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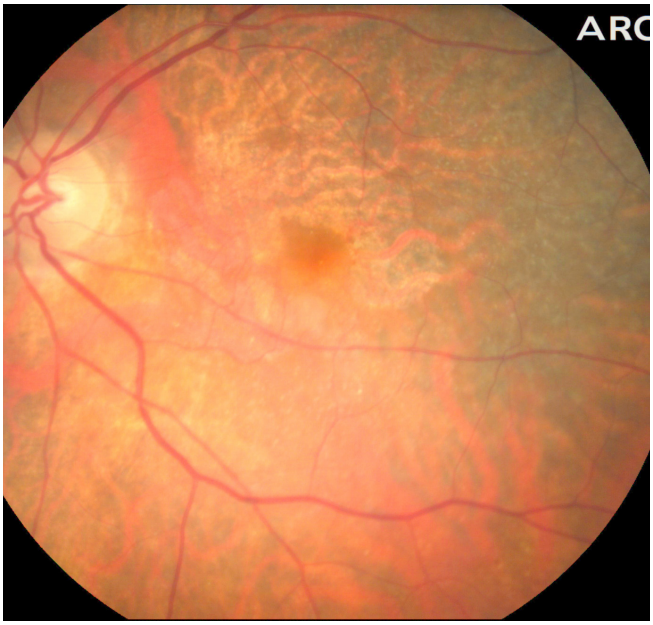
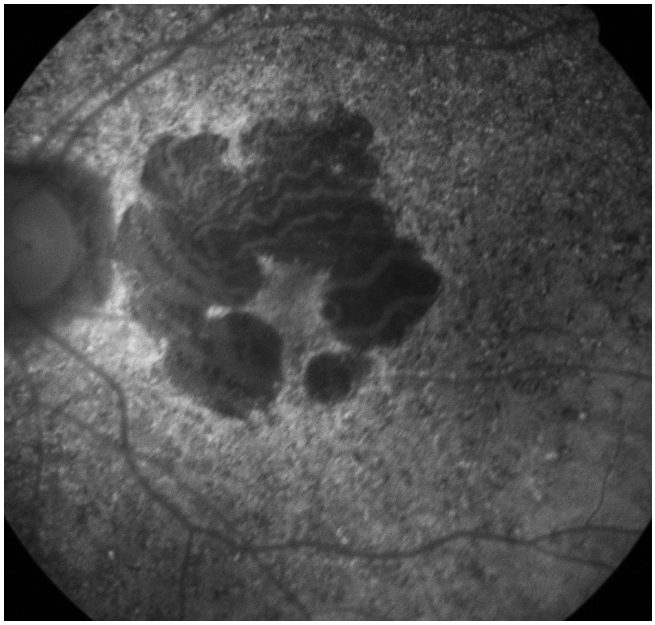
Auto-Fluorescence in Clinical Practice using a modified Canon Retinal Camera

Fundus Auto-Fluorescence (FAF) imaging is probably the most exciting new affordable clinical technology to arrive in optometric practice since the OCT.

Lipofusin is a pigment which glows, or Auto-fluoresces, with a reddish golden yellow colour if excited with a blue green light. We can thus evaluate the amount of Lipofuscin present in the retina. This is the basis of FAF imaging. Lipofuscin exhibits a broad emission spectrum from 500 to 750 nm with peak emission at about 630 nm.



Picture (A) Apparantly "normal" retinal appearance



Picture (B) Same eye, FAF demonstrates significant geographic atrophy

The RPE digests the by-products of the visual cycle generally very efficiently by phagocytosis. There is a small proportion of the degradation products from the phagocytosed outer segments of the rods and cones and mitochondria that cannot be eliminated. This accumulates in the lysosymes of the RPE as Lipofuscin and related products.

Lipofuscin deposition normally increases with age, but may also occur from RPE cell dysfunction or an abnormal metabolic load on the RPE. The dominant fluorophore in lipofuscin is believed to be A2-E, a compound that may interfere with normal RPE cell and photoreceptor function, by producing toxic oxidative radicals, when stimulated by light. After the age of 70 lipofuscin levels level out or sometimes even decline. We are not sure exactly why this occurs, but may be due to evacuation of the lipofuscin into the extravascular space as precursor material leading to drusen formation.

