Clinical applications of fundus autofluorescence in retinal disease

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Abstract

Fundus autofluorescence (FAF) is a non-invasive retinal imaging modality used in clinical practice to provide a density map of lipofuscin, the predominant ocular fluorophore, in the retinal pigment epithelium. Multiple commercially available imaging systems, including the fundus camera, the confocal scanning laser ophthalmoscope, and the ultra-widefield imaging device, are available to the clinician. Each offers unique advantages for evaluating various retinal diseases. The clinical applications of FAF continue to expand. It is now an essential tool for evaluating age related macular degeneration, macular dystrophies, retinitis pigmentosa, white dot syndromes, retinal drug toxicities, and various other retinal disorders. FAF may detect abnormalities beyond those detected on funduscopic exam, fluorescein angiography, or optical coherence tomography, and can be used to elucidate disease pathogenesis, form genotype-phenotype correlations, diagnose and monitor disease, and evaluate novel therapies. Given its ease of use, non-invasive nature, and value in characterizing retinal disease, FAF enjoys increasing clinical relevance. This review summarizes common ocular fluorophores, imaging modalities, and FAF findings for a wide spectrum of retinal disorders.

Keywords: Fundus autofluorescence, Retina, Imaging, Lipofuscin, Age related macular degeneration, Central serous retinopathy, Macular dystrophy, Retinitis pigmentosa, White dot syndrome, Hydroxychloroquine

Background

Fundus autofluorescence (FAF) is a non-invasive imaging technique that detects fluorophores, naturally occurring molecules that absorb and emit light of specified wavelengths [1]. To produce autofluorescence, a fluorophore absorbs a photon of the excitation wavelength, which elevates an electron to an excited, high energy state. The electron dissipates some energy through molecular collisions, then emits a quantum of light at a lower energy and longer wavelength as it transitions back to ground state. Classically, FAF utilizes blue-light excitation, then collects emissions within a preset spectra to form a brightness map reflecting the distribution of lipofuscin, a dominant fluorophore located in the RPE. FAF may use other excitation wavelengths to detect additional fluorophores, such as melanin with near-infrared autofluorescence.

First described by Delori in the 1980s, FAF has since expanded in both scope and practice [1]. Given the unique findings not identified with funduscopic examination, fundus photography, or fluorescein angiography, FAF is useful in the evaluation of a diverse spectrum of diseases involving the retina and RPE, including degenerative, dystrophic, inflammatory, infectious, neoplastic, and toxic etiologies. This review summarizes the known ocular fluorophores, various imaging modalities, and broad clinical applications of FAF.

Ocular fluorophores: lipofuscin, A2E, and other ocular fluorophores

Lipofuscin

Located in the RPE, lipofuscin is a dominant macular fluorophore that absorbs blue light with a peak excitation wavelength of 470 nm and emits yellow–green light at a peak wavelength of 600–610 nm (Fig. 1) [2]. Lipofuscin is a heterogeneous mixture and derives its autofluorescent properties from bisretinoid compounds, which are metabolic byproducts of vitamin A and the visual cycle.
Bisretinoids are initially formed in photoreceptor outer segments and then deposited in the RPE as lipofuscin, accumulating in RPE lysosomes with age [1]. Lipofuscin also increases in degenerative disorders, including age-related macular degeneration (AMD), and macular dystrophies such as Best and Stargardt disease [3]. The distribution of lipofuscin, and consequently the distribution of autofluorescence, is greatest in the posterior pole but limited in the fovea, and decreases toward the periphery [1, 4].

A2E

N-Retinyl-N-retinylidene ethanolamine (A2E) is the first and best characterized component of lipofuscin, with excitation at 430–450 nm and maximum emission at 560–575 nm [5]. A pyridium bisretinoid, A2E is enzymatically indigestible, accumulates in RPE lysosomes, exerts multiple toxic effects on RPE cells in vitro, and has been implicated in multiple degenerative retinal diseases. When irradiated by blue-light, A2E undergoes photo-oxidation and generates reactive oxygen species [6–10]. In addition, A2E has been shown to interfere with cholesterol metabolism, destabilize cell membranes, damage DNA, and trigger apoptosis [11–13]. However, other studies suggest that A2E may in fact protect the retina from photo-oxidative stress. A2E generates singlet oxygen species much less effectively than its precursor all-trans-retinal, and conversion to A2E may protect the retina from the toxic effects of all-trans-retinal. A2E accumulation may be merely a marker for aberrant visual cycle activity, rather than the source of retinal damage [14, 15]. In addition, A2E is distributed peripherally in human eyes, rather than centrally, like lipofuscin, suggesting that A2E may not be the dominant fluorophore responsible for increased macular FAF over time [16–18]. Given these mixed results, the role of A2E in retinal disease appears complex and requires further study to fully elucidate its function.

Other ocular fluorophores

Other clinically significant fundus fluorophores include vitelliform lesions and optic disc drusen. Vitelliform lesions refer to the clinical finding of round, yellow, retinal lesions reminiscent of an egg yolk. While lipofuscin is located within RPE lysosomes, vitelliform lesions consist of extracellular fluorophores—shed outer segment debris...
in the subretinal extracellular space, which accumulate due to RPE dysfunction and loss of apposition between photoreceptor tips and the RPE [19–21].

Optic disc drusen are deposits of extracellular mitochondria in a filamentous protein matrix and if superficial, may produce increased FAF [22]. These lesions may be associated with visual field defects, optic nerve dysfunction, and various vitreoretinal conditions including retinitis pigmentosa, Alagille syndrome, and pseudoxanthoma elasticum [23].

Structures anterior to the retina, including the cornea and lens, naturally emit autofluorescence and can cause interference, decreasing image resolution in FAF systems. The cornea has an excitation peak at 365–480 nm and an emission peak at 620 nm [24], while the lens has an excitation peak at 420–430 nm and an emission peak at 520 nm [25]. Diseases involving these structures can further impact FAF findings. For example, corneal autofluorescence increases in patients with diabetes and is thought to result from the accumulation of advanced glycation products [26, 27]. Cataracts increase light absorption and scatter by the lens, leading to poor contrast autofluorescence images that improves with cataract extraction and intraocular lens placement [28, 29].

