**Fundus Autofluorescence Imaging**

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Fundus auto fluorescence (FAF) imaging is a novel imaging method that allows topographic mapping of lipofuscin distribution in the retinal pigment epithelial (RPE) cell monolayer as well as of other fluorophores that may occur with disease in the outer retina and the sub neurosensory space. Excessive accumulation of lipofuscin (LF) granules in the lysosomal compartment of RPE cells represents a common downstream pathogenetic pathway in various hereditary and complex retinal diseases including age related macular degeneration (AMD). FAF imaging has been shown to be useful in understanding the pathophysiological mechanisms, diagnostic, phenotype-genotype correlation, identification of predictive markers of disease progression, and monitoring of novel therapies.

Most ocular media and tissues exhibit fluorescence emission upon excitation by a suitable wavelength of light. Light is absorbed by fluorophores, causing electrons to become excited to higher electronic state. The electrons remain in an excited state for a nanosecond, and their energy is emitted as they return to their ground state. Ocular fluorophores are endogenous and are visible in the cornea, lens and retinal pigment epithelium.

Ocular fluorophores exhibit marked changes in their fluorescence properties with regards to age and pathology. Hence they can be used as indicators of ageing or as diagnostic tools in diseases.

Fluorescence of the retinal pigment epithelium is mainly related to lipofuscin, a fluorescent pigment that is absent in fetal and newborn retinal pigment epithelium. Lipofuscin continuously accumulates in the retinal pigment epithelium as a result of incomplete digestion of spent rod outer segment disk. It is potentially noxious, acting as a photo sensitizer in blue light and generating free radicals both in isolated granules and within the retinal pigment epithelium (RPE). Lipofuscin has several distinct fluorescent components, of which A2E the red emitting fluorophore is very important as it is responsible for retinal pigment epithelial apoptosis, mediated via inhibition of lysosomal digestion of proteins and blue light mediated disruption of lysosomal membranes.

A 40 % increase in the fluorescence intensity of lipofuscin is commonly considered as an indicator of ageing. Abnormally high lipofuscin content in the retinal pigment epithelium has been demonstrated in a variety of inherited retinal disorders such as Bests' disease, Stargardts disease, Fundus flavimaculatus and in age related macular degeneration.

Intact human lipofuscin granules exhibit a broad band excitation spectrum from 300 to 620 nm, and an

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**Fig 1 Normal fundus autofluorescence imaging.**

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OPHTHALMIC INSTRUMENTATION
emission spectrum that peaks in the yellow-orange region. Melanin, the other main chromophore of the retinal pigment epithelium, though nonfluorescent, exhibits age-related fluorescence properties probably due to the combination with lipofuscin. The excitation spectrum of melano-lipofuscin fluorescence is 364 nm and emission maximum at 540 nm² (Fig 1).

Using a confocal scanning laser ophthalmoscope characteristic patterns of fundus autofluorescence in normal subjects and in patients with different retinal disorders have been described ⁶ ⁷.

Fundus autofluorescence can also be studied using the fundus camera to a certain extent.

**Fundus Auto Fluorescence Changes in early Age-related Macular Degeneration:**

Alteration in the fundus autofluorescence in age-related macular degeneration can be classified into 9 phenotypic patterns (Fig 2) including normal, minimal change, focally increased, patchy pattern, focal plaque like, linear, lace-like, reticular and speckled ⁸.

**Normal Pattern:** of autofluorescence is characterized by

1. Homogenous background autofluorescence
2. Gradual decrease in fundus autofluorescence towards inner macula and fovea due to masking effect of yellow macular pigment.
3. Normal pattern may be seen even in the presence of soft/hard drusen.

**Minimal Change Fundus Autofluorescence Pattern:** is characterized by limited irregular increase or decrease in background fundus autofluorescence without any obvious topographic pattern.

The **Focally Increased Pattern:** is characterized by the presence of at least one area < 200 μm in diameter with markedly increased fundus autofluorescence. This area will be much brighter than the surrounding background autofluorescence. This spot has well-defined borders and is usually surrounded by an area of gradually decreasing fundus autofluorescence (dark halo). These areas may correspond to areas of visible alterations in colour fundus photographs such as focal hyper pigmentation or drusen.

**Patchy Pattern:** is characterized by a larger ill defined area (>200 μm) of markedly increased fundus autofluorescence surrounded by progressively increasing background autofluorescence. These areas may correspond to a large soft drusen or areas of hyper pigmentation.

**Linear Pattern:** is characterized by the presence of at least one linear area with markedly increased fundus autofluorescence, typically well demarcated corresponding to hyper pigmented lines on fundus photograph.

**Lace-like Pattern:** shows multiple branching linear structures of increased fundus autofluorescence forming a lacy pattern corresponding to hyper pigmentation in the colour image.

