Graefes Arch Clin Exp Ophthalmol (2008) 246:105–114
DOI 10.1007/s00417-007-0639-9

RETINAL DISORDERS

Ocular fundus auto-fluorescence observations at different wavelengths in patients with age-related macular degeneration and diabetic retinopathy

Martin Hammer · Ekkehart Königsdörffer · Christiane Liebermann · Carsten Framme · Günter Schuch · Dietrich Schweitzer · Jürgen Strobel

Received: 30 January 2007 / Revised: 27 April 2007 / Accepted: 7 May 2007 / Published online: 26 July 2007

Abstract
Background Post-translational protein modification by lipid peroxidation products or glycation is a feature of aging as well as pathologic processes in postmitotic cells at the ocular fundus exposed to an oxidative environment. The accumulation of modified proteins such as those found in lipofuscin and advanced glycation end products (AGEs) contribute greatly to the fundus auto-fluorescence. The distinct fluorescence spectra of lipofuscin and AGE enable their differentiation in multispectral fundus fluorescence imaging.
Method A dual-centre consecutive case series of 78 pseudophacic patients is reported. Digital colour fundus photographs as well as auto-fluorescence images were taken from 33 patients with age related macular degeneration (AMD), 13 patients with diabetic retinopathy (RD), or from 32 cases without pathologic findings (controls). Fluorescence was excited at 475–515 nm or 476–604 nm and recorded in the emission bands 530–675 nm or 675–715 nm, respectively. Fluorescence images excited at 475–515 nm were taken by a colour CCD-camera (colour-fluorescence imaging) enabling the separate recording of green and red fluorescence. The ratio of green versus red fluorescence was calculated within a representative region of each image.
Results The 530–675 nm auto-fluorescence in AMD patients was dominated by the red emission (green vs. red ratio, g/r=0.861). In comparison, the fluorescence of the diabetics was green-shifted (g/r=0.946; controls: g/r=0.869). Atrophic areas (geographic atrophy, laser scars) showed massive hypo-fluorescence in both emission bands. Hyper-fluorescent drusen and exudates, unobtrusive in the colour fundus images as well as in the fluorescence images with emission >667 nm, showed an impressive green-shift in the colour-fluorescence image.
Conclusions Lipofuscin is the dominant fluorophore at long wavelengths (>675 nm or red channel of the colour fluorescence image). In the green spectral region, we found an additional emission of collagen and elastin (optic disc, sclera) as well as deposits in drusen and exudates. The green shift of the auto-fluorescence in RD may be a hint of increased AGE concentrations.

Keywords Fundus auto-fluorescence · Age-related macular degeneration · Diabetic retinopathy · Colour-autofluorescence imaging

None of the authors has any financial relationship. All primary data are under full control of the authors and may be reviewed upon request.

M. Hammer (✉) · E. Königsdörffer · C. Liebermann · D. Schweitzer · J. Strobel
Department of Ophthalmoloy, University of Jena,
Bachstr. 18,
07740 Jena, Germany
e-mail: martin.hammer@med.uni-jena.de

C. Framme · G. Schuch
Department of Ophthalmoloy, University of Regensburg,
Franz-Josef Strauss-Allee 11,
93053 Regensburg, Germany

Introduction

Ocular fundus autofluorescence imaging has gained a growing interest in recent years [3, 5, 6, 10, 17, 18, 39, 40, 47–52]. Intrinsic tissue fluorescence was found to arise from posttranslationally modified proteins, lipoproteins, and nondegradable metabolic waste products as well as from cofactors of the cellular energy metabolism such as NADH and FAD. Thus, alterations of the tissue fluorescence may be an early indicator for ageing and disease on a

molecular level. This especially holds for the postmitotic cells of the retina and the retinal pigment epithelium (RPE) [22, 41]. Therefore, fluorescence imaging may be helpful in the diagnostics of diseases such as age-related macular degeneration (AMD) as well as diabetic retinopathy. More precise diagnostics in combination with new and available medication may lead to a better treatment of patients with macular diseases.

Several studies using fluorescence imaging by the Heidelberg retina angiograph (HRA), have focussed on the observation of fundus autofluorescence (AF) in the junctional zone of geographic atrophy (GA) in AMD patients. Correlations have been found between areas of increased AF and the progress of GA [28] as well as the loss of retinal sensitivity [29, 30]. These observations support the hypothesis that lipofuscin, which accumulates in the lysosomal compartment of the RPE cells and is known to be fluorescent [6, 11], interferes with the visual function and, finally, triggers apoptosis [14, 41–43, 46]. However, different fluorophores with different functions in the cells or adverse effects to the cells may contribute to the fluorescence. These may be oxidized poly-unsaturated fatty acids from phagozytosed rod outer segments [22], adducts of their byproducts to proteins [31], glycosylated proteins [45], Schiff base products of all-trans-retinal and ethanolamine as well as their epoxids [26, 43] or furanoids [9], and FAD. Although the age pigment lipofuscin (which itself is a mixture of different compounds) is considered to be the strongest fluorophore at the ocular fundus, all the other substances mentioned above can not be ruled out and will contribute to the fluorescence. In 1995, Arend et al. [1] observed a fluorescence of drusen at shorter wavelengths than that of lipofuscin. In atrophic lesions, they found an emission maximum at 520 nm due to collagen fluorescence. Solbach et al. [39] and Staudt et al. [44] discussed a fluorescence of dursen and other intra- and extracellular compartments, also not originating from lipofuscin. Furthermore, fluorescence [13, 14] is shown in advanced glycation end products (AGEs), which are formed by the nonenzymatic Maillard-reaction from proteins and glucose, particularly under diabetic conditions [45], and was also found in AMD [20, 31], particularly when associated with drusen [53], and may be regarded as a general feature of aging [25].

Thus, native fluorescence of the ocular fundus may be related to various pathologic processes which may be differentiated if we succeed in the discrimination of the fluorophores. Fluorophores may differ in their excitation and emission spectra as well as in their lifetimes. Fluorescence lifetime imaging of the fundus, though technically challenging, is under development [32, 35], and initial clinical investigations have been published [33, 34, 36]. Fluorescence spectra were measured in vivo by Delori et al. [5, 6]. However, their instrumentation does not provide an autoflourescence image. This was the incentive to develop a fundus fluorescence imaging technique with video resolution providing two spectral channels [13] referred to as colour AF. In contrast to other imaging studies describing the lateral pattern of AF [2, 3, 10, 18, 23], this technique may contribute spectral information and, thus, give hints to the chemistry of the fluorescence. Here, we present the initial results of colour AF, HRA AF imaging, as well as far red AF [40] obtained from consecutive case series of patients recruited at the Departments of Ophthalmology at the Universities of Regensburg and Jena, suffering from AMD or diabetic retinopathy, respectively. Persons without ocular fundus pathologies served as a control group.