As an independent prescriber I work closely with local vitreo-retina and medical-retina specialists.

One of our main concerns was to try to identify which of our early dry macular degeneration patients were at high risk of visual loss and needed aggressive management. Auto-Fluorescence gives us an additional tool to categorise our dry ARMD patients into high and low risk groups, after taking into account all the other risk factors.

The systems currently commercially available up to now have been Confocal Laser Scanning Ophthalmoscopes (HRA, Spectralis and Optos 200TX systems).

These produce excellent images but unfortunately this comes at a high price. They also occupy a considerable amount of valuable floor space.

A few years ago I commissioned Pat Falvey of ARC Optical to develop a system to enable us to assess Auto-Fluorescence using our existing retinal camera systems.

We wanted to be able to offer this additional service without removing the patient from the eye examination hair and having to take them to a different room. We already have floor space restraints imposed by all the existing high technology we currently utilise. I have been using the system for the last year or two with impressive results.

ARC OPTICAL

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Confocal laser ophthalmoscopes, such as the Spectralis system use the same exciting(488nm - argon laser) and emmission filter setting(500-520 nm) as is used for fluoroscein angiography. For this reason, cSLO FAF must be done before fluorescein angiography as any traces of fluorescein will mask the relatively weak lipofuscin autofluorescence signal.

With the fundus camera systems excitation is usually in the green spectrum (535 to 580 nm) and emission is recorded in the yellow-orange spectrum (615 to 715 nm. As a result fluorescein angiography has a negligible effect on any autofluorecence imaging taken afterwards

The fluorescence that Lipofuscin shows is very weak, about 100x weaker than the retinal fluorescence seen during fluorescein angiography. As a result, any camera system must be very sensitive. In addition, the flash intensity cannot be made too high or there will be backscatter from the crystalline lens which will degrade the image.

Modern retinal camera systems use a longer excitation wavelength which reduces the absoption by the crystalline lens of which fluoresces between 500-550nm.

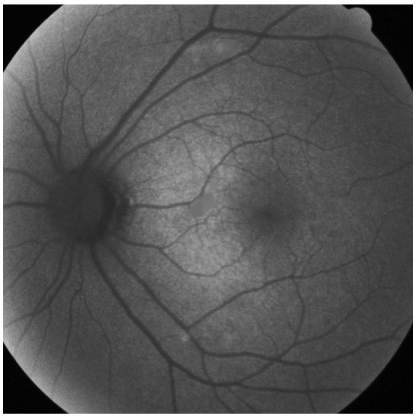
The optic disc and blood vessels appear dark in an autofluorescence picture, more so with the cSLO than the retinal camera version. The optic disc has no lipofuscin and the retinal vessels mask the autofluorescence from the deeper layers. Blood does not absorb the longer wavelengths used in the fundus camera systems as much as the shorter wavelengths used with cSLO systems. As a result the blood vessels do not appear at such a high contrast as the very black definite vessels seen in cSLO images. The same applies to the optic disc which shows some fluorescence with the longer wavelengths.

The FAF is less intense in the foveal region probably due to the protective effect of the macular pigments, lutein, zeaxanthin and the RPE melanin. Another possible explanation is that the cone phagocytosis may produce less lipofuscin than that of the rods. The longer wavelengths are not absorbed so much by the macular pigments so the macular region does not appear so dark with fundus camera systems as with the cSLO.

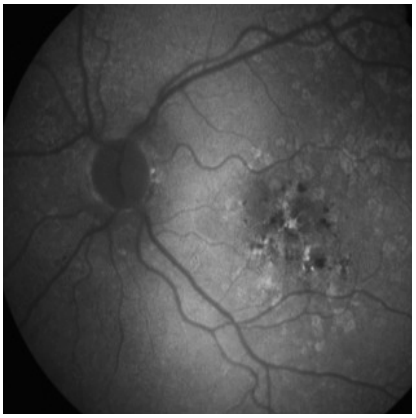
Disc Drusen are often better visualised with the modified retinal camera system rather than the cSLO, especially if buried deeper in the optic nerve. The autofluorescence associated with CSR and CNV is also better seen with the retinal camera system than the cSLO.

