# Classification of Fundus Autofluorescence Patterns in Early Age-Related Macular Disease

*Almut Bindewald,*[1] *Alan C. Bird,*[2] *Samantha S. Dandekar,*[2] *Joanna Dolar-Szczasny,*[3] *Jens Dreyhaupt,*[4] *Frederick W. Fitzke,*[5] *Wilma Einbock,*[6] *Frank G. Holz,*[1] *Jork J. Jorzik,*[7] *Claudia Keilhauer,*[8] *Noemi Lois,*[9] *Juliane Mlynski,*[8] *Daniel Pauleikhoff,*[10] *Giovanni Staurenghi,*[11] *and Sebastian Wolf*[6]

**Purpose.** To describe and classify patterns of abnormal fundus autofluorescence (FAF) in eyes with early nonexudative age-related macular disease (AMD).

**Methods.** FAF images were recorded in eyes with early AMD by confocal scanning laser ophthalmoscopy (cSLO) with excitation at 488 nm (argon or OPSL laser) and emission above 500 or 521 nm (barrier filter). A standardized protocol for image acquisition and generation of mean images after automated alignment was applied, and routine fundus photographs were obtained. FAF images were classified by two independent observers. The $\kappa$ statistic was applied to assess intra- and interobserver variability.

**Results.** Alterations in FAF were classified into eight phenotypic patterns including normal, minimal change, focal increased, patchy, linear, lacelike, reticular, and speckled. Areas with abnormal increased or decreased FAF signals may or may not have corresponded to funduscopically visible alterations. For intraobserver variability, $\kappa$ of observer I was 0.80 (95% confidence interval [CI]0.71–0.89) and of observer II, 0.74. (95% CI, 0.64–0.84). For interobserver variability, $\kappa$ was 0.77 (95% CI, 0.67–0.87).

**Conclusions.** Various phenotypic patterns of abnormal FAF can be identified with cSLO imaging. Distinct patterns may reflect heterogeneity at a cellular and molecular level in contrast to a nonspecific aging process. The results indicate that the classification system yields a relatively high degree of intra- and interobserver agreement. It may be applicable for determination of novel prognostic determinants in longitudinal natural history studies, for identification of genetic risk factors, and for monitoring of future therapeutic interventions to slow the progression of early AMD. (*Invest Ophthalmol Vis Sci.* 2005; 46:3309–3314) DOI:10.1167/iovs.04-0430

Age-related macular disease (AMD) is now the leading cause of severe visual loss in all industrialized countries.[1–3] As populations age, the prevalence of AMD will increase and its significance will grow, unless effective interventions are developed. Present treatments to reduce or avoid loss of visual function from advanced AMD remain limited. Evidence for treatments that could slow the progression of disease at an earlier stage is just beginning to accumulate.

Severe visual loss results from choroidal neovascularization (CNV), pigment epithelial detachment, or geographic atrophy (GA) of the retinal pigment epithelium (RPE).[4] Early manifestations of AMD precede these advanced stages of the disease. The former are characterized by extracellular deposits (i.e., drusen), accumulating in the inner aspects of Bruch's membrane (BM).[5] Depending on the size and morphology, hard drusen are distinguishable from soft drusen. High-risk drusen characteristics have been identified in natural history studies.[4,6–8] Further manifestations of early AMD include focal hypo- or hyperpigmentation at the level of the RPE. Previous terminology for early manifestations includes so-called age-related maculopathy (ARM) in contrast to AMD for advanced atrophic or neovascular forms of AMD.[9] Herein, the term age-related macular disease (AMD) is used, as proposed by an author (ACB) in an earlier study,[3] and early stages are distinguished from late forms of AMD.

With the advent of confocal scanning laser ophthalmoscopy (cSLO) it is possible to visualize fundus autofluorescence (FAF) and its spatial distribution in vivo.[10–14] It represents a tool to evaluate the RPE during aging and in ocular disease.[15] As shown by spectrophotometric investigations by Delori et al.,[11] FAF is mainly derived from lipofuscin (LF) in the RPE. Excessive accumulation of LF represents a common pathogenetic pathway in various monogenetic and complex retinal diseases and is believed to precede photoreceptor degeneration.[16–18] Recent studies have described FAF changes in early and advanced atrophic AMD.[13,19] Excessive LF accumulation may precede the development of GA and the enlargement of preexisting GA.[20]

Lois et al.[21] and Delori et al.[15] have described changes in FAF in eyes of patients with drusen in the absence of atrophic or neovascular complications. Of note, alterations in FAF were not necessarily associated with corresponding funduscopically

From the [1]Department of Ophthalmology, University of Bonn, Bonn, Germany; the [2]Department of Clinical Ophthalmology, Moorfields Eye Hospital, London, United Kingdom; the [3]First Eye Hospital, Medical University of Lublin, Lublin, Poland; the [4]Institute of Medical Biometry and [7]Department of Ophthalmology, University of Heidelberg, Heidelberg, Germany; the [5]Institute of Ophthalmology, University College of London, London, United Kingdom; the [6]Department of Ophthalmology, Inselspital, Bern, Switzerland; the [8]Department of Ophthalmology, University of Würzburg, Würzburg, Germany; the [9]Department of Medical Retina and Vitreo-retinal Surgery, University of Aberdeen, Aberdeen, Scotland, United Kingdom; the [10]Department of Ophthalmology, St. Franziskus Hospital, Münster, Germany; and the [11]Department of Ophthalmology, University of Brescia, Brescia, Italy. The authors constitute The International Fundus Autofluorescence Classification Group (IFAG) and are listed in alphabetical order.