Materials and methods

In two study centres a total of 84 pseudophacic patients were examined. Thirty-nine patients (mean age 78.9±6.4 years) were suffering from AMD. Inclusion criteria were an age of 60 years or older and AMD diagnosed according to the international classification and grading system [4]. Thirteen patients (mean age 68.6±6.1 years) were diagnosed with diabetic retinopathy according to ETDRS report number 10 [12]. The 32 persons in the control group (mean age 61.0± 17.8 years) did not show any pathologies at the fundus. All patients gave informed consent to the study which followed the tenets of the Declaration of Helsinki and the guidelines of a local ethics committee.

Fundus AF images were acquired using three different techniques described in detail elsewhere [13, 16, 40]. Briefly, HRA AF imaging (Heidelberg Engineering, Dossenheim, Germany) used the 488 nm argon laser line for fluorescence excitation. Fluorescence emission was observed at wavelengths greater than 500 nm. For AF imaging with the Funduscamera FF 450 (Carl Zeiss Meditec, Jena, Germany) different filter combinations were used (for review of excitation and emission wavelengths of the different imaging techniques see Table 1). First, the fluorescence was excited with a broadband filter 476–604 nm and recorded the emission in the far red spectral region (675–715 nm) according to Spaide et al. [40] (far red AF). A digital 12 bit CCD-camera F-view (Soft Imaging Systems, Münster, Germany) was used for image acquisition. Second, the filters used were those for fluorescein angiography, with which the FF 450 fundus camera was equipped (excitation: 475–515 nm, emission >530 nm). However, the AF images were recorded with a colour 3-CCD camera Hitachi HV-C20A (Hitachi, Tokyo, Japan) or Panasonic 555E (Panasonic, Secaucus, NJ, USA) [13]. Thus, we obtained colour AF images with a green (530–570 nm) and a red (570–675 nm) channel. Although this is

Table 1 Excitation and emission wavelengths of HRA AF, far red AF, and colour AF

Wavelength/method	Excitation	Emission
HRA AF	488 nm	>500 nm
Far red AF	476–604 nm	675–715 nm
Colour AF	475–515 nm	530–570 nm
		570–675 nm

a very raw spectral separation, it allows detecting spectral differences within an image. These were highlighted by the pixel by pixel calculation of the ratio of the intensity recorded in both channels. Averaging of that ratio over a representative area of the image provided a general estimation parameter of the fluorescence spectra. That average over a 70×70 pixel area temporal to the macula and apart from pathologic lesions and retinal vessels was calculated from each colour AF image. Statistical analysis included the calculation of the Pearson correlation coefficient as a measure of the linear association between the patient's age and the green vs. red fluorescence intensity ratio as well as students t-test of the mean ratios of the three patient groups. The SPSS software (version 13.0.1, SPSS Inc., Chicago, IL, USA) was used for statistics.

Additionally, digital colour fundus photographs were recorded. Fluorescein angiography was performed if indicated. The in vivo investigations were parallelled by fluorescence microscopy at histologic sections of choroid and RPE from a donor eye of an AMD patient. These cryosections were a gift from Dr. Gaillard from Northern Illinois University at DeKalb. Fluorescence micrographs were obtained using FITC filters (excitation: 450–490 nm, emission: >515 nm) with an Axioplan microscope (Carl Zeiss, Jena, Germany).

Results

Figure 1 shows images of a healthy 72-year-old subject from the control group. The colour AF image in Fig. 1b demonstrates a homogeneous orange fluorescence all over the fundus with the exception of the optic disc and the retinal vessels. As in all colour AF images, the fluorescence of the optic disc is greenish, whereas the vessels appear in dark red. The green versus red ratio image in Fig. 1c, representing shorter wavelength fluorescence as higher gray scale values and longer wavelengths as lower gray scale values irrespective of the absolute fluorescence intensity, demonstrates the homogeneity of the fluorescence. The decrease of the fluorescence in the macula (Fig. 1b,d) is not seen in the ratio image since it results from the absorption of the excitation light by the macular pigment. Diminished excitation, however, decreases the fluorescence emission intensity but leaves the emission spectrum unchanged. In the far red fluorescence image in Fig. 1d, the optic disc appeared dark. The green fluorophore does not emit at 675–715 nm.

The ratios of the fluorescence intensities in the green and red channels, calculated from the colour AF images recorded

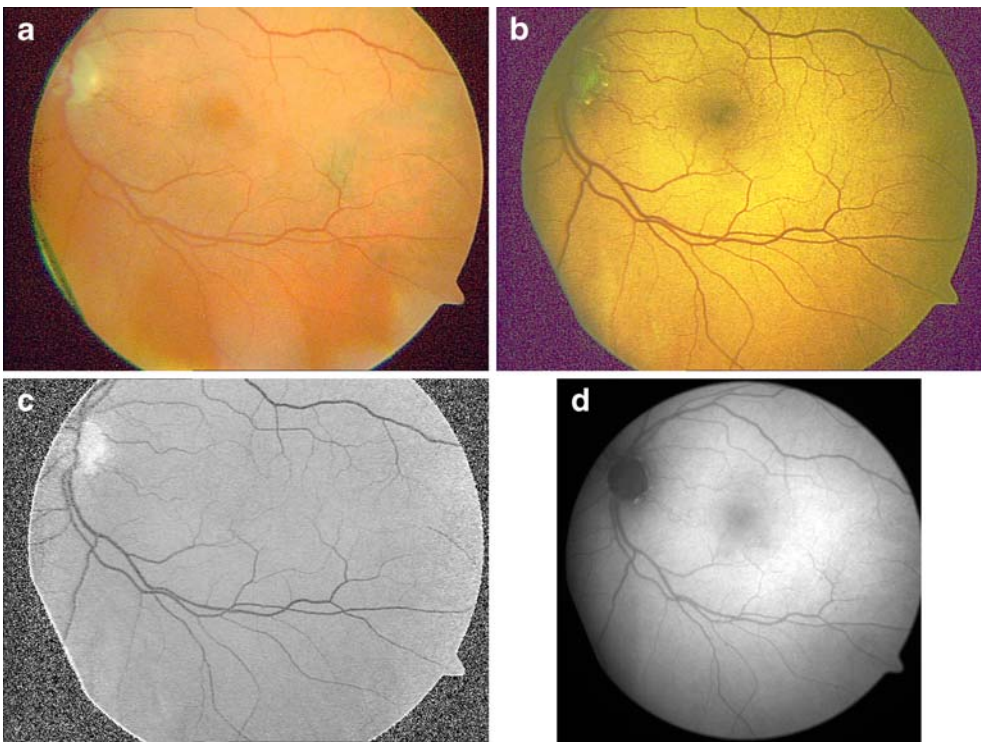

Fig. 1 Normal fundus (control group), age 72 years. **a** Fundus photograph. **b** Colour AF. **c** Green vs. red ratio from **b**. **d** Far red AF

in Jena, versus the patient's age are shown in Fig. 2. The images from the Regensburg study arm were not suitable for quantitative analysis because of image compression. For the control group, Fig. 2 shows a slight increase of the green vs. red fluorescence (r=0.404) with age which, however, was not significant (p=0.108). The AMD patients showed a steeper, but also not significant, increase of the ratio with age (r=0.662, p=0.152). There was no correlation of the green vs. red fluorescence ratio with age in the diabetic group (r=0.196). Often, the fundus AF of diabetic patients appeared green-shifted (compare Figs. 1b and 7b). Consequently, the average of the green vs. red ratio over all diabetics (0.946±0.047) was slightly higher than that of the controls (0.869±0.054) and the AMD patients (0.861±0.054). This difference, however, was not significant (p=0.212 and p=0.304, respectively).