In order to minimize interference from the lens and cornea, fundus cameras have adopted barrier filters with red-shifted wavelengths. Confocal scanning laser ophthalmoscopes (cSLO) utilize confocal optics in the form of a spatial pinhole that collects light from a single optical plane at the level of the fundus while eliminating out-of-focus light [30–32].

Melanin
Melanin is an ocular pigment located in the both RPE cells and in uveal melanocytes. In the fundus, melanin is distributed primarily in the fovea, macula, and periphery [4]. Within RPE cells, melanin granules are located apically and lipofuscin basolaterally [33, 34]. In contrast to lipofuscin, melanin has a peak excitation at a longer wavelength of 787 nm and is the primary fluorophore in near-infrared autofluorescence [35]. On conventional FAF, melanin absorbs the short-wavelength excitation beam, decreasing the overall autofluorescent signal [5].

Melanin protects the retina from light-induced damage in several ways [36]. Melanin located in anterior segment structures such as the iris absorb and block visible light and UV radiation, shielding the retina from excessive light energy. In addition, RPE melanin acts as an antioxidant, protecting against free radicals, redox-reactive heavy metals, photo-oxidation, and lipofuscin accumulation [37–41], although this effect decreases with age and melanin may even gain pro-oxidant properties over time [40]. Individuals with lightly pigmented irises [42–44] and decreased RPE melanin content [45] demonstrate a higher incidence and severity of age-related macular degeneration.

Rhodopsin
Rhodopsin is a visual pigment concentrated in rod photoreceptor outer segments that absorbs the excitation beam and decreases autofluorescence [46]. However, with continued exposure to light, rhodopsin undergoes photo-isomerization and loses its absorptive capabilities, resulting in a progressive increase in autofluorescent signal [47]. Termed the bleaching effect, rhodopsin absorption and photo-isomerization is seen with short-wavelength, but not near-infrared, excitation beams. Compared to dark-adapted eyes in which rhodopsin is maximally active, FAF can increase over 30 % after exposure to light that strongly bleaches rhodopsin (Fig. 2) [48]. Changes in optical density due to the bleaching effect are decreased in retinal dystrophies involving photoreceptor dysfunction, including cone-rod dystrophy, Stargardt disease, and choroideremia [49].

Commonly used fundus autofluorescence (FAF) imaging systems
Commercially available FAF systems include fundus cameras, confocal scanning laser ophthalmoscopes (cSLO), and ultra-widefield technologies (Table 1). Limitations of FAF include a low signal strength (two orders of magnitude less than the peak signal of fluorescein angiography) and autofluorescence artifact from anterior segment structures. In addition, the blue-light excitation beam may cause patient discomfort and be potentially toxic to the retina, although there have been no formal studies demonstrating the adverse effects of FAF. The various imaging modalities have utilized different strategies to address these shortcomings.

Fundus camera
The fundus camera is a digital system that captures autofluorescence using a single flash of light (Fig. 3a) [50]. Commercially available models are produced by Topcon®, Zeiss®, and Canon® (Table 1). To reduce autofluorescence of the lens and cornea, Spaide introduced the ‘modified Topcon’ filter set with red-shifted wavelengths, with an excitation spectra of 535–585 and a 615–715 nm emission barrier filter [51].

The use of the red-shifted wavelengths by fundus cameras decreases absorption by macular pigments, allowing detection of subtle perifoveal RPE changes [50]. This excitation spectra also decreases absorption by the crystalline lens, preserving image quality in cases of cataract. In fact, fundus cameras demonstrate reduced image degradation and higher rates of successful image acquisition
in patients with cataracts compared to cSLOs [52, 53]. Fundus cameras also provide better detection of exudative retinal disease such as choroidal neovascularization or central serous chorioretinopathy compared to cSLOs [54]. However, even with modification, fundus cameras capture more reflected and scattered light compared to confocal systems [53, 55]. Scattered light from structures outside the retinal plane may falsely increase the FAF signal, a phenomena termed pseudo-autofluorescence [54].

Because the excitation spectra of fundus cameras differs from that of fluorescein angiography, the two imaging modalities can be used in any order without interference [55]. Additional advantages of fundus cameras include color imaging capability and greater resistance to motion artifact in cases of poor fixation. The single flash exposure of flood light illumination is more comfortable for the patient but produces images with low contrast, although photographers may manually acquire several images for image averaging or utilize image manipulation methods to improve contrast. Fundus cameras are less expensive than confocal scanning laser ophthalmoscopes (cSLOs), but require post-purchase modifications such as the installation of filters.

**Confocal scanning laser ophthalmoscope (cSLO)**

cSLO systems utilize a system of mirrors to focus a low power laser in a two-dimensional raster pattern onto the fundus (Fig. 3b). Platforms use a blue excitation wavelength of 488 nm and detect emission wavelengths of 500–700 nm [56]. Confocal optics reduce light detection to a single optical plane, eliminating scattered light and interference from structures outside the retina, such as the crystalline lens [57]. cSLO systems offer real-time averaging, where typically nine to sixteen images are averaged to produce high-contrast and high-resolution images. There is no limit to the number of images averaged [50]. However, real-time averaging may lead to loss of information, especially in patients with poor fixation and excessive eye movement [50]. cSLO imaging cannot be preceded by fluorescein angiography, which has a similar excitation and emission spectra. A substantial fraction of the excitation beam is also absorbed by macular pigments, which have absorption spectra similar to the excitation beam wavelength [58].

Commercially available cSLO systems include the Heidelberg Spectralis® and the Nidek F-10®. The Heidelberg retinal angiograph® (HRA), now available as the Heidelberg Spectralis®, uses a barrier filter of 500 nm and offers 20–55 degree images. Other cSLO systems include the Zeiss prototype SM 30 4024® (ZcSLO) with a barrier filter of 521 nm and the Rodenstock cSLO® (RcSLO) with a barrier filter of 515 nm, though these are no longer commercially available. A comparison of the HRA®, ZcSLO®, and RcSLO® demonstrates that HRA® takes the brightest images, while the RcSLO® captures the darkest images with the lowest contrast [59]. The level of background noise was lowest for HRA® and highest for ZcSLO® [59].

