system, the retina is scanned with a low-power laser projected from a point source to produce retinal autofluorescence. The reflected light passes through a small aperture (confocal pinhole) located at the focal point of the lens in the device to prevent light scattering, thus providing clear fundus images. The cSLO obtains several images, an average of the sections is created, and the pixel values are normalized to yield a clear image.[20] Although the field of view is 30 degrees, a larger area can be imaged by changing the lens through the system.[20] The cSLO prevents autofluorescent structures anterior to the retina from blocking retinal autofluorescence.

At present, there are many cSLOs that provide FAF images, including the Zeiss SM 30 4024 (ZcSLO, Zeiss, Oberkochen, Germany), Rodenstock cSLO (RcSLO; Rodenstock, Weco,

Düsseldorf, Germany), Heidelberg Retinal Angiography System (HRA classic, HRA 2, Spectralis®, Heidelberg Engineering, Dossenheim, Germany), F-10 (Nidek, Aichi, Japan), and Optomap® Panoramic 200 Tx (Optomap; Optos, Scotland).

The excitation light of the HRA 2 is blue solid-state diode laser with a wavelength of 488 nm and acquires FAF images with a 500 nm barrier filter.[21] The Spectralis® (Spectralis SD-OCT, Heidelberg Engineering GMbH, Heidelberg, Germany) device synchronizes cSLO and optical coherence tomography (OCT) images, unlike the HRA 2.

The fluorophores in the retina emit fluorescence in a wide spectrum of wavelengths. Therefore, depending on the excitation wavelength, the fluorophores producing the autofluorescence change and the FAF image may vary. The most commonly used excitation light is blue light (488 nm) and is called blue autofluorescence (also known as short-wave autofluorescence, blue-AF, short wave-AF). In blue autofluorescence, excitation is at 488 nm (blue light) and emission is between 500 and 800 nm with a peak at 630 nm. A peak is observed at this wavelength because LF emits at 630 nm.[22]

Near-infrared autofluorescence (NIR-AF) uses a 787 nm excitation wavelength and 800 nm emission filter. The main fluorophore for NIR-AF is melanin. Fluorescence is more pronounced in the choroid and RPE due to the high density of melanin.[23] In NIR-AF imaging, RPE atrophy appears as a reduced signal, but a low-level signal may be detected due to melanin in the choroid.

Recently, the use of green light (504 nm and 532 nm) has been introduced and adapted to wide-field retinal imaging systems. Because green light is absorbed less by macular pigments than blue light, it can provide a better evaluation of the LF signal in the macula.[24] Therefore, green autofluorescence can reveal changes in fovea more clearly. The lower energy of the green excitation light also makes the patient more comfortable during imaging.[25]

**Fundus Autofluorescence Appearance of the Healthy Fundus**

1. Appearance of the healthy fundus on short-wave (blue) autofluorescence:

In the healthy fundus, diffuse autofluorescence is most intense between 5 and 15 degrees from the fovea. The optic nerve and retinal vessels are hypoautofluorescent because the optic nerve does not contain LF and blood blocks autofluorescence.

Xanthophylls (lutein and zeaxanthin) protect the photoreceptor and RPE cells in the fovea by filtering blue light, eliminating free radicals, and masking the natural autofluorescence of the subfoveal RPE cells.[26] The blue light used in short-wave autofluorescence is absorbed by xanthophylls.[27] These pigments are dense in the fovea, resulting in hypoautofluorescence in this area. In addition, the density of melanin in the fovea also causes light to be absorbed.[28]

2. Healthy fundus appearance with NIR-AF:

Due to the density of melanin, the fovea appears hyperautofluorescent. The RPE cells in the macula are more cylindrical and contain less LF and more melanin, unlike the periphery.[28] Similar to short-wave autofluorescence imaging, the optic disc and retinal vessels are also hypoautofluorescent on NIR-AF.