Besides those general spectral features of AF images, different excitation- and emission wavelengths reveal different symptoms of AMD or diabetic retinopathy, respectively. Figure 3 shows images of an AMD patient (74 years) with minimal classical choroidal neovascularisation (CNV) demonstrated by fluorescein angiography (Fig. 3b). The HRA-AF may be assigned to the minimal change pattern according to the international classification [3]. Additionally, in the colour AF drusen may be recognised which are not seen in the HRA image (see arrows) and, thus, are not highlighted by hyper-fluorescence but by a spectral shift towards shorter wavelengths. This was found to be a typical feature of hyperfluorescent drusen and sometimes even of nonfluorescent drusen.

The condition of a 73-year-old patient after successful photodynamic therapy (PDT) of the CNV is documented in Fig. 4. The fluorescein angiography (Fig. 4b) shows a nonperfused central area and, thus, the closure of the CNV. HRA AF (Fig. 4c) demonstrates an annular hyper-fluorescence at the margin of the PDT area. The colour AF image shows a lipofuscin-like orange fluorescence of this annulus. Two haemorrhages are clearly seen nasal and superior to the laser lesion. The areas appearing hypofluorescent in the HRA AF image, however, exhibit green fluorescence in the colour AF image. This is even more pronounced in fibrinous scars secondary to AMD (Fig. 5d). As the case of a 74-year-old patient demonstrates, this fluorescence is not seen in the HRA AF image (Fig. 5c).

A case of geographic atrophy (in a 71-year-old) is shown in Fig. 6. The atrophic zone is characterised by a decreased fluorescence in the HRA AF as well as in the colour AF image. The colour AF, however, demonstrates a remaining green fluorescence in the atrophy. Interestingly, areas of recent atrophy progression (see arrows) are circumvented by a green-shift of the AF.

The images in Fig. 7 show findings in a 64-year-old patient suffering from diabetic retinopathy. The overall colour AF (Fig. 7b) appears more greenish than that of the AMD patients and controls. The atrophic area secondary to laser photocoagulation is hypofluorescent in the far red AF image (Fig. 7d) but, again, shows a remaining green fluorescence in the colour AF. Hard exudates are seen as hyperfluorescent spots. The green vs. red ratio image (Fig. 7c) demonstrates a spectral shift towards shorter wavelengths for those exudates.

Fluorescence micrographs of the RPE-choroid complex from the donor eye of an AMD patient (Fig. 8) revealed two strongly fluorescent structures: the RPE and Bruch's membrane. While the RPE showed a golden fluorescence, the emission of Bruch's membrane was green. Additional to the overall thickening of Bruch's membrane (Fig. 8a), basal laminar deposits (Fig. 8b) were also found to be hyper-

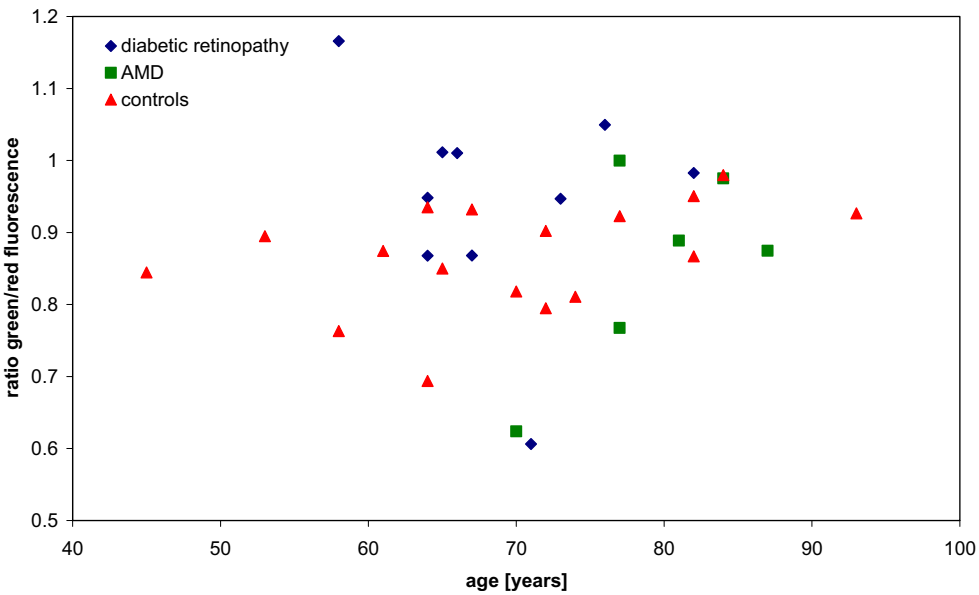

Fig. 2 Ratio of the fluorescence in the green and in the red channel versus the patient's age (averaged over a 70×70 pixel area temporal to the macula and apart from pathologic lesions and retinal vessels)

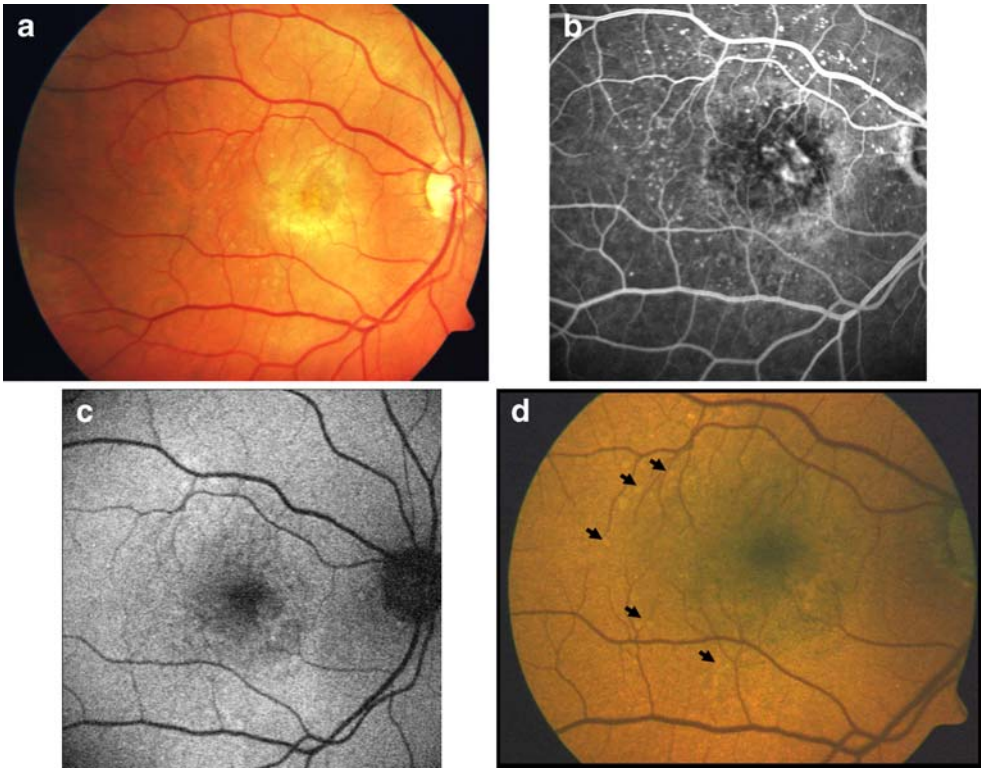

Fig. 3 AMD patient (74 years) with minimal classical choroidal neovascularisation. **a** Fundus photograph. **b** Fluorescein angiography. **c** HRA-AF. **d** Colour AF, soft drusen with green-shifted AF (*arrows*)

fluorescent. Drusen exhibited different fluorescence patterns: Fig. 8c demonstrates a nearly nonfluorescent druse whereas other drusen showed granular green hyperfluorescence (Fig. 8d).