**Reticular Pattern:** is characterized by the presence of multiple small areas <200 μm in diameter of
decreased fundus autofluorescence with progressively decreasing fundus autofluorescence from the centre of the lesion toward the surrounding background fluorescence. This pattern is associated with cluster of small soft drusen, hard drusen and areas of pigmentary changes (Fig. 3).

**Speckled Fundus Autofluorescence Pattern**: has the simultaneous presence of a variety of fundus autofluorescence abnormalities in a larger area of the fundus autofluorescence image.

**Geographic atrophy** in dry age-related macular degeneration is characterized by areas of retinal atrophy. Due to atrophy of retinal epithelium cells and lack of lipofuscin, fundus autofluorescence imaging in patients with geographic atrophy shows decreased fundus autofluorescence intensity over the atrophic patches (Fig 4 a & b).

Geographic atrophy (GA) represents the atrophic late-stage manifestation of “dry” AMD. During the natural course of the disease, atrophy slowly enlarges over time and the fovea itself is typically not involved until later (“foveal sparing”). Due to the distinct changes of the topographic distribution of RPE LF, the signal is markedly reduced over atrophic areas. The high – contrast difference between atrophic and non atrophic retina permits precise quantification of the atrophic areas on FAF images using customized image analysis software. This allows accurate assessment of progression of atrophy and can be used in longitudinal observations, including interventional trials. (Fig 5)

The identification of elevated levels of FAF intensities in the junctional zone of atrophy is of particular interest as these changes precede cell death. Studies using FAF imaging have reported distinct phenotypes in the distribution and appearance of these areas, while there was a high degree of intraindividual symmetry 6, 7.

Current data on spread of atrophy suggest that there is a linear growth of atrophy over time and that the best
predictor would be the growth rate in the previous year. Using FAF imaging, it has been shown that the extension of areas of increased autofluorescence surrounding atrophy patches correlates with atrophy progression over time. Further analysis within the Fundus Autofluorescence in Age-related Macular Degeneration (FAM) Study summarizing the data of 195 eyes demonstrated that the great variability and range of atrophy enlargement between patients is largely dependent on the specific pattern of FAF abnormalities at baseline outside the atrophic patches. Eyes with the banded and the diffuse FAF pattern showed a more rapid enlargement compared with eyes without FAF abnormalities and the focal FAF pattern. Within the diffuse pattern group, eyes with a diffuse trickling pattern exhibited an even higher spread rate (median 3.02 mm²/year) compared to the other diffuse types. As there is high degree of intraindividual symmetry, genetic determinants rather than non-specific ageing changes may be involved.

In the junctional zone surrounding atrophy, areas of increased fundus autofluorescence intensities and excessive retinal pigment epithelium lipofuscin load can be identified. This area of increased fundus autofluorescence has been clearly shown to precede new patches of atrophy or enlargement of preexisting atrophic patches. Atrophy enlargement varies between 0 and nearly 14 mm²/year with a mean rate of progression between 1.74 mm²/year and 2.79 mm²/year. Very small areas show slower spread of atrophy, but variations in atrophy enlargement cannot be totally explained by baseline atrophy (Fig. 4).

Areas of atrophy can be accurately delineated, quantified with image analysis software and atrophy progression rates can be calculated. Hence it is an easy, feasible, noninvasive imaging technique to review patients with geographic atrophy over time.

Patients with neovascular age-related macular degeneration with choroidal neovascular membranes if of recent onset shows areas of hyperfluorescence on fundus fluorescein angiography corresponding to areas of normal AF with adjacent areas of increased fundus autofluorescence. Preserved AF indicates viable retinal pigment epithelium initially which has implications for treatment interventions and long term visual prognosis. However in patients with choroidal neovascular membrane of long duration the fundus autofluorescence over the affected area is decreased indicating loss of retinal pigment epithelium and photoreceptors (Fig 6).

Theoretical consideration would suggest that FAF imaging may give important clues in choroidal neovascularisation (CNV) secondary to AMD. It may be helpful to assess the integrity of the RPE, which may influence the development and behavior of new vascular complexes as well as photoreceptor viability and potential therapeutic success.

Patients with early CNV secondary to AMD tend to have patches of continuous or normal auto fluorescence...
corresponding with areas of hyper fluorescence on the comparative fluorescein angiograms, implying that RPE viability is preserved at least initially, during CNV development. By contrast, eyes with long standing CNV typically exhibit more areas of decreased signal.

Comparing FAF finding with the classification of occult and classic CNV based on fluorescein angiography, focal areas of decreased FAF were reported to be more prevalent in classical CNV in comparison to larger occult CNVs. Mc Bain and associates confirmed this findings and speculated that typical low FAF signals at the site of the CNV are related to absorption phenomena caused by the CNV growing in the subretinal space, rather than being related to severe damage to the RPE. However a more recent study could not demonstrate any significant effect in FAF alterations between occult and classic CNVs secondary to AMD. A continuous pattern of preserved auto fluorescence in the central macula was observed in most patients, and this was correlated with better visual acuity, shorter symptom length, and smaller lesion size.