Less of the light used in NIR-AF is absorbed by media opacities due to its long wavelength. Geographic atrophy lesions appear brighter than non-atrophic areas and the lesion margins in the fovea are more clearly distinguishable. There is less contrast in short-wave autofluorescence imaging. **Figure 1** shows FAF images of a normal fundus.

**Clinical Use of Fundus Autofluorescence Images**

FAF images can be examined to obtain information about the RPE. Hypoautofluorescence indicates a decrease in RPE cells and/or low concentration of LF. RPE atrophy appears hypoautofluorescent.[29] In addition, the presence of fibrosis, intraretinal fluid, pigment, and blood are also factors that impede autofluorescence. Situations and conditions that cause hyper- and hypoautofluorescence are presented in **Table 1**.

Increased autofluorescence is observed in conditions with LF accumulation, such as Stargardt disease, Best disease, and adult vitelliform macular dystrophy. Hyperautofluorescence is also observed in the presence of drusen and macular edema.[26] In eyes with geographic atrophy, areas of hyperautofluorescence can be observed surrounding the atrophic area. Changes in autofluorescence have also been demonstrated in the peripheral fundus of eyes with AMD.[30]

**Use of Fundus Autofluorescence in Dry Age-Related Macular Degeneration**

According to the Beckman classification, AMD was classified as the early, intermediate, and late stage.[31] In this classification, the presence of small drusen (drupelet, <63 µm) is considered a normal age-related change. The presence of medium-sized drusen (≥63 to <125 µm) was defined as early AMD, while the intermediate stage is defined as the presence of large drusen (≥125 µm) or medium-sized drusen together with pigmentary changes.

**Imaging Pigmentary Changes with Fundus Autofluorescence**

Hyperpigmentation in AMD can cause typical focal, linear, and/or lace-like hyperautofluorescence in FAF images.[32] Changes in FAF are thought to be due to the presence of

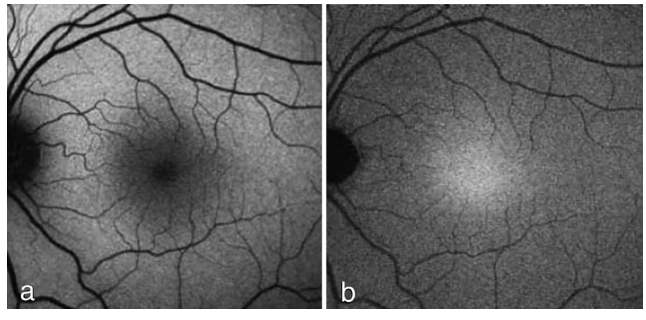


**Figure 1.** Short-wave (blue) (a) and near-infrared (b) fundus autofluorescence images of a normal fovea

melanolipofuscin.[32] Melanolipofuscin accumulation may not be uniform. Autofluorescence may vary depending on whether its melanin content is high or low.

Hypopigmented areas show hypoautofluorescence due to RPE degeneration or reduced LF.[33]

**Fundus Autofluorescence Imaging in Early and Intermediate AMD**

The imaging of drusen, which is an early finding of AMD, is important as it can provide clues regarding progression to the late stage. Drusen has subtypes such as soft, refractile, basal laminar, and cuticular drusen.[34] Reticular pseudodrusen (RPD) located under the retina is another subtype of drusen. Drusen can be differentiated using multimodal imaging methods including color fundus photography, fundus FA, near-infrared reflectance, FAF, and OCT.[34]

On FAF imaging, drusen can have different appearances.[33] A hyperautofluorescent appearance can be seen due to LF within the drusen or in the RPE overlying the drusen. A hypoautofluorescent appearance is caused by regressed drusen or degenerated RPE cells.