This may be explained by differences in the excitation and barrier filters between the 2 systems, meaning that slightly different fluorophores may be involved, including at a reduced level, collagen and elastin. Also the non-confocal nature of the fundus camera means that slightly out of focus autofluorescence will be imaged better.

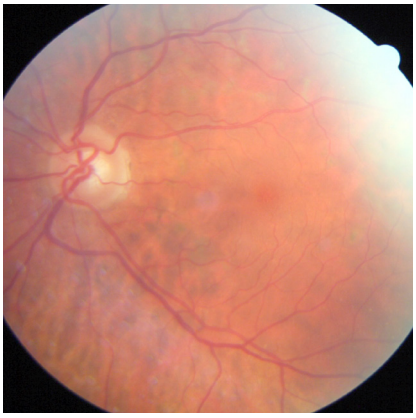
The fundus camera also allows for greater control of camera settings such as focus, flash intensity, gamma settings etc. This may make for better AF images. It does mean that slightly more operator intervention can be needed at times.



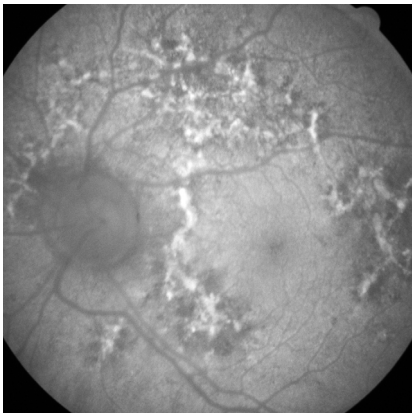
Picture (C) Normal retinal autofluorescence appearance



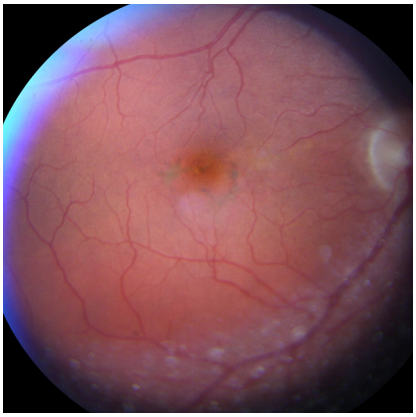
Picture (D)Showing retinal autoflourecence appearance in AMD whith both hyper and hypo-fluoresent lesions



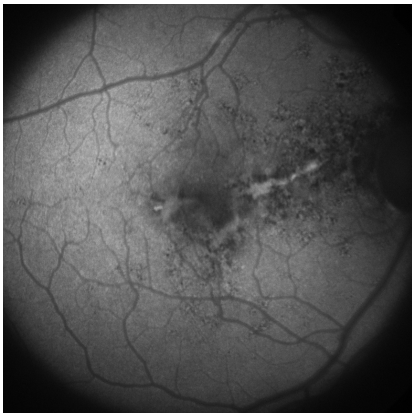
Picture (E) Apparently normal retinal appearance



Picture (F) FAF shows otherwise



Picture (G) Early pigment mottling



Picture (H) FAF shows the fuller picture

Anything that reduces the amount of the exciting wavelength reaching the retina, will reduce the FAF signal seen. Examples include extra-vascular blood and lenticular opacification.

In the normal retina of a 40-year old the AF retinal photo has a uniform grey background colour Picture (C). In a “sick” retina the Lipofuscin first builds up into clumps of Hyper-Autofluorescence, which shows as brighter white patches against the uniform grey background Picture (D). As the RPE cells deteriorate further, the hyper-fluorescent white areas often become Hypo-fluorescent dark patches as the RPE atrophies. As the atrophy progresses the dark, lipofuscin-free patches grow in size, reflecting the true visual state of the retina, not always obvious when viewed by other means Picture (E).

In early ARMD focally increased AF appears to indicate the likely progression to Geographic Atrophy, rather than CNV as the mechanism of further visual loss.

A reduction in AF indicates loss of either RPE or photoreceptor/photoreceptor outer segments. This loss often appears subtle on biomicroscopy or standard retinal photography and can be easily missed(see picture A). AF photography shows such defects up clearly, giving us a better understanding of outer retinal health (picture B) which clearly shows areas of extensive atrophy surrounded by "hot-spots" of lipofuscin accumulation, indicating areas likely to atrophy in near future.