Supported by AMD Research Priority program Grants SPP 1088, Ho 1926/1-2, Ho 1976/3-1, and Wo 478/10-1 from the German Research Foundation, a Medical Research Council Grant, and The Foundation for Fighting Blindness.

Submitted for publication April 16, 2004; revised December 17, 2004, and May 10, 2005; accepted July 18, 2005.

Disclosure: **A. Bindewald**, None; **A.C. Bird**, None; **S.S. Dandekar**, None; **J. Dolar-Szczasny**, None; **J. Dreyhaupt**, None; **F.W. Fitzke**, None; **W. Einbock**, None; **F.G. Holz**, None; **J.J. Jorzik**, None; **C. Keilhauer**, None; **N. Lois**, None; **J. Mlynski**, None; **D. Pauleikhoff**, None; **G. Staurenghi**, None; **S. Wolf**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Frank G. Holz, Department of Ophthalmology, University of Bonn, Ernst-Abbe-Str. 2, 53127 Bonn, Germany; frank.holz@ukb.uni-bonn.de.

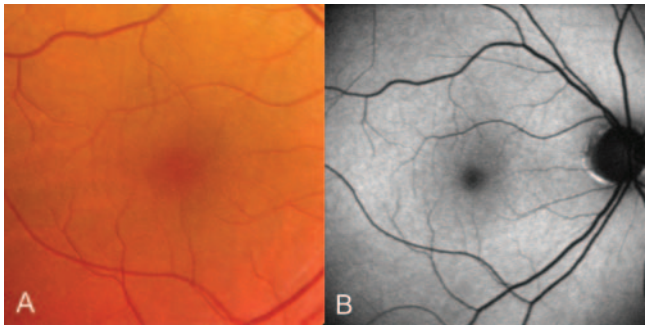


**FIGURE 1.** FAF image (**B**) with a homogeneous background fluorescence and a gradual decrease in the inner macula toward the foveola due to the masking effect of macular pigment (*normal pattern*). Only small hard drusen are visible in the corresponding fundus photograph (**A**).

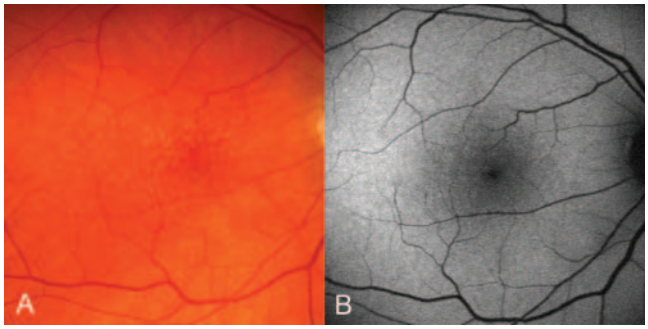


**FIGURE 2.** Fundus photograph (**A**) and FAF image (**B**) with only minimal variations from the normal background FAF (*minimal change pattern*). There is very limited irregular increase or decrease in FAF intensity due to multiple small hard drusen.

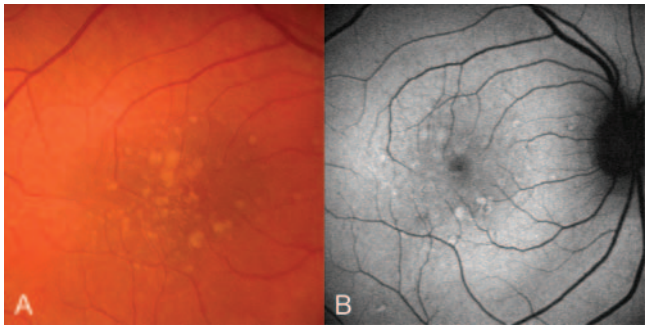


**FIGURE 3.** FAF image (**B**) showing the *focal increased pattern* with several well-defined spots with markedly increased FAF. (**A**) Fundus photograph of the same eye with multiple hard and soft drusen.