Small and medium-sized drusen can produce variable FAF images and may sometimes be overlooked.[33] Different appearances on FAF suggest that the drusen contents may also be different. Soft drusen appear as areas of hyperautofluorescence that is slightly more pronounced at the periphery than the center on FAF imaging. Cuticular drusen are punctate and hypoautofluorescent. Drusenoid pigment epithelium detachment shows a patchy pattern of hyper- and hypoautofluorescent areas.[35]

Reticular pseudodrusen, which differs from other drusen types by its subretinal location, is thought to be a risk factor for progression to late stage AMD. FAF imaging is known to be more sensitive than color fundus photography in demonstrating the presence of RPD.[36] Studies have shown that the presence of reticular drusen is an important risk factor for late stage AMD.[37,38] RPD appear as small, yellowish-white, round or oval lesions on fundus examination. On FAF imaging, they appear as multiple clusters of small (50-400 µm diameter, usually <200 µm), regularly arranged, homogeneous round or oval areas with low-contrast hypofluorescence.[33] They are located mostly in the superior part of the fovea, their prevalence increases with age, and they are more common in women.[33] In early AMD, the presence of RPD is called the "reticular pattern" due to its characteristic appearance in FAF imaging.[33] Although it is not clear why reticular drusen appear hypoautofluorescent, it is thought to be due to the accumulation of subretinal deposits that block the LF in the RPE.[39] **Figure 2** shows RPD imaged by cSLO.

**Table 1. Causes of hypo- and hyperautofluorescence**

| |
|---|
| **Hypoautofluorescence** |
| Reduction or absence of lipofuscin in the retinal pigment epithelium |
| Retinal pigment epithelium atrophy (geographic atrophy) |
| Hereditary retinal dystrophies |
| Increased melanin in the retinal pigment epithelium (retinal pigment epithelial hypertrophy) |
| Presence of fluid, cells, or extracellular material anterior to the retinal pigment epithelium |
| Intraretinal fluid (macular edema) |
| Presence of cells containing melanin |
| Presence of intraretinal and subretinal lipids |
| New intraretinal and subretinal hemorrhage |
| Fibrosis and scars |
| Retinal vessels |
| Luteal pigment (lutein and zeaxanthin) |
| Media opacities (vitreous, lens, anterior chamber, cornea) |
| **Hyperautofluorescence** |
| Increased LF accumulation in the retinal pigment epithelium |
| Lipofuscinopathies (Stargardt disease, Best disease, adult vitelliform macular dystrophy) |
| Age-related macular degeneration (hyperautofluorescent lesion at the geographic atrophy margin suggests the lesion may enlarge) |
| Fluorophores anterior or posterior to the retinal pigment epithelium |
| Intraretinal fluid (macular edema) |
| Subretinal fluid separating the retinal pigment epithelium and photoreceptors (due to insufficient outer segment turnover) |
| Conditions involving lipofuscin-containing macrophages in the subretinal space (choroidal tumors such as nevus and melanoma) |
| Drusen |
| Old intraretinal and subretinal hemorrhages |
| Choroidal vessels in eyes with retinal pigment epithelium and choriocapillaris atrophy |
| Conditions with a decrease in luteal pigment (idiopathic macular telangiectasia type 2) |
| Displacement of luteal pigment (cystoid macular edema) |
| Optic nerve drusen |
| Artifacts |