The Nidek F-10® digital ophthalmoscope offers blue light FAF at 490 nm, fluorescein angiography, and indocyanine green angiography [60]. In addition, infrared imaging at 700 nm using Retro-mode on the Nidek F-10®, which relies on laterally scattered light, is more sensitive for drusen than conventional color fundus photography [61, 62].
Table 1  Fundus autofluorescence imaging modalities. Excitation wavelengths, barrier filters, fields of view, advantages, and disadvantages of commercially available FAF systems. Although some systems use multiple wavelengths, only the FAF excitation wavelength is provided

| Imaging modality | Fundus autofluorescence imaging systems | Excitation wavelength | Barrier filter (nm) | Field of view | Advantages | Disadvantages |
|------------------|----------------------------------------|-----------------------|--------------------|--------------|------------|--------------|
| Fundus camera    |                                        |                       |                    |              |            |              |
|                  |                                        |                       |                    |              | Better for visualizing exudative retinal disease, red-shifted wavelengths decrease absorption by macular pigments and reduce lens interference, can be used with FA, color imaging, decreased motion artifact, more comfortable for patient | No real-time averaging, poor contrast, capture more reflected and scattered light, prone to pseudo-autofluorescence |
| Topcon TRC-50DX  | 535–585 nm                             | 615–715               | 20, 35, 50         |              | Non-mydriatic, also offers FA, ICG | |
| Zeiss Visucam 224/524 | 510–580 nm                             | 650–735               | 30, 45             |              | Non-mydriatic, Visucam 524 with FA and optional ICG | |
| Canon CR-2 plus AF (non mydriatic) | 530–580 | 640 | 35, 45 | | Non-mydriatic, also offers cobalt setting | |
| Confocal scanning laser ophthalmoscope (cSLO) | | | | | Confoal optics reduces interference from the lens, real-time averaging, high contrast, high resolution, decreased scattered light | Excitation beam is absorbed by macular pigments, cannot be preceded by fluorescein angiography, fixation loss, monochromatic, patient discomfort |
| Heidelberg retinal angiograph (HRA 2) | 488 nm | 500 | 20, 30, 55 | | No longer commercially available | |
| Heidelberg spectralis | 488 nm | 500 | 20, 30, 55 | | Also offers red-free, FA, ICG, simultaneous FA/ICG, infrared reflectance, multicolor imaging, dual wavelength technology can calculate macular pigment density, spectral domain OCT | |
| Zeiss prototype SM 30 4024 (ZcSLO) | 488 nm | 521 | 20, 40 | | No longer commercially available | |
| Rodenstock (RcSLO) | 488 nm | 515 | 20, 40 | | No longer commercially available | |
| Nidek F-10 | 490 nm | 510 | 40, 60 | | Also offers multicolor imaging, retro-mode, FA, ICG | |
| Optos ultra-widefield | 532 nm, 633 nm | 540 | 200 | | Detects peripheral findings, non-mydriatic, brief image acquisition time, can be used with FA | Disadvantages vary by system and lens |
| Widefield cSLOs | | | | | Decreased absorption by macular pigments, also offers color fundus, red-free, FA, ICG | No real-time averaging, poor contrast, distortion of peripheral retina, view limited in superior and inferior quadrants, lid/lash artifact |
Table 1 continued

| Imaging modality                      | Fundus autofluorescence imaging systems | Excitation wavelength | Barrier filter (nm) | Field of view | Advantages                                                                 | Disadvantages                                                                 |
|---------------------------------------|----------------------------------------|-----------------------|--------------------|---------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Staurenghi lens                        | N/A                                    | N/A                   | 150                |               | Lens attaches to cSLO                                                      | Requires placement of contact lens                                                                                     |
| Heidelberg ultra-widefield lens       | N/A                                    | N/A                   | 105                |               | Lens attaches to HRA or Spectralis. High contrast, non-distorted images, no lid/ lash artifact, can be used with fluorescein angiography | Smaller field of view, view limited in nasal and temporal quadrants                                                     |

cSLO confocal scanning laser ophthalmoscope, ICG indocyanine green angiography, FA fluorescein angiography, OCT optical coherence tomography
FAF images are monochromatic and lack the color information of fundus photography. cSLO systems such as the Heidelberg Spectralis® do offer reflectance-based MultiColor® imaging, which can be used in conjunction with SD-OCT. This technology simultaneously acquires reflectance images at three different wavelengths: blue (486 nm), green (518 nm), and infrared (815 nm), and then superimposes the images to form a final multicolored image [63]. Recently, LaRocca and colleagues introduced a “true color” SLO using a supercontinuum laser [64]. However, these techniques use fundus reflectance imaging, and there are currently no color options for FAF.

cSLO systems can be used reliably to quantify macular pigment density, which consists of lutein, zeaxanthin, and meso-zeaxanthin [65]. With an absorption spectrum of 400–540 nm and peaking at 460 nm, macular pigments filter blue light and also act as antioxidants to protect the retina [66, 67]. Changes in macular pigment density may reflect visual function and retinal diseases such as age-related macular degeneration [68, 69]. To measure macular pigment, cSLOs such as the Heidelberg Spectralis® utilize dual-wavelength autofluorescence, which uses lipofuscin autofluorescence as an indirect measure of macular pigment density. Averaged images are obtained at two excitation wavelengths, 488 and 514 nm, using a barrier filter above the macular pigment absorption wavelength (e.g. 530 nm). These images are then digitally subtracted from each other to construct a map of macular pigment density [70]. Variations of this FAF technique include using only one high absorption wavelength to obtain images, using a fundus camera instead of a cSLO, and inferring macular pigment density by comparing foveal and para-foveal autofluorescence [70]. Besides FAF, other methods used to measure macular pigment density include motion photometry, heterochromatic flicker photometry, resonance Raman spectroscopy, and fundus reflectance [65].

Although cSLO and fundus cameras use different excitation and emission wavelengths, the two systems are largely concordant on retinal pathology given the wide autofluorescent spectra of lipofuscin. While the fundus camera has lower cost and shorter image acquisition time, studies suggest superior images are obtained with cSLO in 70% of cases [50].

**Optos ultra-widefield system**

The Optomap Ultra-Widefield® system by Optos® combines confocal scanning laser technology with an ellipsoid mirror to achieve up to 200 degrees of view (82.5% of retinal surface area) of the ocular fundus (Fig. 3c) [71]. The Optos® system simultaneously uses two excitation wavelengths of red (633 nm) and green (532 nm) light with an emission filter of >540 nm [48]. Similar to the fundus camera, the longer wavelength spectra of this system reduces absorption by macular pigment and allows for a clear image after fluorescein angiography.