Discussion

The data presented here show that different physiological as well as pathological features may be highlighted in fundus

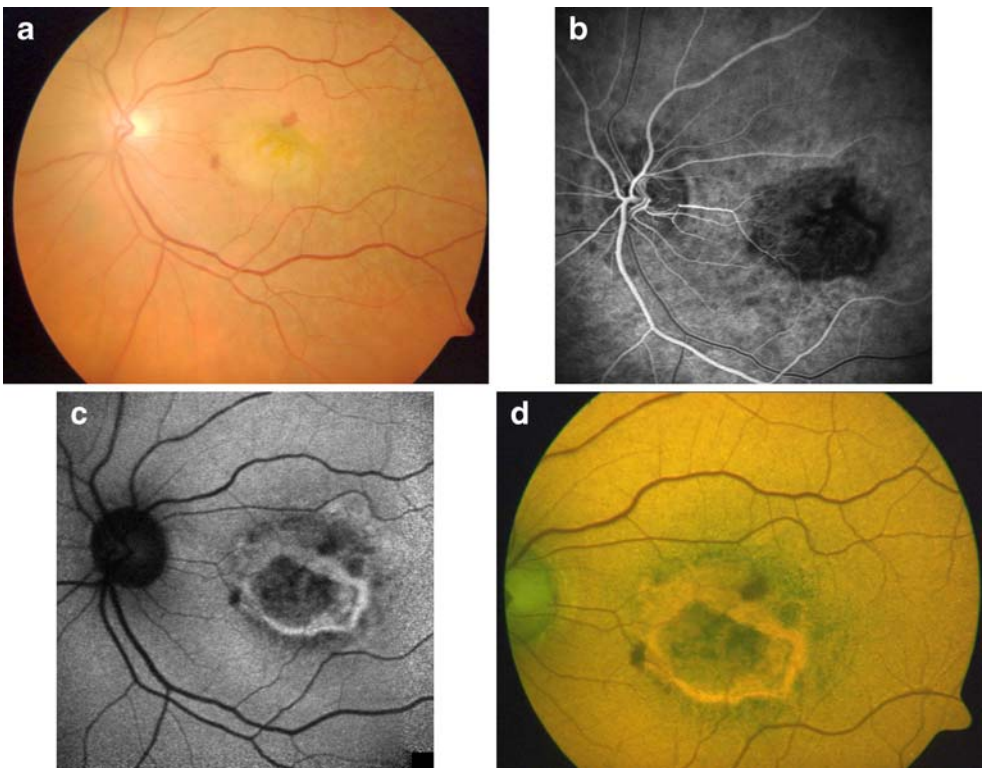

Fig. 4 AMD patient (73 years) after PDT of the CNV. **a** Fundus photograph. **b** Fluorescein angiography. **c** HRA-AF. **d** Colour AF

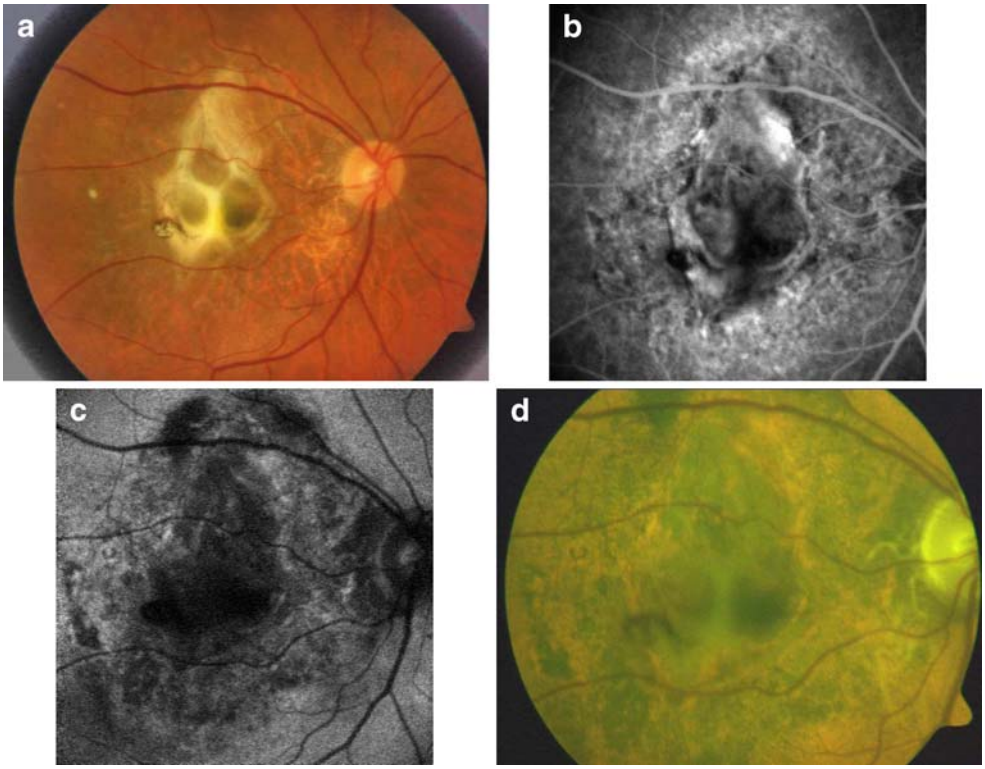

Fig. 5 Patient (74 years) with a central scar secondary to AMD. **a** Fundus photograph. **b** Fluorescein angiography. **c** HRA-AF. **d** Colour AF

AF images by the choice of different wavelength ranges for excitation and emission. Generally speaking, the far red fluorescence may be solely attributed to lipofuscin. Spectrally shorter emitting fluorophores are not seen in the emission range 675–717 nm applied here. The HRA AF also predominantly represents lipofuscin fluorescence since this is the principal fluorophore at the ocular fundus. The application of colour AF, however, reveals the existence of additional fluorophores. Most noticeable in the healthy controls is the green fluorescence of the optic disc. Since