In contrast to the data in patients with advanced atrophic AMD, the predictive value of areas with increased FAF intensities remains unclear. Looking at 125 eyes with soft drusen and no history of laser treatment, a longitudinal analysis (mean follow up: 18 months) within the FAM study reported 9 eyes with development of advanced exudative AMD during the review period. Six of these 9 eyes exhibited the so-called “patchy” FAF pattern at baseline, which may represent a high-risk marker for progression to advanced AMD.

**FAF in Acute and Chronic Recurrent Central Serous Chorioretinopathy:**

Definite FAF patterns can be made out in both acute and chronic CSR. AF is therefore an interesting tool to apply to differentiate between acute and chronic CSR. In acute CSR, decreased AF is due to blockage caused by oedema. Whereas in chronic recurrent forms irregular and increased AF is observed reflecting reactive RPE changes secondary to RPE defects and neurosensory detachment. Another change observed is the decreased AF at the point of leakage due to SRF blocking the AF or RPE atrophy at the leakage point itself as is presumed in chronic recurrent cases.

**FAF in STGD-FFM (Stargardts- Fundus Flavimaculatus):**

Although previous reports found high levels of AF in all patients with Stargardts fundus flavimaculatus disease some may have normal or low levels of FAF (Fig 8). There seems to be a relationship between patterns of AF and peripheral functional abnormalities. Patients with low levels of AF at the centre of macula, including the fovea and normal / low levels of AF.
temporally and nasally had peripheral rod and cone dysfunction. Thus it appears that patients with this pattern of autofluorescence have more widespread disease. However there appeared to be no relationship between the degree of AF at macula and macular dysfunction as detected by PERG, since all patients had marked PERG abnormalities independent of levels of AF. (Fig. 9)

**FAF in Macular Holes:**

Autofluorescence imaging is useful for the diagnosis and staging of macular holes and is comparable with the results of fluorescein angiography. AF imaging demonstrates the bright fluorescence of macular holes with an appearance similar to that obtained in fluorescein angiography. In contrast macular pseudo holes showed no AF. The attached operculum in stage 2 and the detached operculum in stage 3 macular holes showed focally decreased AF. The associated retinal elevation and cuff of SRF were less fluorescent compared with the background AF of the normal fellow eyes. Following successful surgical treatment, the AF of macular holes was no longer visible.

Being noninvasive and rapid, AF imaging may become a useful alternative to fluorescein angiography in the assessment and differential diagnosis of full thickness macular holes.

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**References**


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**Fig. 10:** Fundus autofluorescence imaging in macular holes demonstrating increased autofluorescence at areas of the hole.
Patients and Patience

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Several attributes make up a successful practitioner of Ophthalmology. Good clinical sense and better common sense are among them. But if you ask me the most important one would be patience. It is like Patience begets Patients. Patience is most needed while asking questions and listening to the answers. But some patients very definitely test the patience of even the most patient of you.

Classically you allow the patient to talk about his/ her symptoms in his/ her own words. But there are some whose words just will not stop. The Verbal Diarrhoea Type. They will go on and on about the background of their illness till you want to interfere. “And she was telling me about that beauty parlour lady who stays in the corner house. Mind you, I never listen to gossip. It seems this lady... blah...blah... blah... and then I suddenly felt a pain in my left eye and I asked her to stop”. By that time you would want to ask her to stop. But you just grin and bear it. But sometimes real significant facts do come out of the jumble.

Then there is the Know All Type who many a time is a relative of Mrs. Malprop. “Then Dr. So and so told me that I had BP of the eye and asked me to use ‘Toilet’ eye drops twice a day. My sister also has BP of the eye but uses ‘Latrine Post’ eye drops”. She would blithely confess. Or it would be about the ‘plain glasses’ given to him for his ‘far sightedness’ by the doctor in Bangaluru. They may provide some lighter moments.

The Forgetful Type can be equally testing. In spite of you printing “Bring this with you on the next visit”, the previous prescription is invariably forgotten. And so are the present glasses. “I thought I will check my eyes while waiting for the appointment with my beautician. That is why I didn’t bring my glasses”. And when you ask if they remember the power of the glasses, fifty percent will say ‘point five’ and the other fifty percent ‘two point five’.

The Doubting Type can put gray hairs on your scalp. ‘Read the last line’, you would say. “The very last?” ‘Yes’. (Is there any other ‘last’?). “At the bottom?” ‘Yes, yes’. “The Smallest line?” ‘Yes’, you would say tearing out the (grey) hairs by the handful. And he will read ‘To be held in good light 14 inches from the eye’! And he is the one who will call you up at 11 pm to ask if he should put two or three drops each and which eye to put the drops first, right or left. Some years back I had a post-op. patient who used to come with a list (yes, list of doubts written down). ‘Can I go up the stairs?’ ‘Can I go down the stairs?’ were two of them. I was sorely tempted to answer the first in affirmative and the second in the negative.

In a Lighter vein

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