Ultra-widefield imaging allows for improved detection and analysis of many pathologic retinal conditions with peripheral findings, including diabetic retinopathy and other retinal vascular diseases, age-related macular degeneration, and myopic degeneration [72]. The Optos® system has several important features, including the ability to acquire images through a native non-dilated pupil, a brief image acquisition time (250 ms), and the option of pseudocolor fundus photography [48]. However, use of the ellipsoid mirror in the Optos® system distorts the peripheral retina, creating a topographic mismatch, and the view is limited superiorly and inferiorly [73]. The Optos® system is also limited by
also result from a window defect due to loss of rhodopsin, which normally absorbs the excitation wavelength and decreases autofluorescence. Rhodopsin attenuation and hyper-autofluorescence can occur physiologically as seen in the bleaching effect. In disease, photoreceptor degeneration and loss of rhodopsin unmasks the autofluorescent signal of the underlying RPE, creating hyper-autofluorescent lesions such as in white dot syndromes and other pathologies [80]. Fluorophores other than lipofuscin, including optic disc drusen, can also form hyper-autofluorescent lesions.

In contrast, hypo-autofluorescence can arise from decreased lipofuscin or blockage by material anterior to the RPE/photoreceptors. Perhaps the most striking example of hypo-autofluorescence due to decreased lipofuscin is the absence of autofluorescence at the site of RPE tears, where the RPE is completely denuded [81]. Other pathologies involving decreased lipofuscin include RPE atrophy in GA and visual cycle defects in fundus albipunctatus. Meanwhile, blockage can occur from any material anterior to the RPE/photoreceptors, including physiologic structures such as blood vessels and macular pigments, and pathologic structures such as media opacities, intraretinal or subretinal hemorrhage, and fibrosis.

**Age-related macular degeneration**

Affecting 6.5% of Americans over 40 years old, AMD can be associated with vision loss in both non-neovascular and neovascular forms of disease [82]. FAF can be an important tool to monitor non-neovascular AMD—especially GA, the second most important etiology of severe vision loss in AMD after choroidal neovascularization [83].

In early non-neovascular AMD, FAF may show hyper and hypo-autofluorescent areas that reveal more widespread disease than appreciated with fundoscopy or color photography. Hyperpigmented lesions may represent melanin granules, which correlate with hypo-autofluorescence, or melanolipofuscin granules, which correlate with hyper-autofluorescence [84]. Depigmented, hypo-autofluorescent areas correspond to RPE atrophy, which may signal early geographic atrophy [84, 85]. In 2005, the International FAF Classification Group defined eight phenotypic FAF patterns associated with early non-vascular AMD: normal, minimal change, focal increase, patchy, linear, lace-like, reticular, and speckled [86].

**Drusen**

Drusen have an extremely variable appearance on FAF depending on size, composition, and health of the overlying RPE and ellipsoid layer [87]. Large drusen are more likely to result in FAF changes, while small drusen may be iso-autofluorescent and remain undetected [88]. Intermediate drusen (63–125 µm in diameter) demonstrate a
Causes of hyper-autofluorescence

- Increased RPE lipofuscin
- Subretinal autofluorescent material
  - Vitelliform dystrophies
  - Central serous chorioretinopathy
  - Macular telangiectasia type II
- Window defect: loss of luteal or photopigment
- Other autofluorescent material
  - Optic disc drusen
  - Astrocytic hamartoma

Causes of hypo-autofluorescence

- Reduced or absent RPE lipofuscin
- Reduced visual cycle
- Media opacities
- Macular pigments
- Intraretinal and/or subretinal hemorrhage
- Fibrosis and scar tissue

- RPE atrophy
- RPE tear
- Hereditary
- RDH5 retinopathy
- Lutein
- Zeaxanthin
- Pigment
- Spicules

Fig. 4 a Causes of hyper-autofluorescence. b Causes of hypo-autofluorescence
pattern of central hypo-autofluorescence with an annulus of hyper-autofluorescence, likely due to central RPE atrophy surrounded by abnormal RPE [89]. Cuticular drusen, associated with vitelliform macular detachment and described as multiple dense nodules creating a “stars in the sky appearance” with fluorescein angiography, appear hypo-autofluorescent with FAF [90]. Crystalline drusen also appear hypo-autofluorescent due to associated RPE atrophy [85].

Hyper-autofluorescent lesions include large, soft confluent drusen and drusenoid pigment epithelial detachments (PED) [88]. Drusenoid PEDs typically appear as a foci of hyper-autofluorescence bordered by a hypo-autofluorescent halo, but may produce intermediate to decreased signal if there is overlying RPE atrophy or fibrovascular scarring. They are associated with a high risk for disease progression [91].

First described as dot-like spots seen on blue light photography, reticular pseudodrusen are subtle accumulations of material above the RPE and visualized best on FAF, near-infrared FAF, or SD-OCT [92, 93]. They appear as small, round, elongated foci of hypo-autofluorescence bounded by interspersed hyper-autofluorescence in a reticular pattern [94]. Reticular pseudodrusen are associated with a high risk for progression to advanced disease involving choroidal neovascularization and/or geographic atrophy [95].

Geographic atrophy
Geographic atrophy is the hallmark of advanced non-neovascular AMD. Lesions typically occur parafoveally, with foveal sparing. RPE atrophy and consequent loss of intrinsic fluorophores produces an area with a low to extinguished FAF signal with sharply demarcated borders. Lesions that include the fovea are more difficult to visualize on FAF due to low baseline concentration of lipofuscin and absorption interference by macular pigment.

Geographic atrophy may be surrounded by perilesional hyper-autofluorescence, which represent areas of ongoing RPE cell dysfunction, vertically superimposed RPE cells, and variable progression to atrophy [79, 96]. Phenotypes of perilesional hyper-autofluorescence include: none, focal, diffuse, banded, and patchy (Fig. 5). The diffuse phenotype is further delineated into reticular, branching, trickling, fine granular, and fine granular with peripheral punctate spots (GPS) patterns. Diffuse (especially the trickling pattern) and banded phenotypes are associated with a higher risk for disease progression.