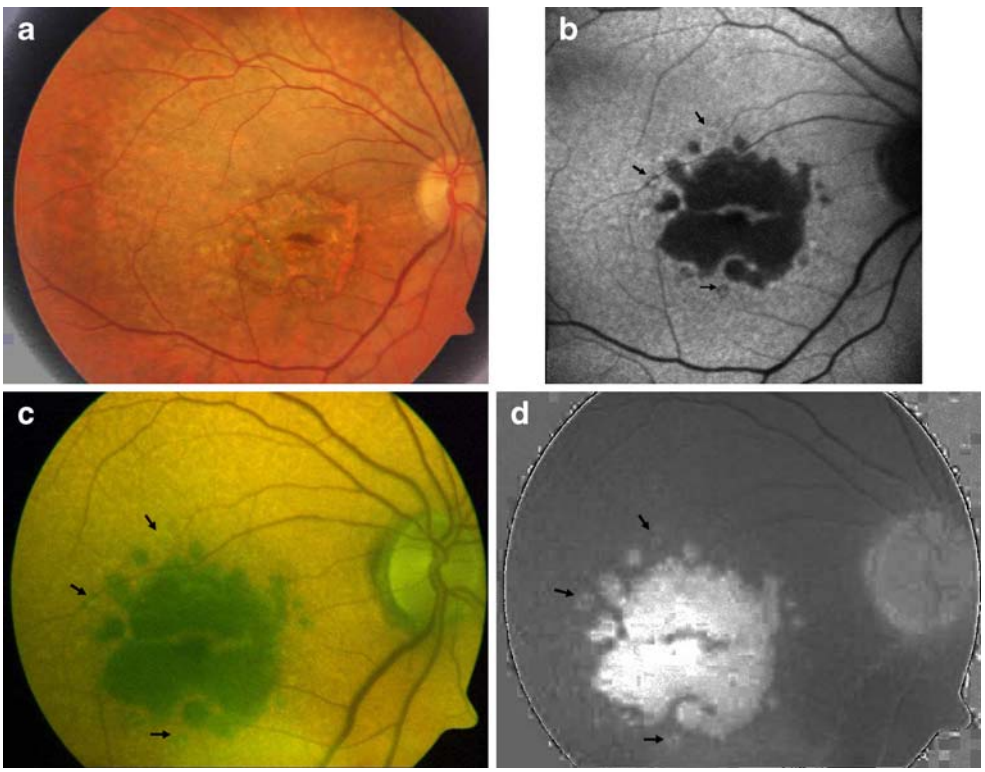

Fig. 6 Patient with geographic atrophy (71 years). Areas of atrophy progression (*arrows*) showing green-shift of the colour AF. **a** Fundus photograph. **b** HRA-AF. **c** Colour AF. **d** Green vs. red ratio from **c**

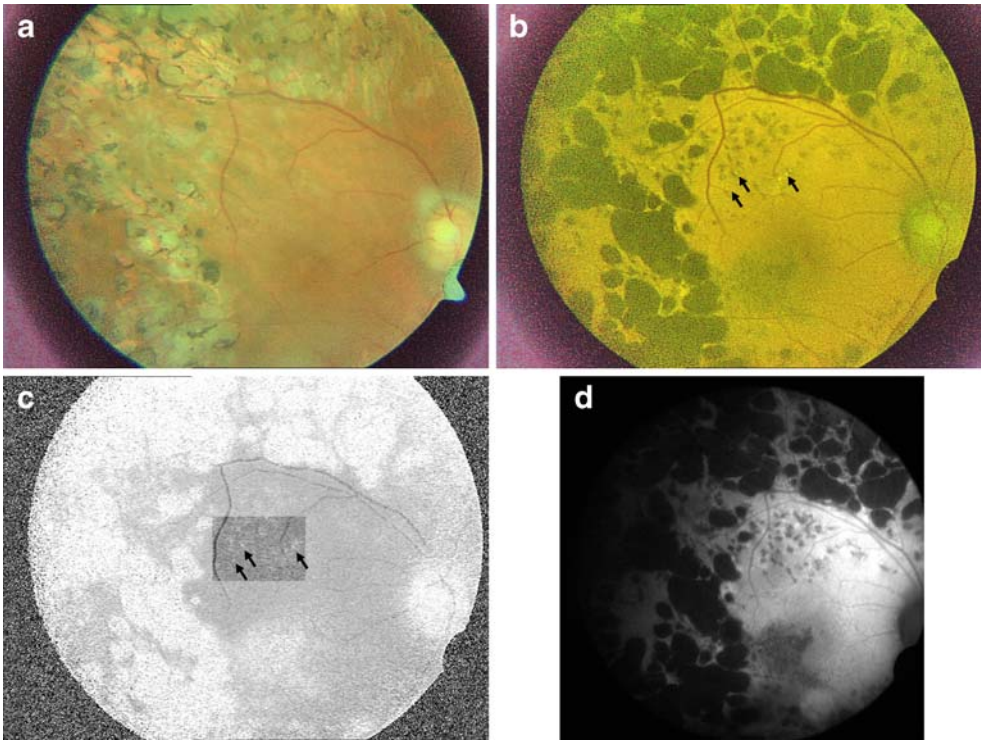

Fig. 7 64-year-old patient suffering from diabetic retinopathy. **a** Fundus photograph. **b** Colour AF. *Arrows* (in **b** and **c**) are hard exsudates. **c** Green vs. red ratio from **b**. **d** Far red AF

the total exclusion of reflected light from the fluorescence measurement was ensured in preceding experiments [13], this fluorescence has to be attributed to collagen. The emission maximum of collagens was found at 500 nm upon excitation at 446 nm [33, 37]. The fluorescence of the optic disc is not seen in the HRA AF images. Because of the excavation of the papilla nervi optici, this is out of focus of the confocal scanning system in retinal imaging and, thus,