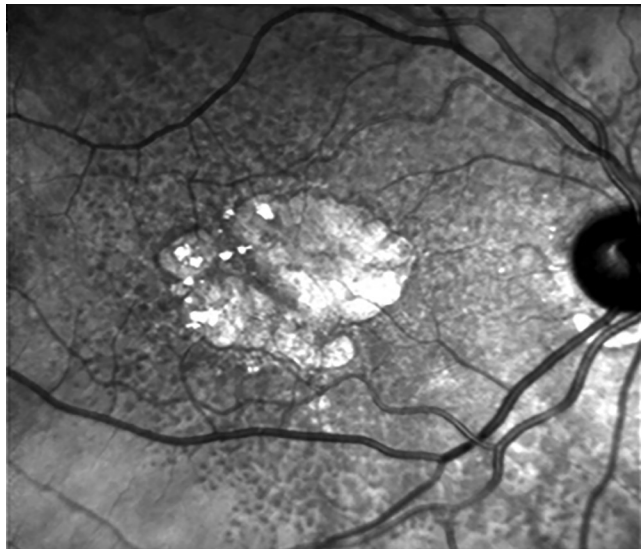

**Figure 2.** Confocal scanning laser ophthalmoscope image of reticular pseudodrusen

The frequency of RPD in eyes with geographic atrophy suggests that this finding is associated with the disease. However, the mechanism of RPD formation and its effect on disease progression have not been determined.

FAF changes in cases of early AMD were classified in an international study. The International Fundus Autofluorescence Classification Group (IFAG)[34] identified eight different autofluorescence patterns in these patients: normal, minimal change, focal increase, patchy, linear, lace-like, reticular, and speckled. Based on this study, it was predicted that in areas of abnormal autofluorescence, damage started at the RPE level. This classification of early AMD can provide clues about the prognosis of the disease.

The most common finding in intermediate AMD is areas with spots of increased autofluorescence (87.9%).[40] Punctate areas of decreased autofluorescence (26.7%) and linear areas of increased autofluorescence (19.8%) are also observed to a lesser extent.[40]

FAF findings have been reported to provide insight regarding neovascular transformation.[41] Analysis using FAF was reported to be the most sensitive method for identifying conversion to neovascularization compared to other imaging methods (color fundus photography, FA, indocyanine green angiography, and OCT.[41] Batioglu et al.[33] showed that the patchy, linear, and reticular FAF patterns were at high risk of transformation into choroidal neovascular membranes. FAF phenotypes identified in early and intermediate AMD provide information about disease prognosis and may be useful for informing the patient and determining follow-up intervals.

**Use of Fundus Autofluorescence in Geographic Atrophy**

The presence of geographic atrophy is a nonspecific finding of late AMD. In FAF imaging, geographic atrophy appears as well-defined areas of hypoautofluorescence. There may be a single or multiple hypoautofluorescent areas. In geographic atrophy, RPE loss results in LF loss, thus the hypofluorescent appearance. **Figure 3** shows a FAF image of geographic atrophy.

Geographic atrophy is frequently seen in the central or parafoveal macula, sometimes progressing to the peripapillary region.[42] Generally, patchy areas of geographic atrophy in the parafoveal area merge to form horseshoe or ring patterns, but over time may also affect the spared central zone. Geographic atrophy area is easier to measure with FAF than other imaging methods. This is because the lesion margins can be clearly determined. The contrast between the lesion and the normal retina in FAF imaging allows atrophic areas to be measured with advanced computer software. With these programs, enlargement of the atrophy area over time can be determined (**Figure 4**).

The presence or absence of foveal involvement can be determined by FAF imaging with 72-93% sensitivity and 59-88% specificity.[43] Both FAF methods should be compared when assessing whether geographic atrophy involves the fovea. Because the short wavelength used in blue autofluorescence is absorbed by pigments in the fovea, the boundaries of foveal involvement can be more clearly observed with the long wavelength used in NIR-AF. **Figure 5** shows blue and infrared FAF images of geographic atrophy.

FAF imaging can reveal hyperfluorescent areas around geographic atrophy. This suggests that cell death may occur in those areas.[42] The hyperautofluorescent areas may be punctate or large irregular areas.