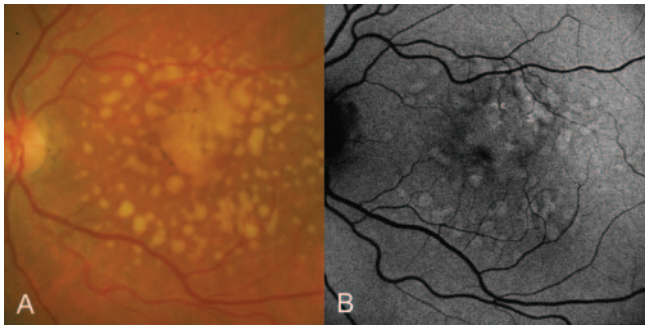


**FIGURE 4.** The FAF image (**B**) shows multiple large areas (>200 μm diameter) of increased FAF (*patchy pattern*) corresponding to large, soft drusen and/or hyperpigmentation in the fundus photograph (**A**).

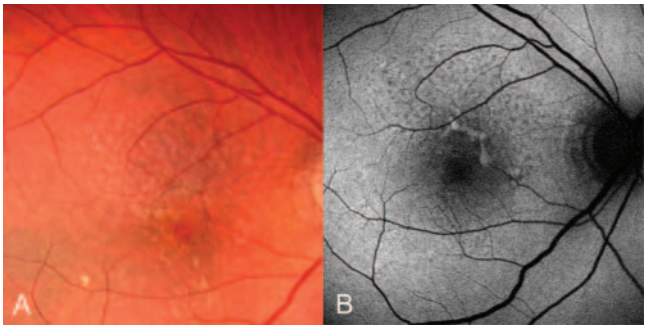


**FIGURE 5.** The *linear pattern* is characterized by the presence of at least one linear area with markedly increased FAF, visible in this FAF image (**B**). A corresponding hyperpigmented line is visible in the fundus photograph (**A**).

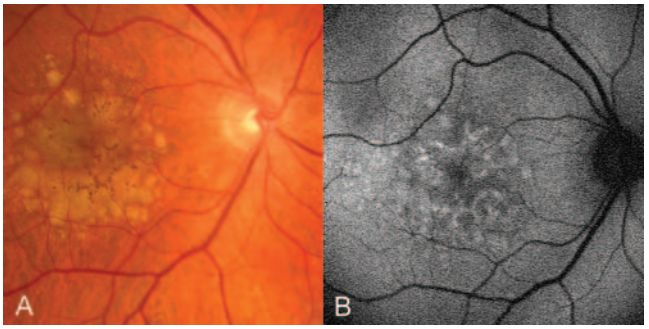


**FIGURE 6.** In this FAF image (**B**) multiple branching linear structures of increased FAF form a *lacelike pattern*. This pattern of increased FAF may correspond to hyperpigmentation on the fundus photograph (**A**) or to no visible abnormality.

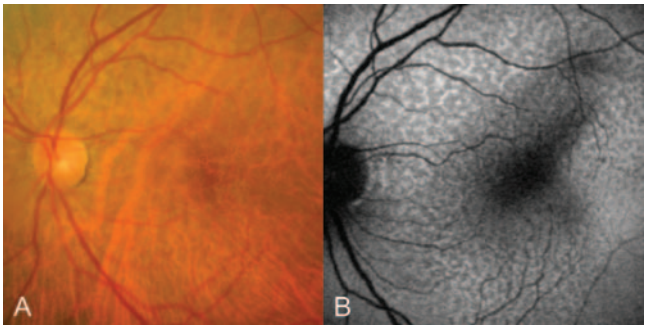


**FIGURE 7.** Multiple specific small areas of decreased FAF with brighter lines between characterize the *reticular pattern* as shown in this FAF image (**B**). The reticular pattern not only occurs in the macular area but is found more typically in a superotemporal location. There may be visible reticular drusen in the corresponding fundus photograph (**A**).

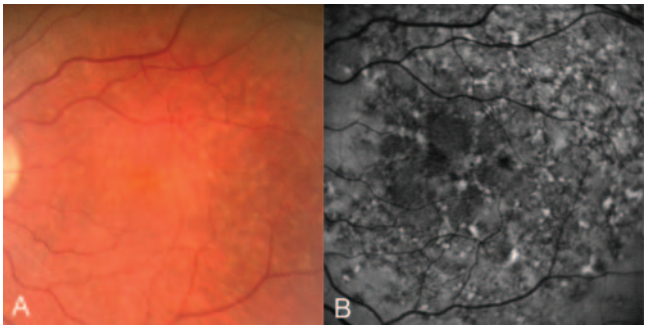


**FIGURE 8.** This FAF image (**B**) shows a variety of FAF abnormalities in a larger area of the FAF image that characterize the *speckled pattern*. There seem to be fewer pathologic areas in the corresponding fundus photograph (**A**).

visible drusen or irregular pigmentations, indicating that FAF imaging gives information over and above the normal fundus photography used in fluorescein angiography and allows the characterization of age-related changes in the RPE.

To describe various abnormal FAF patterns in early AMD and to develop an FAF classification system, a workshop was organized by the FAM-Study group (Fundus Autofluorescence in Age-Related Macular Degeneration) in Frankfurt, Germany, on July 30, 2003, that was funded by the German Research Council (Deutsche Forschungsgemeinschaft; DFG). All participating members from five tertiary ophthalmic centers recorded FAF images in patients with early AMD as part of ongoing natural history studies.