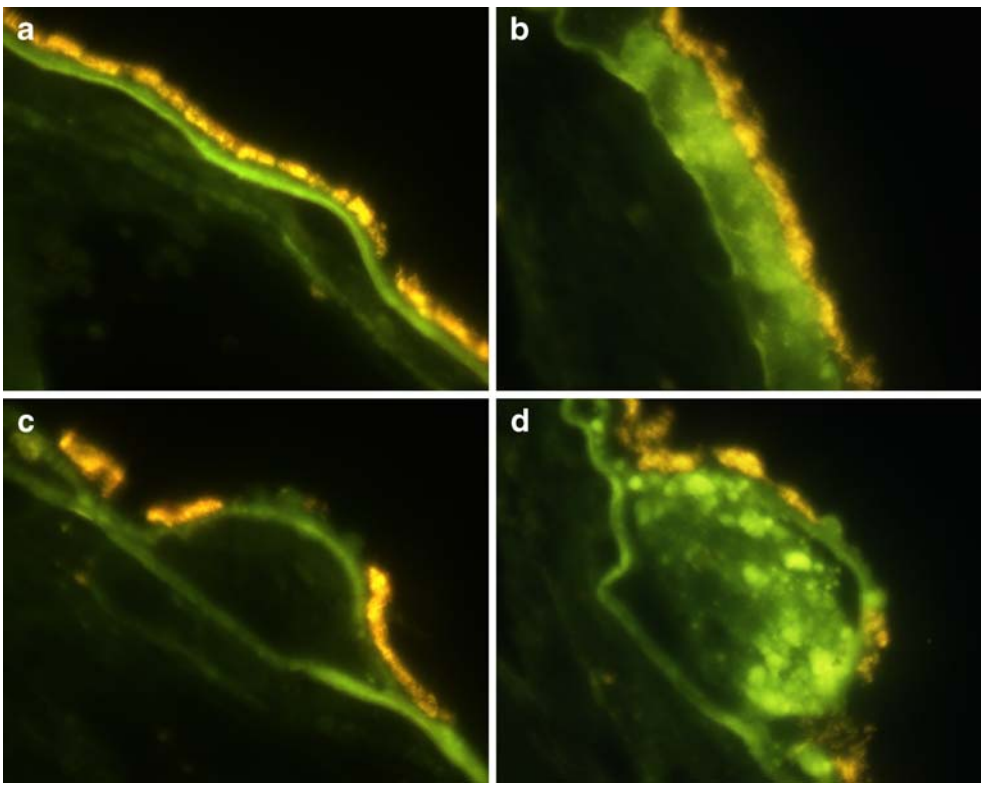

Fig. 8 AF micrographs of RPE and choroid of a donor eye from an AMD patient. **a** Normal fundus. **b** Thickened Bruch's membrane with basal-laminar deposits. **c** Druse. **d** Druse filled with green fluorescent material

the detection of its fluorescence is suppressed by the confocal field diaphragm. There is no AF seen at the optic disc in the far red images because of the lack of lipofuscin.

The reddish appearance of retinal vessels in the colour AF images results from the spectral transmission of the haemoglobin. Because this is much higher for red than for green light, the green fluorescence emission from behind the vessels is absorbed by haemoglobin while the red one may transmit the vessels.

The general orange fundus AF strongly resembles that of the RPE in a histological cross section of RPE and choroid from an AMD patient (Fig. 8). Hence, in fundus AF imaging we primarily observe the golden fluorescence of the RPE rather than the green fluorescence of Bruch's membrane (Fig. 8), which is covered by RPE lipofuscin fluorescence in the en face observation in vivo. The dominance of one fluorophore in the healthy controls is emphasised further by the homogeneity of the ratio of green vs. red fluorescence emission (Fig. 1c). The fluorescence emission itself is depressed in the fovea due to an attenuation of the excitation light by the macular pigment (Fig. 1b,c); however, there was no alteration of the spectral composition of the emission. The increase of the ratio of the green versus red fluorescence with age in the control group and, more pronounced, in AMD patients may reflect either a thickening and structural change of Bruch's membrane or a decrease of the lipofuscin content of the RPE [8].

The colour AF images of diabetic patients were often found to be green-shifted. Although the green vs. red fluorescence intensity ratio was not significantly different in diabetic patients versus nondiabetics in our study population, the shift was highly visible in most of the cases. There were some green-shifted hyperpigmentations showing coincidence with hard exsudates in the fundus photograph (Fig. 7), but the general appearance of the green fluorescence was not a focal, but a global one. Thus, this green-shift may indicate systemic processes rather than local pathologies of the eye. One explanation may be the general glycation of proteins in diabetics [45]. Advanced glycation end products showed a fluorescence maximum at 495 nm upon excitation at 446 nm [33, 37]. Thus, the observed green-shift may indicate enhanced levels of AGEs at the fundus of diabetic patients. On the other hand, the increase of the green vs. red ratio with age in the controls and, more pronounced, in AMD patients (Fig. 2) also may result from a thickening of Bruch's membrane [24]. This membrane emits green fluorescence light upon blue light excitation (Fig. 8) and its thickness increases with age [27]. Collagen as well as AGEs may contribute to the green fluorescence, and further investigations are necessary in order to elucidate the role of either fluorophore. Here, fluorescence lifetime imaging, which can be applied in vivo [33, 34], may be helpful.

The AF of drusen is a matter of debate since Arend et al. [1] found their fluorescence to be green-shifted in some cases. This is consistent with our in vivo (Fig. 3d) as well as in vitro (Fig. 8d) findings. The accumulation of green fluorescent material in drusen as well as in basal laminar deposits (Fig. 8b) found here, confirms the observations of Marmorstein et al. [24]. It may be attributed to protein glycation. Handa et al. [15] and Ishibashi et al. [20] demonstrated the abundance of pentosidine and carboxymethyllysine, two of the most studied collagen AGE products, in soft macular drusen as well as basal laminar and linear deposits. Their possible role in the pathogenesis of AMD was pointed out by these authors. Smith et al. [38] found a remarkable co-localisation of drusen with focal increased AF. They discussed their findings in the context of lipofuscin accumulation in the RPE over large soft drusen. Our results, however, suggest that hyperfluorescence at the site of drusen does not necessarily result from the RPE lipofuscin but may be emitted by the drusen themselves. Since this fluorescence is shifted towards shorter wavelengths, the AF of drusen can be distinguished from that of lipofuscin by colour AF. Besides hyperfluorescent drusen, Delori et al. [7] found drusen with an annular fluorescence pattern. This pattern was not found in our investigation possibly due to a much smaller lateral resolution of our imaging technique. However, Delori's finding of a greater contrast of the annular pattern upon an excitation at 550 nm (lipofuscin only) compared to 470 nm, exciting different fluorophores, is consistent with the differentiation of drusen- and lipofuscin-AF possible with our technique.

A hyperpigmented annulus around a lesion secondary to PDT in neovascular AMD (Fig. 4) was found in some but not all of our patients. Since we did not take AF photographs prior to PDT, we do not know whether that annulus resulted from the treatment or already existed before PDT as a feature of the CNV. From its orange appearance in colour AF, however, we think that the fluorescence results from an increased lipofuscin content of the RPE. Alternatively, the fluorescence may arise from melanin showing far red fluorescence [21]. Both phenomena may be explained by the phagocytosis of melanin and lipofuscin, released during PDT, by the RPE cells at the margin of the PDT area. On the other hand, it may also arise from nonproliferating RPE cells and, thus, may indicate either just enhanced metabolic activity of the RPE or a lipofuscin load which is critical to the cells. This issue has to be addressed by further investigations. The PDT lesion itself (Fig. 4) appeared hypofluorescent in the HRA AF. In contrast, weak green fluorescence in and around the lesion in colour AF possibly revealed a beginning fibrinous process which may result in an AMD scar as seen in Fig. 5. Here, the scar, which is hypofluor-

escent in the HRA AF, showed green fluorescence, obviously due to collagen and elastin, in colour AF.

Hyper-AF in the junctional zone of geographic atrophy was first demonstrated by Holz et al. [18]. It is still a matter of debate whether this hyperfluorescence is predictive for the progression of the GA or not [19, 28, 29]. This question can only be answered by extensive longitudinal studies. What we see, however, from colour AF is a green-shift circumventing regions of recent progression of the atrophic zone (Fig. 6). Where this shift coincides with hyperfluorescence, it possibly indicates an alteration of the composition of the lipofuscin. Whether this has an impact on disease progression should be investigated in subsequent studies.