FAF is a valuable imaging method in terms of geographic atrophy progression.[44] The extent of the hyperfluorescent areas

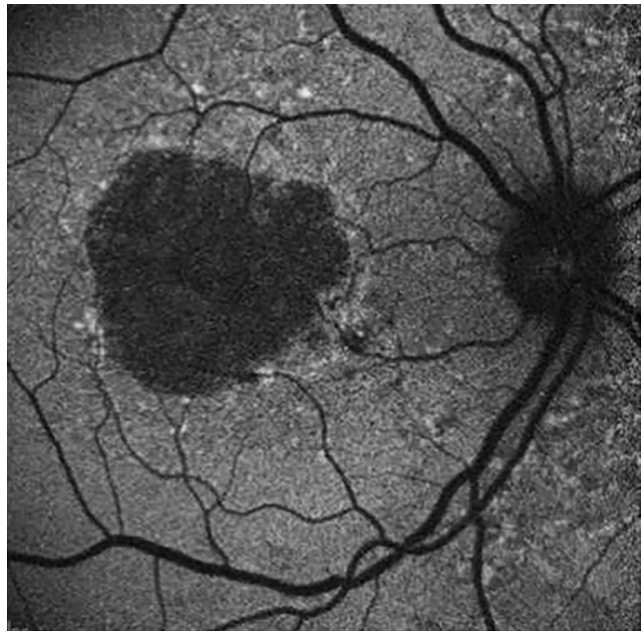

**Figure 3.** Blue autofluorescence image of geographic atrophy

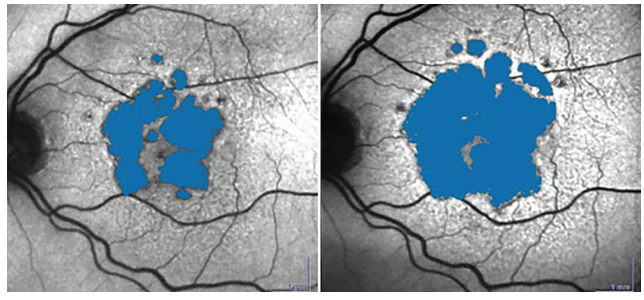

**Figure 4.** Area calculation from fundus autofluorescence images of geographic atrophy. Expansion of the geographic atrophy over time can be seen

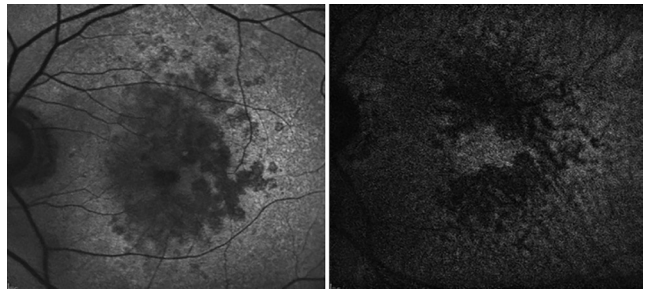

**Figure 5.** Blue (left) and near-infrared (right) fundus autofluorescence images of geographic atrophy

around the geographical atrophy was shown to be positively correlated with disease progression.[45] Schmitz-Valckenberg et al.[46] reported that retinal sensitivity was also reduced in these areas of hyperautofluorescence.

Different classifications have been described for FAF patterns surrounding geographic atrophy. Lois et al.[47] classified geographic atrophy as focal, increased, reticular, combined, and homogeneous. Subsequently, the FAM study group[30] developed a classification for FAF patterns around geographic atrophy. The researchers examined FAF patterns in four different groups: focal, band, patchy, and diffuse. The diffuse pattern describes a phenotype extending over a wider area than the geographic atrophy boundaries and is examined in five groups: granular, branching, trickling, reticular, and granular + peripheral punctate dots. Some of the described FAF patterns have been associated with a faster rate of progression. Holz et al.[48] showed that the enlargement rate was lowest in eyes with no hyperautofluorescence around the geographic atrophy and highest in those with the diffuse and band patterns. In addition, they reported that the "diffuse trickling" pattern, a subgroup of diffuse pattern, had the highest progression rate.[48] Similarly, Batıoğlu et al.[49] observed high rates of progression of the diffuse trickling and band patterns. **Figure 6** shows an example of the diffuse and band patterns.