An international FAF classification system may be useful for other groups performing FAF imaging in the context of AMD research and may help in the performance of meta-analyses of similar studies in the future. There is also a potential for identifying prognostic determinants based on FAF imaging and for identifying genetic factors.[22]

## Methods

FAF images were recorded with confocal scanning laser ophthalmoscopes (Heidelberg Retina Angiograph, HRA classic and HRA 2, Heidelberg Engineering, Dossenheim, Germany; or the prototype SM 30-4024; Carl Zeiss Meditec, Inc., Jena, Germany), the optical and technical principles of which have been described previously.[10,12,20] A small pinhole aperture suppresses light originating from outside the focal plane to enhance image contrast compared with nonconfocal images. For excitation at 488 nm an argon blue or optically pumped solid-state laser is used, and emission is recorded above 500 nm (HRA) or 521 nm (Carl Zeiss Meditec, Inc.) with a barrier filter. For acquisition of FAF images, standard operation procedures were developed including focusing of the retinal image in reflection and red-free mode, sensitivity adjustment, and acquisition of 30° × 30° FAF images. The images encompassed the entire macular area. For the HRA, the best nine single images were aligned, and a mean image was generated to amplify the FAF signal using image analysis software (Heidelberg Eye Explorer [HEE]; Heidelberg Engineering). With the cSLO a series of images of each eye was recorded at standard video scanning rates on VHS videotape. Later, images were digitized at a 768 × 576 resolution with a frame grabber (Millenium; Matrox Imaging Products Group, Dorval, Quebec, Canada).

Eyes from patients older than 55 years with drusen and/or focal hypo- or hyperpigmentation appearing in color fundus photographs in the absence of other retinal disease and with media clear enough to allow FAF imaging were included. The study adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from each subject after an explanation of the nature and possible consequences of the study. The study was approved by the institutional human experimentation committee or institutional review board of each participating center. Abnormal FAF was defined as either an increased or decreased FAF signal compared with the normal background FAF outside such areas. FAF images of normal eyes show a typical decrease in FAF intensity in the macular area due to absorption by macular pigment in the neurosensory retina anterior to the LF-containing RPE cell monolayer.[19,23] There is also a lower signal along large retinal vessels (absorption) and at the optic disc (absence of autofluorescent material).

In the FAM Study FAF images are recorded in various manifestations of AMD including early stages and advanced atrophic and neovascular forms of the disease. Patients are examined according to standardized operation procedures for visual acuity testing, fundus photography, and FAF imaging.[13] Blood samples are taken after written informed consent has been received for further molecular genetic analyses.

For the classification of fundus images, we used the definitions recently published by the International ARM Epidemiologic Study Group.[9] The macula was defined as the part of the retina centered on the foveola with a diameter of approximately 5500 μm. The inner macula was defined as the area within a circle of 3000 μm and the outer macula as the area within a circle of 6000 μm centered on the foveola.

To assess interobserver variability for the novel FAF classification, two independent observers (observers I and II) classified 100 eyes of 100 patients with funduscopically visible drusen and/or focal hypo- or hyperpigmentation with regard to the predominant FAF pattern at the posterior pole. Analyses were repeated after 1 month's time to determine intraobserver variability. For both intra- and interobserver variability, $\kappa$ statistics were calculated.[24]

## Results

In eyes with early AMD, topographic alterations in FAF were classified into eight different patterns. Besides normal FAF, these were termed minimal change, focal increased, patchy, linear, lacelike, reticular or speckled patterns. The characteristics of each pattern are given in the following sections.

### Normal Pattern

A normal FAF pattern is characterized by a homogeneous background autofluorescence with a gradual decrease in the inner macula toward the foveola due to the masking effect of yellow macular pigment (Fig. 1). The absence of abnormal alterations may be seen on FAF images, even in the presence of soft or hard drusen.

### Minimal Change Pattern

Eyes with only minimal variations from the normal pattern appearance showed very limited irregular increase or decrease of background FAF without an obvious topographic pattern (Fig. 2).

### Focal Increased Pattern

This pattern is defined by the presence of at least one spot (<200 μm diameter) of markedly increased FAF much brighter than the surrounding background fluorescence. The borders are well defined, with no gradual decrease of FAF observed between the background and the area with focal increased FAF. Some areas of focal increased FAF may be surrounded by a darker-appearing halo. On color fundus photographs, these areas may or may not correspond to visible alterations, such as focal hyperpigmentation or drusen (Fig. 3).

### Patchy Pattern

This pattern is characterized by the presence of at least one larger area (>200 μm diameter) of markedly increased FAF. These areas are brighter than the surrounding background fluorescence. The borders of the areas are typically less well defined than the previous pattern, and there is a gradual increase in FAF from the background to the patchy area. Again, these areas of increased FAF may or may not correspond to large, soft drusen and areas of hyperpigmentation (Fig. 4).