Fig. 5 Perilesional hyper-autofluorescence patterns in geographic atrophy in age-related macular degeneration. Geographic atrophy can be classified based on perilesional phenotypic FAF patterns. The subtype may impact prognosis, with diffuse and banded phenotypes portending greater risk for progression to advanced disease. Republished with permission of Association for Research in Vision and Ophthalmology, from “A subgroup of age-related macular degeneration is associated with mono-allelic sequence variants in the ABCA4 gene”, Lars G. Fritsche, Monika Fleckenstein, Britta S. Fiebig, Steffen Schmitz-Väckenberg, Almut Bindewald-Witsch, Claudia N. Keilhauer, Agnes B. Renner, Friedenike Mackensen, Andreas Maner, Daniel Pauleikhoff, Christine Adrion; Ulrich Mansmann; Hendrik P. N. Scholl; Frank G. Holz, Bernhard H. F. Weber, vol 53, 2012; permission conveyed through Copyright Clearance Center, Inc.
Given the potential for understanding and predicting disease progression, FAF evaluation of atrophic AMD is an important clinical and research modality [97].

**Choroidal neovascularization**

Neovascular AMD is defined by the presence of choroidal neovascularization, which is located either above the RPE (type 2), or under the RPE (type 1). Type 3 neovascularization is located intraretinally and originates from the deep retinal capillary plexus [98].

Early choroidal neovascularization is not readily detectable on FAF, reflecting intact RPE and photoreceptor layers [99]. Classic choroidal neovascularization appears hypo-autofluorescent due to blockage of the RPE by the type 2 fibrovascular complex in the subretinal space [100]. Occult type 1 neovascularization is also hypo-autofluorescent due to associated atrophy of the overlying RPE. Choroidal neovascularization may be bordered by hyper-autofluorescence in 38% of cases due to associated RPE proliferation or photoreceptor loss resulting in a window defect [101]. Hemorrhages and exudates are initially hypo-autofluorescent due to excitation light absorption, but then may become hyper-autofluorescent after undergoing organization.

Fundus autofluorescence patterns in non-neovascular AMD may predict the development of choroidal neovascularization. Batoglu et al. found that the patchy pattern of early non-neovascular AMD had the strongest correlation with progression to neovascular AMD, with 30.4% of eyes developing choroidal neovascularization the mean follow-up period of 29.2 months [102, 103]. Linear and reticular patterns were also associated with increased risk for choroidal neovascularization [102].

**RPE tears**

RPE tears are a well-known complication of neovascular AMD, most commonly associated with large (greater than 600 microns in height) fibrovascular PEDs [104]. RPE tears can occur spontaneously or following photodynamic therapy or anti-VEGF therapy due to accelerated contraction of the neovascular complex exerting traction on the RPE monolayer [105, 106].

RPE tears appear as a well-demarcated area of hypo-autofluorescence due to absent RPE, with adjacent hyper-autofluorescence in the form of rolled redundant RPE. Over time, tears remodel and resurfacing occurs, with recovery of autofluorescence extending centripetally from the borders toward the center (Fig. 6). The process of resurfacing correlates with visual improvement and may benefit from treatment with anti-VEGF, though studies differ (Fig. 7) [105, 107, 108].

**Differential diagnosis of age-related macular degeneration**

The wide variation in clinical presentation and severity of AMD, as well as its considerable overlap with other macular dystrophies, presents an interesting diagnostic challenge. Although drusen are the hallmark of AMD, they are not pathognomonic for the disease. Small drusen <63 µm represent normal aging rather than AMD [109]. Drusen should be differentiated from the irregular flecks of late onset Stargardt disease, which are characterized by intense hyper-autofluorescence on FAF. Drusen should also be differentiated from vitelliform lesions such as in Best dystrophy and adult onset vitelliform macular dystrophy, discussed below. Macular dystrophies that present with drusen or drusen-like deposits include Malattia Leventinese, Sorsby fundus dystrophy, and
North Carolina macular dystrophy, all of which have an earlier age of onset and autosomal dominant inheritance. Geographic atrophy can resemble central areolar choroidal dystrophy, late onset cone dystrophy, and central serous chorioretinopathy [110] and can be differentiated with the aid of FAF.

**Central serous chorioretinopathy**

CSCR most often presents as a serous retinal detachment that results from choroidal dysfunction and idiopathic leakage from the RPE into the subretinal space. During the initial presentation of CSCR, 72–96 % of cases show hypo-autofluorescence corresponding to the focal leakage site on fluorescein angiogram and to the area of serous retinal detachment, due to blockage by subretinal fluid [111–113]. A subset of patients may show localized RPE detachment, corresponding with focal hyper-autofluorescence. As the disease progresses, subsequent FAF images show granular hyper-autofluorescence, with increased number and size of hyper-autofluorescent dots corresponding to subretinal precipitates on OCT (Fig. 8a, b) [113]. This material represents photoreceptor debris and macrophages accumulating in the subretinal space due to separation of the neurosensory layer from the RPE [114, 115]. The hyper-autofluorescent precipitates gravitate inferiorly or collect at the borders of the detachment.

In chronic cases lasting longer than 6 months, 85 % showed hypo-autofluorescent atrophic gravitational tracts that correlate to prior dependent subretinal fluid (Fig. 8c) [111, 114].

Near-infrared FAF is more sensitive for CSCR than conventional short-wavelength FAF, with short-wavelength FAF revealing 83.2 % of abnormalities compared to 100 % by near-infrared FAF [116]. Ultra-widefield FAF can aid in the diagnosis and monitoring of CSCR [117]. Pang et al. found that 57 % of CSCR cases had extensive involvement of the peripheral retina. Abnormal hyper-autofluorescent lesions in the periphery could indicate active disease and warrants further evaluation by SD-OCT and indocyanine green angiography [118].

**Macular dystrophies**

**Stargardt disease**

Stargardt macular degeneration is the most common hereditary juvenile macular dystrophy. The disorder most commonly results from an autosomal recessive mutation in the ABCA4 gene, leading to defective outer segment degradation, lipofuscin accumulation, and central degeneration of the RPE and photoreceptor layer. Rare cases of autosomal dominant Stargardt disease result from mutations in the Elongation of Very Long Chain Fatty Acids (ELOVL4) gene [119]. Clinically, Stargardt disease...
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presents as foveal atrophy surrounded by flecks of yellow deposits, peripapillary sparing, and associated central vision loss [120].