From the data presented here, we suggest that the spectral resolution of the ocular fundus autofluorescence as in colour AF may provide additional information on pathologic features in AMD as well as in diabetic retinopathy. A further advantage of this technique over HRA AF is the possibility of recording the fluorescence image with a single flash of the fundus camera. Thus, there is no need for image registration or correction.

This study has several limitations. First, only pseudophacic patients were included. In phacic eyes, the fluorescence of the lens may interfere with that of the fundus. This is a disadvantage of colour AF compared to the HRA AF, largely suppressing the lens fluorescence by confocal imaging, and far red AF, excluding the lens fluorescence by the choice of the excitation wavelength. However, techniques to compensate for the lens AF are currently under development. A second limitation of the study is that it was cross-sectional. Thus, the data leave us with some questions on the natural history of AMD or diabetic retinopathy, which have to be addressed by subsequent longitudinal investigations.

In conclusion, HRA AF as well as far red AF document the lipofuscin distribution over the ocular fundus. The same AF pattern is seen in the red channel of the colour AF. The green channel adds information on fluorophores emitting between 530 and 570 nm. That may be collagen and AGEs. The diagnostic merit of colour AF, however, has to be established in detailed studies to be performed subsequently.

Acknowledgements The authors gratefully acknowledge the gift of histological sections from a donor eye of an AMD patient by Dr. Elizabeth Gaillard from Northern Illinois University at DeKalb.

References

1. Arend O, Weiter JJ, Goger DG, Delori FC (1995) In vivo Fundus Fluoreszenzmessungen bei Patienten mit altersbedingtrer Makuladegeneration. Der Ophthalmologe 92:647–653
2. Bindewald-Wittich A, Han M, Schmitz-Valckenberg S, Snyder SR, Giese G, Bille JF, Holz FG (2006) Two-photon-excited fluorescence imaging of human RPE cells with a femtosecond Ti: sapphire laser. Invest Ophthalmol Vis Sci 47:4553–4557
3. Bindewald A, Bird AC, Dandekar SS, Dolar-Szczasny J, Dreyhaupt J, Fitzke FW, Einbock W, Holz FG, Jorzik JJ, Keilhauer C, Lois N, Mlynski J, Pauleikhoff D, Staurenghi G, Wolf S (2005) Classification of fundus autofluorescence patterns in early age-related macular disease. Invest Ophthalmol Vis Sci 46:3309–3314
4. Bird AC, Bressler NM, Bressler SB, Chisholm IH, Coscas G, Davis MD, de Jong PTVM, Claver CCW, Klein BEK, Klein R, Mitchell P, Sarks JP, Sarks SH, Soubrane G, Taylor, Vingerling JR (1995) An international classification and grading system for age-related maculopathy and age-related macular degeneration. Surv Ophthalmol 39:367–374
5. Delori FC (1994) Spectrometer for noninvasive measurement of intrinsic fluorescence and reflectance of ocular fundus. Applied Optics 33:7439–7452
6. Delori FC, Dorey KC, Staurenghi G, Arend O, Goger DC, Weiter JJ (1995) In vivo fluorescence of the ocular fundus exhibits retinal pigment epithelium lipofuscin characteristics. Invest Ophthalmol 36:718–729
7. Delori FC, Fleckner MR, Goger DG, Weiter JJ, Dorey CK (2000) Autofluorescence distribution associated with drusen in age-related macular degeneration. Invest Ophthalmol Vis Sci 41:496–504
8. Delori FC, Goger DG, Dorey CK (2001) Age-related accumulation and spatial distribution of lipofuscin in RPE of normal subjects. Invest Ophthalmol Vis Sci 42:1855–1866
9. Dillon J, Wang Z, Avalle LB, Gaillard ER (2004) The photochemical oxidation of A2E results in the formation of a 5,8,5′,8′-bis-furanoid oxide. Exp Eye Res 79:537–542
10. Einbock W, Moessner A, Schnurrbusch UE, Holz FG, Wolf S (2005) Changes in fundus autofluorescence in patients with age-related maculopathy. Correlation to visual function: a prospective study. Graefes Arch Clin Exp Ophthalmol 243:300–355
11. Eldred GE, Katz ML (1988) Fluorophores of the human retinal pigment epithelium: separation and spectral characterization. Exp Eye Res 47:71–86
12. ETDRS Study Group (1991) Grading diabetic retinopathy from stereoscopic color fundus photographs - an extension of the modified airlie house classification - ETDRS Report Number 10. Ophthalmology 98:786–806
13. Hammer M, Nagel E, Schweitzer D, Richter S, Schweitzer F, Konigsdorffer E, Strobel J (2004) Spektrale Differenzierung in Eigenfluoreszenzbildern des Augenhintergrunds von Patienten mit altersabhängiger Makuladegeneration. Ophthalmologe 101:1189–1193
14. Hammer M, Richter S, Guehrs KH, Schweitzer D (2006) Retinal pigment epithelium cell damage by A2-E and its photo-derivatives. Mol Vis 12:1348–1354
15. Handa JT, Verzijl N, Matsunaga H, Aotaki-Keen A, Lutty GA, te Koppele JM, Miyata T, Hjelmeland LM (1999) Increase in the advanced glycation end product pentosidine in Bruch's membrane with age. Invest Ophthalmol Vis Sci 40:775–779
16. Holz FG (2001) Autofluoreszenz-Imaging der Makula. Der Ophthalmologe 98:10–18
17. Holz FG, Bellman C, Staudt S, Schutt F, Volcker HE (2001) Fundus autofluorescence and development of geographic atrophy in age-related macular degeneration. Invest Ophthalmol Vis Sci 42:1051–1056
18. Holz FG, Bellmann C, Margaritidis M, Schutt F, Otto TP, Volcker HE (1999) 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 237:145–152
19. Hwang JC, Chan JW, Chang S, Smith RT (2006) Predictive value of fundus autofluorescence for development of geographic atrophy in age-related macular degeneration. Invest Ophthalmol Vis Sci 47:2655–2661