### Linear Pattern

The linear pattern is defined by the presence of at least one linear area of markedly increased FAF. The borders of these areas are typically well demarcated with no gradual decrease in FAF observed between the background and the linear structure. Linear structures of increased FAF usually correspond to hyperpigmented lines on the color fundus photograph (Fig. 5).

### Lacelike Pattern

This type shows multiple-branching linear structures of increased FAF that form a lacelike pattern (Fig. 6). The borders

**TABLE 1.** Interobserver Variability between Two Independent Observers, Using the Novel Fundus Autofluorescence Classification

| | Normal | Minimal Change | Focal Increase | Patchy | Linear | Lacelike | Reticular | Speckled |
|---|---|---|---|---|---|---|---|---|
| Normal | — | — | — | — | — | — | — | — |
| Minimal change | 2 | **9** | — | — | — | — | — | — |
| Focal increased | — | — | **4** | 1 | — | — | 1 | 1 |
| Patchy | — | — | — | **23** | — | 1 | — | — |
| Linear | — | — | — | — | **3** | 1 | — | 1 |
| Lacelike | — | — | — | 1 | — | **2** | — | — |
| Reticular | — | — | — | — | — | — | **15** | 1 |
| Speckled | — | 2 | 1 | 2 | 1 | — | 2 | **26** |

$\kappa = 0.77$, 95% CI, 0.67; 0.87.

may be difficult to define, as a gradual decrease of FAF is occasionally observed from the center of the linear areas toward the surrounding background. A lacelike pattern of increased FAF may correspond to hyperpigmentation on the color image or to no visible abnormality.

### Reticular Pattern

The reticular pattern is defined by the presence of multiple small areas (<200 $\mu$m diameter) of decreased FAF. The borders of these areas are typically difficult to determine because there is a decrease in FAF from the center of the lesions toward the surrounding background fluorescence. The reticular pattern was found to occur not only in the macular area but also more typically in a superotemporal location. This pattern of decreased FAF may be associated with funduscopically visible numerous small soft drusen, hard drusen, or areas with pigmentary changes or no visible abnormality in the fundus photograph (Fig. 7). The funduscopic appearance, especially in the superotemporal aspect, resembles previous descriptions of reticular pseudodrusen.[25]

### Speckled Pattern

The speckled FAF pattern is characterized by the simultaneous presence of a variety of FAF abnormalities in a larger area of the FAF image. The changes extend beyond the macular area and may cover the entire posterior fundus. Typically, these abnormalities include multiple small areas of irregularly increased and decreased FAF. The small areas of focal increased FAF may be punctuate or resemble linear structures. The corresponding abnormalities visible on color fundus photographs include hyper- and hypopigmentation and multiple subconfluent and confluent drusen (Fig. 8).

Classification of 100 FAF images by two independent observers resulted in an agreement in 82% of the cases. The most frequent pattern found was the speckled pattern (26%) followed by the patchy pattern (23%). In 15%, a reticular FAF pattern was predominant, whereas other patterns were found in the following frequency: minimal change pattern, 9%; focal increased pattern, 4%; linear pattern, 3%; and lacelike pattern, 2%. Absolute frequency data are given in Table 1. In 18% of the cases, there was a disagreement in the evaluation between observers I and II, and in 13 of these 18 images at least one of the observers had a disagreement between the first and the second evaluation (intraobserver variability). The interobserver variability resulted in $\kappa = 0.77$ (95% confidence interval [CI], 0.67–0.87).

The intraobserver variability of observer I was $\kappa = 0.8$ (95% CI, 0.71–0.89). In 84% of the images, the same pattern was classified at both evaluation time points: normal pattern, 1%; minimal change pattern, 8%; focal increased pattern, 4%; patchy pattern, 25%; linear pattern, 4%; lace-like pattern, 3%; reticular pattern, 16%; speckled pattern, 23%. No conformity was found in 16%. Observer II classified the identical FAF pattern at both evaluation time points in 79% of the images: minimal change pattern, 7%; focal increased pattern, 7%; patchy pattern, 20%; linear pattern, 4%; lacelike pattern, 3%; reticular pattern, 16%; speckled pattern, 22% ($\kappa = 0.74$; 95% CI, 0.64–0.84).

## DISCUSSION

Using cSLO FAF imaging, we have identified and classified various patterns of abnormal FAF in eyes with early manifestations of AMD. FAF imaging gives information over and above conventional fundus photography and fluorescence angiography and represents a valuable tool in the evaluation of the RPE during ageing and ocular disease. In general, refined phenotyping with novel diagnostic tools is not only important for identifying prognostic determinants, but, with the emerging field of phenomics, it also is a prerequisite for determining specific genetic factors, particularly in complex, multifactorial diseases such as AMD.[26,27] We propose that the classification system presented herein can be used in other studies of AMD that include FAF imaging. Pooling of material from this and other studies could facilitate molecular genetic analyses that require a high number of patients to identify one or several gene mutations conferring risk for the development of certain AMD manifestations. In addition, better defined phenotypes may allow the identification of specific high-risk characteristics that may be helpful to design and monitor future interventional trials for patients at particular high risk of disease progression and severe visual loss.

