**Fundus Autofluorescence with a Confocal Scanning Laser Ophthalmoscope**

**INTRODUCTION**

Among the different modalities dedicated to imaging the human retina, fundus autofluorescence (FAF) is emerging as a promising, non-invasive method of documenting age and disease related changes in the retinal pigment epithelium (RPE) and adjacent structures.

Until recently, FAF imaging referred primarily to the photographic attempts at recording optic nerve head (ONH) drusen, hyaloid translucent bodies partially buried in the anterior part of the optic nerve head. These are calcified, intracellular mitochondria of optic nerve axons¹ and emit light near the 520-530 nm range² when excited by short wavelength light.

Traditionally, such photography was carried out using film based systems, and push-processing was often used in order to bring out the relatively faint outlines of such deposits. In addition, a poor filter combination, or even aged filters, could create the appearance of autofluorescence which was actually pseudofluorescence, adding confusion to the debate.²

With the advent of digital photography and the ability to enhance images electronically and evaluate them immediately, attempts at documenting ONH drusen were made easier (Figure 1), but without any appreciable improvement in image quality. The immediacy of the results was attractive, but the high flash levels were difficult for the patient and the post-acquisition image enhancement was time consuming.³

The introduction of confocal scanning laser ophthalmoscopy (cSLO) (Heidelberg Engineering, Heidelberg, Germany), improved the ability to record the different levels of autofluorescence as seen in Figure 1 without the need for flash photography.

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**Figure 1:** Color and FAF images of bilateral ONH drusen taken with a Zeiss 450+ fundus camera equipped with a JVC KY F70-B digital camera. Fundus camera fluorescein angiogram (FA) filters were used, flash level was at 25 and the 'Boost' setting was selected. (a,b) Color photographs exhibiting the classic features of ONH drusen seen in a clinical examination. (c) Un-enhanced FAF of the right eye, showing faint fluorescence in a round pattern. (d) A similar photograph taken of the left eye, but the image has been enhanced post-acquisition in an attempt to bring out more detail.

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² Priel E: Personal experience.

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fluorescence originating from the RPE in-vivo. This has allowed documentation of new photographic patterns, in both healthy and diseased eyes,\(^4\) (Figure 2). Over the last few years, a growing body of knowledge has added to our understanding of the autofluorescence emanating from the various components of RPE cells, offering a unique tool for evaluating and following up on their health, function and possible deterioration.\(^5,6\) This technique will be worked out in the coming years and will undoubtedly play a major role in the diagnosis and treatment of retinal diseases originating in the declining viability of the RPE.\(^7\)

**DISCUSSION**

FAF imaging of the faint light emitted from the RPE using fundus camera based digital imaging systems is frustrated by the low level of light reaching the photographic detectors, demanding both higher levels of flash exposure and extensive image enhancement (Figure 3). The use of a cSLO, with its increased light gathering ability and point by point laser illumination, allows easier visualization of the autofluorescent light emitted from the RPE\(^9\)\(^-\)\(^11\) (Figure 4). Recently, with the emergence of FAF imaging as a new field of diagnosis in ophthalmology, newer autofluorescence filters are gradually becoming available for fundus camera based imaging. In combination with averaging algorithms, fundus cameras will have the potential to rival images from cSLOs. Vision loss related to age-related macula degeneration (AMD) has

**Figure 2:** (a,b) FAF images of the same patient as Figure 1, taken with the HRA2. Note the detail of the drusen and high level of fluorescence seen both in the disc area as well as in the rest of the frame. (c,d) Show the late stage FA images of both eyes, with little if any pathology visible in the nerve heads. (e,f) OCT horizontal line scans taken through the middle of the ONH. Both scans demonstrate the relative elevation of both nerve heads.

**Figure 3:** (a) FAF image taken with a Topcon TRC 50 IA fundus camera, with the standard FA filters fitted with a Kodak Megaplus 1.4 digital camera. Flash setting was at 300 ws (max) and the camera gain set to +18db. (b) The same patient, taken with the HRA2. Note the difference in detail between the images generated by the two different systems. (Topcon photos courtesy Denise Krolnik, University of Wisconsin, Madison, Wisconsin.)
been linked to the precursor areas of subretinal change caused by age, mainly in the choroid, Bruch’s membrane and the RPE. As autofluorescence arises mostly from the fluorophore Lipofuscin, contained in the RPE cells, documenting changes in these autofluorescence patterns is considered to be a promising method for obtaining information about the health of the RPE before vision loss occurs. As the RPE ages and as a by-product of cellular breakdown, more and more Lipofuscin accumulates, adding to a stronger autofluorescence signal. As this debilitating dry form of the disease currently has no treatment, documenting the extent and rate of the change will play a major part in studies to find a treatment aimed at halting or preventing deterioration.