20. Ishibashi T, Murata T, Hanagai M, Nagai R, Horiuchi S, Lopez PF, Hinton DR, Ryan SJ (1998) Advanced glycation end products in age related macular degeneration. Arch Ophthalmol 116:1629–1632
21. Keilhauer CN, Delori FC (2006) Near-infrared autofluorescence imaging of the fundus: visualization of ocular melanin. Invest Ophthalmol Vis Sci 47:3556–3564
22. Kopitz J, Holz FG, Kaemmerer E, Schutt F (2004) Lipids and lipid peroxidation products in the pathogenesis of age-related macular degeneration. Biochimie 86:825–831
23. Lois N, Owens SL, Coco R, Hopkins J, Fitzke FW, Bird AC (2002) Fundus autofluorescence in patients with age-related macular degeneration and high risk of visual loss. Am J Ophthalmol 133:341–349
24. Marmorstein AD, Marmorstein LY, Sakaguchi H, Hollyfield JG (2002) Spectral profiling of autofluorescence associated with lipofuscin, Bruch's membrane, and sub-RPE deposits in normal and AMD eyes. Invest Ophthalmol Vis Sci 43:2435–2441
25. Munch G, Thome J, Foley P, Schinzel R, Riederer P (1997) Advanced glycation endproducts in ageing and Alzheimer's disease. Brain Res Brain Res Rev 23:134–143
26. Radu RA, Mata NL, Bagla A, Travis GH (2004) Light exposure stimulates formation of A2E oxiranes in a mouse model of Stargardt's macular degeneration. Proc Natl Acad Sci USA 101:5928–5933
27. Sarks SH (1976) Ageing and degeneration in the macular region: a clinico-pathological study. Br J Ophthalmol 60:324–341
28. Schmitz-Valckenberg S, Bindewald-Wittich A, Dolar-Szczasny J, Dreyhaupt J, Wolf S, Scholl HP, Holz FG (2006) Correlation between the area of increased autofluorescence surrounding geographic atrophy and disease progression in patients with AMD. Invest Ophthalmol Vis Sci 47:2648–2654
29. Schmitz-Valckenberg S, Bultmann S, Dreyhaupt J, Bindewald A, Holz FG, Rohrschneider K (2004) Fundus autofluorescence and fundus perimetry in the junctional zone of geographic atrophy in patients with age-related macular degeneration. Invest Ophthalmol Vis Sci 45:4470–4476
30. Scholl HP, Bellmann C, Dandekar SS, Bird AC, Fitzke FW (2004) Photopic and scotopic fine matrix mapping of retinal areas of increased fundus autofluorescence in patients with age-related maculopathy. Invest Ophthalmol Vis Sci 45:574–583
31. Schütt F, Bergmann M, Holz FG, Kopitz J (2003) Proteins modified by malondialdehyde, 4-hydroxynonenal, or advanced glycation end products in lipofuscin of human retinal pigment epithelium. Invest Ophthalmol Vis Sci 44:3663–3668
32. Schweitzer D, Hammer M, Schweitzer F, Anders R, Doebbecke T, Schenke S, Gaillard ER (2004) In vivo measurement of time - resolved autofluorescence at the human fundus. J Biomed Opt 9:1214–1222
33. Schweitzer D, Hammer M, Schweitzer F, Schenke S (2006) In vivo autofluorescence lifetime imaging at the fundus of the human eye. In: Manns F, Söderberg PG, Ho A (eds) Ophthalmoic technologies XVI. SPIE, San Jose, pp 613808-1–613808-10
34. Schweitzer D, Hammer M, Schweitzer F, Schenke S, Gaillard ER (2003) Evaluation of time-resolved autofluorescence images of the ocular fundus. In: Wagniers GA (ed) Diagnostic optical spectroscopy in biomedicine II. Proceedings of SPIE, Munich, 24-25 June 2003, 5141:8–17
35. Schweitzer D, Kolb A, Hammer M (2001) Autofluorescence lifetime measurements in images of the human ocular fundus. In: Papazoglou TG, Wagnieres, GA (eds) Diagnostic optical spectroscopy in biomedicine. Proceedings of SPIE, October 2001, 4432:29–39
36. Schweitzer D, Kolb A, Hammer M, Anders R (2002) Zeitaufgelöste Messung der Autofluoreszenz - Ein Werkzeug zur Erfassung von Stoffwechselvorgängen. Ophthalmologe 99:776–779
37. Schweitzer D, Schenke S, Hammer M, Schweitzer F, Jentsch S, Birckner E, Becker W, Bergmann A (2007) Towards metabolic mapping of the human retina. Microsc Res Tech. DOI 10.1002/jemt.20427
38. Smith RT, Chan JK, Busuoic M, Sivagnanavel V, Bird AC, Chong NV (2006) Autofluorescence characteristics of early, atrophic, and high-risk fellow eyes in age-related macular degeneration. Invest Ophthalmol Vis Sci 47:5495–5504
39. Solbach U, Keilhauer C, Knabben H, Wolf S (1997) Imaging of retinal autofluorescence in patients with age-related macular degeneration. Retina 17:385–389
40. Spaide RF (2003) Fundus autofluorescence and age-related macular degeneration. Ophthalmology 110:392–399
41. Sparrow JR, Boulton M (2005) RPE lipofuscin and its role in retinal pathobiology. Exp Eye Res 80:595–606
42. Sparrow JR, Cai B (2001) Blue light-induced apoptosis of A2E-containing RPE: involvement of caspase-3 and protection by Bcl-2. Invest Ophthalmol Vis Sci 42:1356–1362
43. Sparrow JR, Villmer-Snarr HR, Zhou J, Jang YP, Jockusch S, Itagaki Y, Nakanishi K (2003) A2E-epoxides damage DNA in retinal pigment epithelial cells. J Biol Chem 278:18207–18213
44. Staudt S, Bellmann C, Schütt F, Lösch A, Holz FG (2000) Various intra- and extracellular fluorophores other than RPE-lipofuscin contribute to variations in spacial distribution and intensity in topographic fundus autofluorescence. Invest Ophthalmol Vis Sci 41(Suppl):168
45. Stitt AW (2001) Advanced glycation: an important pathological event in diabetic and age related ocular disease. Br J Ophthalmol 85:746–753
46. Suter M, Reme C, Grimm C, Wenzel A, Jaattela M, Esser P, Kociok N, Leist M, Richter C (2000) Age-related macular degeneration. The lipofusion component N-retinyl-N-retinylidene ethanolamine detaches proapoptotic proteins from mitochondria and induces apoptosis in mammalian retinal pigment epithelial cells. J Biol Chem 275:39625–39630
47. von Rückmann A, Fitzke FW, Bird AC (1995) Clinical application of in vivo imaging of fundus autofluorescence. Invest Ophthalmol 36:238
48. von Rückmann A, Fitzke FW, Bird AC (1995) Distribution of fundus autofluorescence with a scanning laser ophthalmoscope. Brit J Ophthalmol 79:407–412
49. von Rückmann A, Fitzke FW, Bird AC (1997) Fundus autofluorescence in age-related macular disease imaged with a laser scanning ophthalmoscope. Invest Ophthalmol Vis Sci 38:478–486
50. von Rückmann A, Fitzke FW, Bird AC (1997) In vivo fundus autofluorescence in macular dystrophies. Arch Ophthalmol 115:609–615
51. von Rückmann A, Fitzke FW, Bird AC (1999) Distribution of pigment epithelium autofluorescence in retinal disease state recorded in vivo and its change over time. Graefes Arch Clin Exp Ophthalmol 237:1–9
52. von Rückmann A, Fitzke FW, Fan J, Halfyard A, Bird AC (2002) Abnormalities of fundus autofluorescence in central serous retinopathy. Am J Ophthalmol 133:780–786
53. Yamada Y, Ishibashi K, Ishibashi K, Bhutto IA, Tian J, Lutty GA, Handa JT (2006) The expression of advanced glycation end-product receptors in rpe cells associated with basal deposits in human maculas. Exp Eye Res 82:840–848