In contrast to previous classification schemes using color fundus photographs or angiography,[28] our system is based on FAF imaging, a new, noninvasive method of examining patients with macular disease that was initially introduced by von Rückmann et al.[10] Using spectrophotometric investigations Delori et al.[29] showed that LF granules in the RPE cell monolayer contain the dominant fluorophores responsible for FAF imaging. Excessive LF accumulation represents a common pathogenetic pathway in various monogenetic and complex retinal disorders. LF granules also accumulate with age and may occupy 20% to 33% of the free cytoplasmic space of the RPE cell at ages >70 years.[30] Recent experimental studies have addressed possible molecular mechanisms, to explain how excessive LF may interact with normal cellular functions of RPE cells. A2-E has been identified as a main autofluorescent compound of LF.[31] Toxic properties of A2-E include phototoxic and detergent effects and inhibitory effects on the lysosomal proton pump, with a subsequent increase in lysosomal pH, inhibition of lysosomal enzymes, and impaired degradation of phagocytosed material.[32–35] Identification of other molecular species is now in progress, wherein the LF proteome and posttranslational modifications of LF proteins have been deter-

mined.[36,37] Recently developed animal models that share phenotypic characteristics of AMD, including excessive LF and A2-E accumulation in the RPE, add to the understanding of underlying molecular mechanisms.[38,39] Clinical evidence of adverse effects of LF comes from FAF investigations and the combination with psychophysical studies. Impaired photoreceptor function may occur in association with increased FAF.[40,41] Furthermore, increased FAF in the junctional zone of GA precedes the development of new areas of GA or the enlargement of preexisting atrophic patches.[13]

Alternative fluorophores to RPE LF are present in various anatomic layers of the posterior pole. BM has been shown to possess autofluorescent properties.[11,29,42] However, the excitation and emission spectra are different, although with slight overlap, from those of LF in RPE cells. The same is the case for choroidal components and the sclera. Furthermore, sub–pigment-epithelial fluid in the presence of pigment epithelial detachments or longstanding subretinal hemorrhages may contain fluorophores that induce an increased FAF signal in the wavelength ranges relevant to the method applied herein (Staudt S, *IOVS* 2000:41:ARVO Abstract 873). Because the latter two changes were not present in the eyes examined in our study and based on the spectrophotometric analyses by Delori et al.,[11] it is assumed that the dominant fluorophore responsible for the elevated autofluorescence signals indeed originates from RPE LF. However, this method cannot distinguish between melanolipofuscin or RPE cells that have migrated into the neurosensory retina and LF at the normal RPE cell layer.

Various limitations have to be considered for FAF imaging. Media opacities and, above all, lens opacification may result in FAF images that cannot be analyzed adequately. Yellowish discoloration of the lens in cataract formation is associated with the absorption of the wavelength range used in our study for excitation (i.e., 488 nm). As shown in recent FAF evaluations of eyes with GA, the rate of eyes that could not be imaged or classified adequately was determined to be 90.9%.[43] Furthermore, absolute quantification of the FAF signal is difficult. However, this may not be relevant when looking at variations in the topographic distribution of FAF changes, as in our study, for classifying patterns in association with early AMD. In addition, abnormal FAF findings are readily noted in comparison to normal background fluorescence.[19]

The FAF changes do not necessarily correlate topographically with visible fundus changes in patients with early AMD. Areas of increased FAF may or may not correspond with areas of hyperpigmentation or soft or hard drusen. The autofluorescence signal may be normal, decreased, or increased in corresponding drusen areas. This may reflect the variable composition of drusen compounds including other fluorophores, as well as different reactive alterations in the overlying RPE cell monolayer. Overall, larger drusen were associated more frequently with more pronounced FAF abnormalities than smaller ones. Areas covered with so-called reticular drusen, or reticular pseudodrusen as termed by others,[24,44,45] usually show a unique reticular FAF pattern with multiple small, uniform areas of decreased FAF surrounded by normal FAF.

Areas of hypopigmentation on fundus photographs tend to be associated with a corresponding decreased FAF signal, suggesting the absence of RPE cells or degenerated RPE cells with a reduced content of LF granules. In contrast, areas of hyperpigmentation usually exhibit a higher FAF signal, which may be due to a higher content of autofluorescent melanolipofuscin.[19]

FAF changes remote from funduscopically visible alterations may indicate more widespread abnormalities and diseased areas. It may be speculated that changes seen in FAF imaging on the RPE cell level precede the occurrence of visible lesions as the disease progresses.