Cideciyan et al. synthesized a six stage model of disease pathogenesis that correlates well with FAF findings [121]. Early stages may demonstrate a general increase in lipofuscin and thus increased autofluorescent signal [120, 122]. Intermediate stages show a high variation in mean intensity and texture (microscopic spatial variation in intensity) [121]. The disease then progresses to a pattern of chorioretinal atrophy resulting in macular hypo-autofluorescence, surrounded by hyper-autofluorescent flecks (Fig. 9) [123]. Peripapillary sparing of the hyper-autofluorescent flecks is highly suggestive of Stargardt disease. In advanced stages, complete degeneration and diffuse atrophy of RPE cells and photoreceptor death result in hypo-autofluorescence and vision loss [123].

FAF may show areas of early atrophy and flecks not otherwise appreciated with fundus photography, suggesting its utility in detecting early disease [123]. In addition, FAF correlates well with visual function, with normal macular autofluorescence associated with normal electroretinography findings and good vision [120].

**Best macular dystrophy**

An early-onset macular dystrophy resulting in central vision loss, Best disease arises from autosomal dominant mutations in the BEST1 gene, encoding bestrophin-1. Shed photoreceptor debris and lipofuscin accumulate in the subretinal space, resulting in bilateral yolk-like lesions in the macula [124–126].

The progression of disease is classically separated into five stages of progression: previtelliform, vitelliform, pseudohypopyon, vitelliruptive, and atrophic, with progression at variable rates. In a retrospective, observational study, Querques et al. found that previtelliform lesions showed zero to minimal hyper-autofluorescence. The vitelliform stage had well-circumscribed, homogenous hyper-autofluorescence in the macula. The pseudohypopyon stage showed a gravitational layer of hyper-autofluorescence settling under iso-autofluorescent fluid. The vitelliruptive stage showed a dark lesion bordered by condensations of hyper-autofluorescent material. The atrophic stage was characterized by diffuse, decreased signal due to chorioretinal atrophy [125].

Parodi et al. characterized six different phenotypes of Best disease with FAF, including normal,

**Fig. 8** Fundus autofluorescence of central serous chorioretinopathy. FAF (a) of a son with acute exacerbation of CSCR shows macular detachment due to acute CSCR, with hyper-autofluorescent material at the margin and inferior region of the detachment. SD-OCT (b) of the lesion shows a serous retinal detachment associated with a small pigment epithelial detachment. FAF of the patient's asymptomatic father (c) incidentally revealed an atrophic hypo-autofluorescent gravitational tract from chronic inactive CSCR with hyper-autofluorescent margins.
hyper-autofluorescent, hypo-autofluorescent, patchy, multi-focal, and spoke-like patterns. The patchy subtype occurred most frequently, followed by the hyper-autofluorescent, then hypo-autofluorescent patterns. These findings did not correlate with the stages of progression or best corrected visual acuity [124].

Acute exudative polymorphous vitelliform maculopathy
Occurring as an idiopathic [127] or paraneoplastic syndrome [128, 129], acute exudative polymorphous vitelliform maculopathy (AEPVM) likely results from inflammatory or immune-mediated RPE dysfunction leading to lipofuscin accumulation and exudative retinal

Fig. 9 Fundus autofluorescence of Stargardt disease. While fundus photography (a, b) and clinical examination may have non-specific findings, fundus autofluorescence (c, d) shows hyper-autofluorescent flecks with peripapillary sparing characteristic of the disease. The flecks surround a hypo-autofluorescent central macula corresponding to significant chorioretinal atrophy on SD-OCT (e)
Pattern dystrophies refer to a collection of late-onset, symmetric macular dystrophies with a clinically stable, benign course. Subtypes include adult onset vitelliform dystrophy, “butterfly” pigment dystrophy, reticular dystrophy, multifocal pattern dystrophy simulating fundus flavimaculatus, and fundus pulverulentus [134]. Pattern dystrophies are commonly associated with autosomal dominant mutations in the PRPH2 gene (formerly known as RDS, retinal degeneration slow gene) on chromosome 6, which codes for a membrane glycoprotein on rod and cone photoreceptor outer segments [135]. As a result, yellow, orange, or gray material accumulates subretinally or at the level of the RPE, translating to hyper-autofluorescent lesions [136].

Adult-onset vitelliform dystrophy predominantly results from mutations in the PRPH2 gene and less commonly the BEST1 genes [137]. This disease presents as small bilateral, subfoveal vitelliform lesions with a central clump of pigment that may evolve to RPE atrophy [138]. These vitelliform lesions emit increased autofluorescence [139, 140], while subsequent RPE atrophy results in hypo-autofluorescence [138]. Furino et al. described three FAF patterns, including patchy, ring-like focal, and linear patterns, but did not find a correlation with progression or visual function [141]. Around the same time, a slightly larger study by Parodi et al. identified three separate FAF patterns that progressed from normal, to focal, then to patchy hyper-autofluorescence, which correlated with visual acuity and retinal sensitivity. Of note, near-infrared FAF, which detects melanin, had a higher sensitivity than conventional short-wavelength autofluorescence, visualizing abnormalities in 100 versus 86 % of cases [142].

Multifocal pattern dystrophy simulating fundus flavimaculatus presents as irregular yellow flecks that are concentrated at the posterior pole and vascular arcades and that show hyper-autofluorescence on FAF. Classic “dot and halo” lesions appear as central hypo-autofluorescence surrounded by a hyper-autofluorescent halo [99]. Despite a similar appearance, multifocal pattern dystrophy can be differentiated from Stargardt disease by its autosomal dominant inheritance pattern, adult-onset, benign course (geographic atrophy is atypical with pattern dystrophy), and lack of a dark choroid [143].

Other pattern dystrophies, especially “butterfly” lesions, reticular dystrophy, and fundus pulverulentus have not been well characterized on FAF imaging. However, the FAF findings can be remarkable and are more obvious than can be appreciated with examination by color photography. Moreover, FAF is a much less invasive procedure than fluorescein angiography and therefore is an important tool to reliably determine the diagnosis of macular dystrophies such as pattern dystrophy. Note that there is a high degree of phenotypic variability with pattern dystrophy. Multiple patterns may manifest with the same mutation, or even in the same patient [99]. Further investigations to understand epigenetic and environmental effects on phenotype will help elucidate disease pathogenesis and identify potential treatments.