There are different views about the cause of hyperfluorescence in the area surrounding geographic atrophy. RPE cell hypertrophy, RPE cell shedding into the subretinal space, phagocytosis of melanin and cellular debris, or a combination of all these events has been proposed.[50] Findings observed on FAF imaging are generally consistent with changes in the outer retinal layers on OCT.[50] Evaluating imaging methods together suggests a correlation between LF accumulation and geographic atrophy progression.

One of the current retinal imaging methods used to monitor the progression of geographic atrophy is fluorescence lifetime imaging ophthalmoscopy (FLIO), which measures FAF decay time. FAF times can be recorded *in vivo* with the Heidelberg Engineering ophthalmoscope (Heidelberg, Germany). The working principle is based on time-correlated single photon counting. With pulsed diode laser stimulation, the FAF lifetime and density can be examined in the 30-degree retinal area centered on the fovea. Several retinal diseases, including geographic atrophy, have been studied with FLIO.[51,52,53] Studies examining geographical atrophy with FLIO have shown that different phenotypes display different FLIO patterns.[54] Sauer et al.[55] reported that rapid FAF decay in the macular region was correlated with pigment in the macula. In atrophic areas, the low macular pigment density results in a long FAF lifetime. In addition, long FAF lifetime is seen in scar tissues containing collagen and elastin.[56]

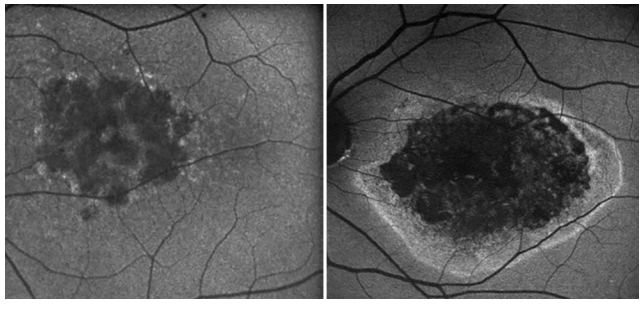

**Figure 6.** *Diffuse trickling* (left) and band (right) patterns

Sauer et al.[57] demonstrated in their study that eyes without hyperfluorescence surrounding the geographic atrophy had better visual acuity and shorter FAF lifetime than those with. The prolonged FAF lifetime in these regions may indicate the onset of change in the RPE cells. A slope of change in FAF lifetime between the unaffected and atrophic areas may be prognostic. All of these hypotheses can be investigated in future studies with large populations.

Deep learning, which is a type of machine learning algorithm, has recently attracted attention due to the ease of classification and diagnosis. There are studies in the ophthalmology literature on the use of fundus cameras and the suitability of the automatic diagnosis of retinal diseases.[58] Matsuba et al.[59] reported in their study that patients with AMD could be detected with high sensitivity using deep learning and Optos imaging, without ophthalmological examination.

In areas with insufficient numbers of medical personnel, wide-field fundus cameras will enable the diagnosis of patients with AMD. A telemedicine system based on imaging methods will be on the agenda in the future. Thus, it will be possible to diagnose AMD, plan treatment and follow-up, interpret FAF images to gain information about progression, and plan the use of new molecules that slow or treat progression in selected patients.

FAF imaging helps the clinician estimate the prognosis of AMD and is a valuable method that provides qualitative and quantitative information about the progression of geographic atrophy. Gaining a more detailed understanding of LF metabolism and identifying eyes at high risk of progression as detected by FAF will guide the use of new molecules in these patients.

**Ethics**

**Peer-review:** Externally peer reviewed.

**Author Contributions**

Concept: F.Ş., Design: F.Ş., N.Ş.K., Data Collection or Processing: N.Ş.K., Analysis or Interpretation: F.Ş., Literature Search: N.Ş.K., Writing: N.Ş.K.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declared that this study received no financial support.

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