Recent efforts to classify and categorize normal and pathological autofluorescence patterns in geographic atrophy have demonstrated that the areas of increased autofluorescence are often seen at the edges of areas of decreased autofluorescence (Figure 5). These areas represent a stage in the life-cycle of the affected RPE cells preceding their final breakdown. Therefore, those areas exhibiting elevated levels of autofluorescence can be considered good prognostic indicators of the expansion of early stages of AMD and other diseases with atrophic lesions (Figure 6).^6^ The high quality of the images taken with the HRA2 cSLO are due to a novel image-acquisition and averaging technique available from within the program, Automatic Real Time Mean (ART-M). This method is an improvement on the previous manual Mean Image program, which called for the manual selection of consecutive images and their averaging by the system’s software, including auto registration. Currently, once the camera is aligned and the retinal landmarks are visible as a dynamic, live image on the screen (while using the fluorescein illumination), the Real Time Mean button (which doubles as the sensitivity control dial on the control panel) is pressed (Figure 7). The system then starts adding up live images according to the pre-set parameter. Once the desired image is seen on the screen, it is acquired (Figure 8). To use the manual Mean Image technique, available on all HRA models, a minimum of two images must be selected to create a Mean Image, but a good rule of thumb is to use at least 9-15 images (Figure 10). Mean Images created from too few original, source images result in poorer quality images (Figure 11). The more images used, the better the resultant Mean Image, but the risk of introducing errant images blurred by eye movement, increases (Figure 12). Therefore, when using this method, one must always evaluate the resultant Mean Image for sharpness and the appearance of double vessels. If such imperfections arise, screen the individual, source images Figure 4: Normal FAF image taken with the HRA2. The darker areas denote areas of decreased autofluorescence: normal around the macula and in the fovea, where the macular pigment absorbs the autofluorescent light. The blood vessels and the ONH appear completely black, as they do not emit light at the autofluorescence imaging wavelength.

Figure 5: Area of round, central atrophy secondary to AMD, characterized by its dark appearance, surrounded by areas of increased autofluorescence due to greater accumulation of lipofuscin, denoting regions susceptible to future spread of atrophy (indicated by arrows).

Figure 6: (a) Color photograph of the right eye of a 42-year-old male patient with chronic central serous chorioretinopathy (CSC). (b) FAF highlights different stages of the disease. Arrow 1 denotes the funnel-shaped area of long-standing atrophy inferior to the macula. Arrow 2 an area of increased autofluorescence nasal to the disc with no atrophy present. Arrow 3 an area of increased autofluorescence adjacent to an atrophic area showing decreased autofluorescence.
Figure 7: Control panel with the Automatic Real Time (ART) button. Pressing it activates the ART acquisition option used for FAF photography.

Figure 8: Screen shot of the HRA2 acquisition window in the ART Mean mode, set to acquire 20 high-speed images which are real-time averaged to create the image seen in the central portion of the window.

Figure 9: Sample FAF photograph showing multiple drusen in the posterior pole, created by averaging the light gathered from 20 single images using the ART Mean option.

Figure 10: Screen shot of a Mean image created manually from the averaging of 16 images.

Figure 11: Screen shot of a Mean image created manually from the averaging of three images. Note the degraded quality of the Mean image, compared to Figure 10.

Figure 12: Screen shot of a Mean image created manually from the averaging of six images. Note the poor quality of the Mean image, including the display of double vessels, as a result of the source images being of different areas of the fundus (seen to the right, highlighted in blue).
for those responsible for the degraded quality and do not use them for creating the Mean Image. In cases where poor fixation or media opacities result in poor Mean Images, attempt to rephotograph several image series until an acceptable result is attained. FAF images must always be acquired prior to injection of the fluorescein dye, and even a small amount of the dye present in the patient’s bloodstream will thwart attempts at FAF photography.

The following images were all taken with a cSLO, the HRA2, prior to injection of the fluorescein dye, during routine angiography sessions at the MOR Institute. Over the past few years, we have developed a ‘feel’ for the truly diagnostic value of the FAF images. The accompanying color images were taken with a Zeiss 450+ fundus camera (Zeiss, Germany), equipped with a JVC KYF 70B digital video camera. The OCT scans were acquired using a Zeiss Stratus OCT 3.

**Conclusion**

From figures 13-26, it can be seen that traditional FA findings in diseases such as AMD, CME, epiretinal membrane (ERM), CSC, macular dystrophies and more, are all highly visible in the FAF images. Parallel, and often complementary, findings are constantly being found during FAF photography, giving autofluorescence imaging increased diagnostic and scientific value. Furthermore, there are many instances where information visible in the FAF image surpasses that which is seen in the FA images. Of special interest are the autofluorescence patterns being formulated for diagnosing and evaluating geographic atrophy associated with AMD. In the coming years, continued cooperation among ophthalmic photographers, interested ophthalmologists and camera manufacturers will place this non-invasive, imaging modality at the forefront of ophthalmic imaging.

**Financial Disclosure**

*Mr. Ethan Priel has been a consultant to Heidelberg Engineering.*

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![Figure 13](image13.png)

**Figure 13:** (a) FAF image showing dark areas of punctuate and confluent atrophy, in exact correspondence to the pattern seen in the FA image (b).

![Figure 14](image14.png)

**Figure 14:** (a) FAF image showing area of blocked background fluorescence by a small, recent intraretinal hemorrhage (arrows). The large, crescent-shaped hyperautofluorescent lesion seen beneath the inferior arcades is an area of older, devitalized blood, whose components emit light at the autofluorescence wavelength. (b) Corresponding FA image shows the spatial relation between the two hemorrhages, but can give no information regarding their relative time of occurrence.