Retinitis pigmentosa

Retinitis pigmentosa (RP) refers to a genetically heterogeneous group of retinal dystrophies characterized by the degeneration of rod photoreceptors. Mutations in the RHO (rhodopsin) gene are most common in autosomal dominant RP, mutations in the USH2A (Usher’s type 2) gene are most common in autosomal recessive RP, and RPGR and RP2 gene mutations are most common in X-linked RP. Patients may exhibit an annular scotoma in the mid-periphery that progressively extends towards the fovea, eventually involving cone photoreceptors and impinging on central vision. Electroretinography (ERG) is the gold standard modality for diagnosis and monitoring of disease progression, but loses clinical value in advanced disease [144]. As such, FAF is a viable supplemental imaging modality to monitor RP and correlate phenotype with genotype [145].

A retrospective, observational case series by Murakami et al. identified three subsets of RP on FAF, where 59 % of patients had a hyper-autofluorescent parafoveal ring not visible on funduscopy, 18 % had abnormal central hyper-autofluorescence extending centrifugally from the fovea, and 24 % had neither pattern [146]. The hyper-autofluorescent ring, known as the Robson-Holder ring, corresponds to the border of inner/outer segment junction disruption (Fig. 10). OCT analysis shows complete photoreceptor loss outside of the ring, with the external limiting membrane in direct apposition to the RPE [147, 148]. The ring itself corresponds to outer segment dysgenesis and lipofuscin production, while normal retina lies within the ring [144, 149–151]. Spatial preservation of retinal sensitivity as measured by multifocal ERG correlates with the radius of the autofluorescent ring, indicating intact retinal sensitivity inside the ring but none
outside [152]. In addition, the size of the ring correlates with visual function as measured by both kinetic and automated perimetry; the more the ring encroached centrally, the more constricted the visual field [153]. Serial imaging of this hyper-autofluorescent ring may help determine the stability or rate of progression of the disease, providing a useful tool for delineating prognosis in different patients [147].

Similar hyper-autofluorescent rings are also seen in several other retinal dystrophies, including Leber congenital amaurosis (LCA), bull’s eye maculopathy, X-linked retinoschisis, Best macular dystrophy, cone dystrophy, and cone-rod dystrophy [149]. This shared phenotype on FAF suggests an underlying common mechanism for the pathogenesis of retinal dystrophies.

**Choroideremia**

Choroideremia is an X-linked recessive defect in the CHM protein, which encodes the rab escort protein (REP1). Beginning in adolescence, male carriers experience night blindness and visual field contractions due to centripetal atrophy of the choroid, RPE, and photoreceptor layer, though the macula is spared [154].

FAF shows bilateral, symmetric, midperipheral zones of hypo-autofluorescence due to RPE atrophy, which have scalloped edges with a preserved area of central stellate autofluorescence (Fig. 11) [155]. The atrophic zones extend with age and eventually involve the fovea [156]. Recent work on gene therapy for choroideremia has shown promising preclinical results and is currently undergoing clinical trials, with the first treatment administered in 2011 [157, 158]. FAF is an accurate structural marker that correlates with visual acuity and age, and may help identify candidates for gene therapy [154].

Interestingly, FAF of asymptomatic female carriers of the CHM mutation show a peripheral speckled pattern of hyper-autofluorescence corresponding to lipofuscin accumulation from photoreceptor degeneration and hypo-autofluorescence due to RPE atrophy [156, 159, 160]. In conjunction with genetic testing, FAF is a useful tool for evaluating female relatives of affected patients.

**Fundus albipunctatus**

Fundus albipunctatus results from an autosomal recessive defect in RDH5, which encodes a retinol dehydrogenase in the RPE [161]. Patients are unable to oxidize 11-cis-retinol to 11-cis-retinal, and this impairment of rhodopsin recycling delays the regeneration of photoreceptor pigments, manifesting as delayed dark adaptation and stationary night blindness [162]. Similar to Stargardt disease, fundus albipunctatus presents as a flecked retina syndrome, with white-yellow subretinal spots in the midperiphery on funduscopy.

As a hereditary defect in the visual cycle, fundus albipunctatus causes decreased lipofuscin production and severely attenuated background autofluorescence [163]. Images appear grainy [164]. FAF may also show hyper-autofluorescent foci corresponding to the white dots, though not all lesions produce autofluorescence (Fig. 12) [165]. SD-OCT through these lesions reveal dome shaped hyperreflective material extending from the RPE
past the photoreceptor layer [156]. Hyper-autofluorescent crescents or concentric parafoveal rings have also been reported [165].

Fundus albipunctatus must be differentiated from diseases with similar presentations. Retinitis punctata albescens is also associated with white punctate retinal lesions.
on the retina and night blindness. This disease is due to an autosomal recessive mutation in RLBP1 encoding CRALBP, which binds to 11-cis-retinol and 11-cis-retinal during the visual cycle [166]. However, retinitis punctata albescens is a severe progressive retinal dystrophy with narrowed vessels, pigimentary degeneration, and visual field loss [166]. Meanwhile, RPE65 encodes an isomerase which acts one step upstream of retinol dehydrogenase in the visual cycle and also results in decreased background autofluorescence on FAF [167]. In contrast to fundus albipunctatus, mutations in RPE65 result in a more severe disease course and are associated with LCA and early onset rod-cone dystrophy [168].

**White dot syndromes**

White dot syndromes refer to a diverse spectrum of inflammatory chorioretinopathies characterized by multifocal, small, yellow-white lesions scattered throughout the posterior pole and/or peripheral fundus. Despite this shared phenotypic presentation, the individual diseases are not related in pathogenesis, management, or prognosis [169].

**Multiple evanescent white dot syndrome (MEWDS)**

This disease affects young, healthy women 20–50 years old and manifests as a self-limited, unilateral presentation with multifocal 100–200 µm white spots scattered in the paramacular and mid-peripheral fundus [170].

On FAF, the multifocal lesions have increased signal strength due to photoreceptor loss and unmasking of natural RPE autofluorescence (Fig. 13) [80, 171]. Hypo-autofluorescent foci at the fovea correspond with foveal granularity on exam and accumulation of hyper-reflective material interdigitating between the RPE and photoreceptor layer on OCT [170]. FAF reveals more lesions than on clinical exam or FA, though less than seen on indocyanine green angiography [172, 173], and FAF may be used in lieu of FA and ICG due to its high sensitivity, greater ease of use, and non-invasive nature of administration.