In the next two photos, at first show an apparently "normal" colour fundus appearance(picture E) in contrast to the definitely "abnormal" FAF photo taken of the same eye minutes afterwards (picture F). This shows the time bomb effect the accumulating lipofuscin has on the retinal pigment epithelium, which before we had FAF imaging we were blissfully unaware of.

In picture G we see early macular pigment mottling, but little else to indicate the changes at RPE level shown by the following FAF image (picture H).

It is the accumulation of lipofuscin in Stargart's Macular Degeneration that is thought to be implicated in the characteristic atrophy seen in this condition (see pitcures J & K). The bright flecks often seen in Stargardts Diseaseare shown histologically to be RPE cells so engorged with Lipofuscin that they become distorted to 10x their normal size.

It is is likely that the damage is caused by the release of oxidative radicals on photostimulation of the lipofuscin pigment. The highest levels of oxidative radicals being produced at high light intensities and shorter blue end of the visual spectrum. There is evidence that the photo-reactivity of lipofuscin increases with age, thereby increasing the oxidative damage it can cause.

In the images of Stargart's patients, you instantly can see the extent of their visual disability, shown by the black areas of RPE atrophy, not necessarily appreciated by viewing their standard retinal images.

Future Therapeutic Applications

It may be that future research into developing therapies to minimise lipofuscin accumulation in the RPE may help us reduce the progression of visual loss in conditions such as ARMD and Stargardt's Disease. FAF imaging may help direct us more accurately to the patients who would most benefit from such therapeutic interventions and also monitor their effectiveness. Theoretically, Stargardt's patients should be advised to avoid vitamin A supplements/beta-carotene and overexposure to sunlight.

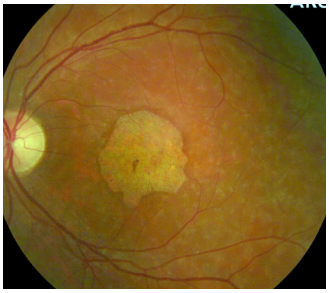
In patients with advanced atrophic AMD, FAF imaging may also be helpful to develop and assess new emerging therapeutic strategies. Visual cycle modulators, which aim to target the detrimental accumulation of toxic by-products of the visual cycle in the RPE, appear as promising pharmaceutical agents to slow down the progression of atrophy.

Dietary reduction of Calorific intake, vitamin E and glutathione supplementation are reported to reduce the production of lipofuscin.

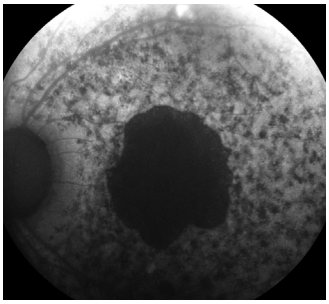
Conclusion

FAF imaging gives us new information in conditions where the health of the RPE plays a key role. FAF records metabolic changes due to the accumulation of toxic fluorophores in the retinal pigment epithelium. Hyper-autofluorescence is a sign of increased lipofuscin accumulation, which may indicate degenerative changes or oxidative injury. Areas of hypo-autofluorescence indicate missing or dead RPE cells.
FAF imaging has a valuable place in a variety of retinal diseases including: age-related macular degeneration, retinitis pigmentosa, central serous chorioretinopathy, Best's disease, cone dystrophies, and Angiod Streaks. There may be a common pathogenic mechanism in these conditions.
We still do not fully understand role of lipofuscin in macular degeneration, but increased autofluorescence may precede development or progression of geographic atrophy in ARMD. Patches of Hyper-FAF are often present in a junctional zone next to existing atrophy and may indicate the likely extension of that atrophy or the development of new areas of atrophy.

Sequential FAF imaging allows us to follow these retinal changes over time. As FAF imaging becomes more commonly used in future years our understanding will increase and it may give us a very valuable tool to monitor the effectiveness of future treatments for these conditions. The Modified Retinal Camera enables us to have this cutting edge diagnostic technology at an affordable price, without taking up ang more valuable floor space in your consulting room.



Picture (J) Starqardt's lesion retinal appearance



Picture (K) FAF shows more extensive atrophy

For more information

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