Based on these cross-sectional observations, we have initiated longitudinal studies to address the hypotheses of possible evolution from one abnormal FAF pattern to another and of gradual peripheral spread during the disease process. Furthermore, the prognostic relevance of individual FAF phenotypes with regard to the incidence of advanced atrophic or exudative manifestations of AMD will be addressed. These studies may also improve our understanding of the pathophysiology and the mechanisms of disease progression.

In summary, we introduce a description and classification system of the various FAF patterns in eyes with early AMD. We encourage other investigators performing research in AMD to include FAF imaging and to use the proposed system to promote standardization of the nomenclature. Such standardization would to facilitate better comparison between studies of AMD and allow for meta-analyses in the future.

## *References*


1. Hyman LG, Lilienfeld AM, Ferris FL, Fine SL. Senile macular degeneration: a case control study. *Am J Epidemiol*. 1983;118: 213–227.
2. Bressler NM, Bressler SB. Preventative ophthalmology: age-related macular degeneration. *Ophthalmology*. 1995;102:1206–1211.
3. Bird AC. Age-related macular disease. *Br J Ophthalmol*. 1996;80: 1–2.
4. Klein R, Klein BE, Jensen SC, Meuer SM. The five-year incidence and progression of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology*. 1997;104:7–21.
5. van der Schaft TL, Mooy CM, de Bruijn WC, Oron FG, Mulder PG, de Jong PT. Histologic features of the early stages of age-related macular degeneration: a statistical analysis. *Ophthalmology*. 1992; 99:278–286.
6. Mitchell RA. Prevalence of age related macular degeneration in persons aged 50 years and over resident in Australia. *J Epidemiol Community Health*. 1993;47:42–45.
7. Holz FG, Wolfensberger TJ, Piguet B, et al. Bilateral macular drusen in age-related macular degeneration: prognosis and risk factors. *Ophthalmology*. 1994;101:1522–1528.
8. Klaver CCW, van Leeuwen R, Vingerling JR, de Jong PTVM. Epidemiology of age-related maculopathy: a review. In: Holz FG, Pauleikhoff D, Spaide RF, Bird AC. *Age-Related Macular Degeneration.* Berlin, Heidelberg: Springer, 2004;1–22.
9. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Surv Ophthalmol*. 1995;39:367–374.
10. von Rückmann A, Fitzke FW, Bird AC. Distribution of fundus autofluorescence with a scanning laser ophthalmoscope. *Br J Ophthalmol*. 1995;79:407–412.
11. Delori FC, Dorey CK, Staurenghi G, Arend O, Goger DG, Weiter JJ. In vivo fluorescence of the ocular fundus exhibits retinal pigment epithelium lipofuscin characteristics. *Invest Ophthalmol Vis Sci*. 1995;36:718–729.
12. Bellmann C, Holz FG, Schapp O, Volcker HE, Otto TP. Topography of fundus autofluorescence with a new confocal scanning laser ophthalmoscope [in German] *Ophthalmologe*. 1997;94:385–391.
13. Holz FG, Bellmann C, Margaritidis M, Schutt F, Otto TP, Volcker HE. Patterns of increased in vivo fundus autofluorescence in the junctional zone of geographic atrophy of the retinal pigment epithelium associated with age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol*. 1999;237:145–152.
14. Bindewald A, Jorzik JJ, Loesch A, Schutt F, Holz FG. Visualization of retinal pigment epithelial cells in vivo using digital high-resolution confocal scanning laser ophthalmoscopy. *Am J Ophthalmol*. 2004;137:556–558.
15. Delori FC, Fleckner MR, Goger DG, Weiter JJ, Dorey CK. Autofluorescence distribution associated with drusen in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2000;41:496–504.
16. Wing GL, Blanchard GC, Weiter JJ. The topography and age relationship of lipofuscin concentration in the retinal pigment epithelium. *Invest Ophthalmol Vis Sci*. 1978;17:601–607.