![Figure 15](image15.png)

**Figure 15:** FAF (a) and FA (b) images of the right eye of a patient with BVO. Note the complete blockage of autofluorescent light from the RPE, caused by the recent hemorrhage. The CME is seen in both imaging modalities.
Figure 16: (a) Color photograph of a patient with AMD, exhibiting areas of atrophy in the posterior pole. (b) FAF image highlights the darker areas of atrophy versus the areas of increased autofluorescence, where future atrophy is likely to occur.

Figure 17: FAF (a) and FA (b) images of the left eye of a patient exhibiting laser applications following a branch vein occlusion (BVO). Note the difference in appearance of the different laser burns and the cystoid macular edema (CME), seen clearly on the FAF image and the cystoid macular edema (CME) (arrows), seen only barely on the FA image.

Figure 18: (a) Color image of the right eye of a 32-year-old male, exhibiting only slight blurring of the perifoveal vessels. (b) FAF image taken on same visit shows a circular, well demarcated hypoautofluorescent pattern consistent with the neurosensory detachment typical of CSC, confirmed by the OCT (c).
Figure 19: (a) FAF photograph of the left eye exhibiting extensive Epiretinal Membrane. The darker areas temporal to the macula and between the arcades correspond to the areas of the thickened Epiretinal Membrane visible in the OCT (b) and in the FFA (c) images.

Figure 20: (a,b) Color photographs of a patient with Stargardt’s disease. (c,d) FAF images display the characteristic pattern of central atrophy, appearing black, as well as the outlying patches displaying increased autofluorescence, indicative of the progressive nature of the disease. (e,f) FA images present the more familiar photographs associated with the disease. Areas of lessened autofluorescence have the potential both to explain decreased retinal function in certain areas as well as help in outlining scotomas.
Photographing Fundus Autofluorescence: Concept, Technology, Images and Interpretation

Figure 21: (a) Indocyanine Green Angiography (ICGA) image outlining an area of an RPE tear, highlighted by the clearer view of the choroidal vessels seen through the area of the denuded RPE. The black line, nasal and alongside the area of the torn RPE (arrows), is the rolled up RPE, appearing black as a result of its increased thickness, hence its ability to block the near-infrared light which usually transverses the RPE in ICGA angiography. (b) FAF image offers a ‘negative image’ of the ICGA, the area of the torn, missing RPE appears darker than the surrounding tissue, while the area of the rolled-up RPE appears brighter than the rest of the RPE, due to the larger amount of lipofuscin present, which results in increased levels of autofluorescence. (c) FA contributes little to an understanding of the findings, offering only the overly bright fluorescence seen in the area of the tear.

Figure 22: (a) Color image showing slight yellowish discoloration in the area of the papillomacular area. (b) FAF image highlights the extent of the serous detachment in this CSC patient. In addition, areas of compromised, scarred RPE are seen throughout the frame, while the area of the rolled-up RPE appears brighter than the rest of the RPE, due to the larger amount of lipofuscin present, which results in increased levels of autofluorescence. (c) FFA image confirms the diagnosis. (d) The OCT scan taken through the central ‘hotspot’ seen in the FAF image. This scan confirms the extent of the detachment. (e) OCT scan taken at the site of the autofluorescence ‘hot spot’ seen superior-nasal to the ONH, and once again shows the serous elevation and the break in the RPE. (f) Locations of the OCT scans seen in d and e.
**Figure 23**: (a) Full thickness macular hole with FAF imaging. (b) Corresponding OCT offers dramatic confirmation. The exposed RPE layer at the bottom of the hole shows the bright, circular pattern seen in the FAF image, which is in sharp contrast to the usually darker appearance of the macular region, due to macular pigment.

**Figure 24**: FAF (a) and FA (b) images outlining multiple, extensive scarring due to high myopia. Note the temporal margins of the central scar exhibiting increased autofluorescence (arrows), a distinction not seen in the FA image.

**Figure 25**: (a,b) Bilateral idiopathic juxtafoveal telangiectasia highlighted in the two FAF images. These demonstrate the ectatic vessels, retinal thickening and intraretinal pigment plaques typically seen in the Type 2 subgroup. (c,d) The FA added serous exudation to the list of findings, confirming the diagnosis.
REFERENCES


8. Clinical Trials.gov Identifier: NCT00393692


Figure 26: (a,b)Color photographs of a 49 year old woman with longstanding bilateral serpiginous choroiditis. These display areas of atrophic scarring and pigment clumping. In addition, in the right eye there are two areas suspected of serpiginous activity: creamy lesions with unsharp borders (arrows). These two areas correspond to the hyperautofluorescence on the FAF image (c: arrows). These findings are consistent with the reactivation pattern of the disease, which occurs at the margins of existing scars. (e,f) FA highlights the scarred areas with their sharp borders, areas of blocked fluorescence due to pigment clumps, and two areas with slightly fuzzy borders (arrows) which once again correspond to the bright areas on the FAF image.