**Punctate inner choroidopathy (PIC)**

A diagnosis of exclusion, PIC refers to the presence of multifocal choroidal lesions in the absence of uveitis or presumed ocular histoplasmosis (POHS). This disease tends to occur in young, myopic women and is associated with the development of choroidal neovascularization and subsequent vision loss [174].

Active PIC lesions present as sub-RPE chorioretinal nodules, which may display minimal hyper-autofluorescence or become hypo-autofluorescent spots as the nodules break through the RPE [175]. These hypo-autofluorescent spots often have a hyper-autofluorescent margin, corresponding to a window defect of surrounding photoreceptor loss on SD-OCT [171, 175]. Meanwhile, the majority of atrophic lesions appear as isolated hypo-autofluorescent spots. A subset of patients present with hypo-autofluorescent spots associated with diffuse hyper-autofluorescent patches, 90 % of which were identified as concurrent MEWDS on multimodal imaging [171]. Similar to the choroidal neovascularization seen in ARMD, choroidal neovascularization in PIC presents as foci of slight hypo- or hyper-autofluorescence with a surrounding hyper-autofluorescent ring [175]. Additional reports have found that FAF and ultra-widefield FAF can be useful for detecting changes in eyes with PIC that are not evident on clinical exam [176, 177].

**Drug toxicity**

**Hydroxychloroquine toxicity**

Hydroxychloroquine is an inexpensive and well-tolerated anti-inflammatory agent. However, the risk for ocular toxicity rises sharply with cumulative dose, especially with doses over 1000 g [178]. In addition, because the drug is not retained in fatty tissues, individuals who are obese or have short stature are at risk for overdosage [179]. In Caucasian populations, hydroxychloroquine toxicity causes irreversible parafoveal photoreceptor loss with foveal sparing, resulting in paracentral scotomas.

FAF shows a hyper-autofluorescent parafoveal ring...
corresponding to photoreceptor damage, which decreases in signal strength over time due to RPE atrophy (Fig. 14b) [180]. However, Asian populations tend to show a pericentral pattern of photoreceptor loss occurring well outside the parafoveal zone, with an odds ratio of 27.1 compared to the parafoveal pattern (Fig. 14d). Pericentral presentations tend to be diagnosed at higher cumulative dosages and at later stages of disease than parafoveal presentations, indicating that pericentral patterns are not as easily recognized on screening [181]. Late manifestations of the disease include bilateral bull's eye maculopathy. If the drug regimen is not halted, the area of depigmentation may spread to involve the central fovea and even the entire fundus.

Screening guidelines for hydroxychloroquine toxicity by the American Academy of Ophthalmology recommend annual examinations starting at 1 year of use and annual evaluation with diagnostic testing including SD-OCT, perimetry, and mf-ERG [179, 182]. Compared to multi-focal ERG, FAF has a sensitivity of 73.7 % and is best used as a component of multi-modal imaging in the screening process [183, 184].

**Didanosine-induced retinal toxicity**

Didanosine (DDI) is a nucleoside reverse transcriptase inhibitor (NRTI) that was previously one of the mainstays for HIV treatment. However, this drug also inhibits the synthesis of mitochondrial DNA (mtDNA), resulting in mitochondrial toxicity and in rare cases precipitating a retinopathy similar to those seen in other mitochondrial disorders [185]. In the six cases described in the literature, adult DDI toxicity presents as sharply demarcated, midperipheral, concentric chorioretinal atrophy [186–189]. Widefield FAF is essential in making the diagnosis and shows a characteristic mottled hyper-autofluorescence and hypo-autofluorescence in the midperiphery that spares the
central macula (Fig. 15) [187]. More advanced cases will show confluent midperipheral hypo-autofluorescent atrophy in each eye.

**Deferoxamine-induced retinal toxicity**

As an iron chelator, deferoxamine is used to treat iron overload, such as in patients requiring chronic transfusions. Although the mechanism is not well understood, deferoxamine may injure the retina through direct toxic effects on RPE cells or chelation-induced iron, zinc, or copper deficiencies [190–192]. Deferoxamine retinopathy has various reported funduscopic manifestations, including pigmentary changes with RPE mottling, vitelliform lesions, and bull’s eye maculopathy [193–195].

FAF is more sensitive than ophthalmoscopy for detecting deferoxamine-induced retinal alterations and can be used to screen patients for toxicity and identify patients at high-risk for vision loss [196]. In a prospective case control study of 197 patients with β-thalassemia who received deferoxamine, 18 patients developed FAF abnormalities. Viola et al. characterized four distinct FAF patterns: minimal change, focal, patchy, and speckled patterns [196]. Patients with the minimal change pattern had the lowest incidence of vision deterioration, while patients with the patchy or speckled pattern had the most severe vision loss [196].

**Conclusion**

Fundus autofluorescence provides information on the metabolic state and overall health of the RPE, and indirectly, the photoreceptor layer. Many imaging systems are available, each utilizing different optical properties that have distinct advantages and disadvantages. FAF is now an important tool for the evaluation of the prognosis and progression of geographic atrophy in AMD and for the phenotypic characterization of retinal dystrophies. FAF is also critical in the diagnosis of white dot syndromes and drug toxicities, among other entities. The applications of this simple, noninvasive imaging modality will continue to evolve and may replace more invasive procedures such as fluorescein angiography.

**Abbreviations**

FAF: fundus autofluorescence; RPE: retinal pigment epithelium; AMD: age-related macular degeneration; cSLO: confocal scanning laser ophthalmoscopy; A2E: N-Retinyl-N-retinylidene ethanolamine; HRA: Heidelberg retinal angiograph; GA: geographic atrophy; PED: pigment epithelial detachment; GPS: fine granular with peripheral punctate spots; CSCR: central serous chorioretinopathy; AEPVM: acute exudative polymorphous vitelliform maculopathy.
Authors’ contributions

MY completed the literature review, drafted the manuscript, and wrote the figure legends. MAK proofread the manuscript and figure legends, and provided important guidance and oversight during the entire drafting process. All authors read and approved the final manuscript.

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Competing interests

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