17. Weiter JJ, Delori FC, Wing GL, Fitch KA. Retinal pigment epithelial lipofuscin and melanin and choroidal melanin in human eyes. *Invest Ophthalmol Vis Sci*. 1986;27:145–152.
18. Dorey CK, Wu G, Ebenstein D, Garsd A, Weiter JJ. Cell loss in the aging retina: relationship to lipofuscin accumulation and macular degeneration. *Invest Ophthalmol Vis Sci*. 1989;30:1691–1699.
19. von Rückmann AV, Fitzke FW, Bird AC. Fundus autofluorescence in age-related macular disease imaged with a laser scanning ophthalmoscope. *Invest Ophthalmol Vis Sci*. 1997;38:478–486.
20. Holz FG, Bellman C, Staudt S, Schutt F, Volcker HE. Fundus autofluorescence and development of geographic atrophy in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2001; 42:1051–1056.
21. Lois N, Owens SL, Coco R, Hopkins J, Fitzke FW, Bird AC. Fundus autofluorescence in patients with age-related macular degeneration and high risk of visual loss. *Am J Ophthalmol*. 2002;133:341–349.
22. Stöhr H, Weber BHF. Genetics of AMD. In: Holz FG, Pauleikhoff D, Spaide RF, Bird AC. *Age-Related Macular Degeneration.* Berlin: Springer; 2004:23–30.
23. Snodderly DM, Brown PK, Delori FC, Auran JD. The macular pigment. I. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. *Invest Ophthalmol Vis Sci*. 1984;25:660–673.
24. Altman D. *Practical Statistics for Medical Research.* Chapman and Hall, 1991:304.
25. Arnold JJ, Sarks SH, Killingsworth MC, Sarks JP. Reticular pseudodrusen: a risk factor in age-related maculopathy. *Retina*. 1995;15:183–191.
26. Gerlai R. Phenomics: fiction or the future? *Trends Neurosci*. 2002; 25:506–509.
27. Elkin PL. Primer on medical genomics part V: bioinformatics. *Mayo Clin Proc*. 2003;78:57–64.
28. Arnold JJ, Quaranta M, Soubrane G, Sarks SH, Coscas G. Indocyanine green angiography of drusen. *Am J Ophthalmol*. 1997;124: 344–356.
29. Delori FC, Goger DG, Dorey CK. Age-related accumulation and spatial distribution of lipofuscin in RPE of normal subjects. *Invest Ophthalmol Vis Sci*. 2001;42:1855–1866.
30. Feeney-Burns L, Hilderbrand ES, Eldridge S. Aging human RPE: morphometric analysis of macular, equatorial, and peripheral cells. *Invest Ophthalmol Vis Sci*. 1984;25:195–200.
31. Eldred GE, Lasky MR. Retinal age-pigments generated by self-assembling lysosomotropic detergents. *Nature*. 1993;361:724–726.
32. Holz FG, Schutt F, Kopitz J, et al. Inhibition of lysosomal degradative functions in RPE cells by a retinoid component of lipofuscin. *Invest Ophthalmol Vis Sci*. 1999;40:737–743.
33. Schutt F, Davies S, Kopitz J, Holz FG, Boulton ME. Photodamage to human RPE cells by A2-E, a retinoid component of lipofuscin. *Invest Ophthalmol Vis Sci*. 2000;41:2303–2308.
34. Sparrow JR, Nakanishi K, Parish CA. The lipofuscin fluorophore A2E mediates blue light-induced damage to retinal pigmented epithelial cells. *Invest Ophthalmol Vis Sci*. 2000;41:1981–1989.
35. Bergmann M, Schütt F, Holz FG, Kopitz J. Inhibition of the ATP-driven proton pump in RPE lysosomes by the major lipofuscin fluorophore A2-E may contribute to the pathogenesis of age-related macular degeneration. *FASEB J*. 2004;18:562–564.
36. Schutt F, Ueberle B, Schnolzer M, Holz FG, Kopitz J. Proteome analysis of lipofuscin in human retinal pigment epithelial cells. *FEBS Lett*. 2002;528:217–221.
37. Schutt F, Bergmann M, Holz FG, Kopitz J. Proteins modified by malondialdehyde, 4-hydroxynonenal, or advanced glycation end products in lipofuscin of human retinal pigment epithelium. *Invest Ophthalmol Vis Sci*. 2003;44:3663–3668.
38. Mata NL, Tzekov RT, Liu X, Wenig J, Birch DG, Travis GH. Delayed dark-adaptation and lipofuscin accumulation in $abcr^{+/-}$ mice: implications for involvement of *ABCR* in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2001;42:1685–1690.
39. Ambati J, Anand A, Fernandez S, et al. An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. *Nat Med*. 2003;9:1390–1397.
40. Scholl HP, Bellmann C, Dandekar SS, Bird AC, Fitzke FW. Photopic and scotopic fine matrix mapping of retinal areas of increased fundus autofluorescence in patients with age-related maculopathy. *Invest Ophthalmol Vis Sci*. 2004;45:574–583.
41. Schmitz-Valckenberg S, Bültmann S, Dreyhaupt J, Bindewald A, Holz FG, Rohrschneider K. Fundus autofluorescence and fundus perimetry in the junctional zone of geographic atrophy in patients with age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2004;45:4470–4476.
42. Arend O, Weiter JJ, Goger DG, Delori FC. In vivo fundus fluorescence measurements in patients with age related macular degeneration [in German]. *Ophthalmologe*. 1995;92:647–653.
43. Bindewald A, Schmitz-Valckenberg S, Jorzik JJ, et al. Classification of abnormal fundus autofluorescence patterns in the junctional zone of geographic atrophy in patients with AMD. *Br J Ophthalmol*. 2005;89:874–878.
44. Klein R, Davis MD, Magli YL, Segal P, Klein BE, Hubbard L. The Wisconsin age-related maculopathy grading system. *Ophthalmology*. 1991;98:1128–1134.
45. Maguire MG, Fine SL. Reticular pseudodrusen. *Retina*. 1996;16: 167–168.