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Retinal Auto Fluorescence (RAF): Clinical applications of Confocal Blue laser RAF

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Last decade has seen refinement in technology to obtain fundus or Retinal Auto-Fluorescence (RAF) images which has greatly facilitated the process of capturing RAF images and also improved the quality of the images, particularly the contrast and the details of the RAF patterns of retinal lesions. These technological advances such as confocal laser scanning ophthalmoscopy and improved optical filters coupled with the availability of descriptive normative data on RAF have established this modality of imaging as a useful everyday clinical tool which can be applied for diagnosis and monitoring on a wide variety of retinal conditions. Hence RAF is now commonly used by many retinal clinicians routinely to aid diagnosis as well as help in management of retinal conditions in judging response to treatment and to predict outcome/ prognosis. Interpretation of autofluorescent patterns is based on understanding of normal distribution of lipofuscin based on observing presence of normal or altered natural autofluorescence in the retina.

Background:

Autofluorescence is the natural ability of a biological structure to emit light of a longer, less energetic wavelength after absorbing light of a shorter and higher energy wavelength. The human retina has autofluorescent properties owing to the presence of molecules, which contain parts called fluorophores that make them autofluorescence after exposure to light of specific wavelengths. The main source of retinal autofluorescence utilised in clinical practice is from lipofuscin located in the retinal pigment epithelium (RPE) cells[1,2,3]. Short (blue) and medium (green) wavelength light can excite lipofuscin related autofluorecence that is captured by commercially available SLO scanners. A confocal system provides the ability to place the excitation light and capture of the emitted light from the same plane giving high contrast images. Patterns of autofluorescence from the retina captured in this way depends on the health of the RPE and also any structures that would normally or abnormally block the transmission of this emitted light through the layers of fundus anterior to the RPE.

Capturing retinal autofluorescence is not new and has conventionally been performed using commercial fluorescein angiography cameras using the standard filters which allow passage of fluorescent light to capture highly autofluorescent lesions such as optic disc drusen without injection of the fluorescein dye.. The fluorescence emitted in many other conditions however is usually of low intensity and this standard, conventional method of capture using non-confocal system is insufficient for capturing it. Instead of using a light bulb and filters for conventional fluorescein angiograms, the incorporation of laser devices to generate accurate excitation wavelengths and specially developed filters in refined digital cameras to capture the low intensity light that is emitted has helped to refine images of high quality with good contrast and resolution. At present RAF images can be obtained with some commercially available camera systems in addition to standard fluorescein cameras. Of the commonly available systems, Topcon utilises modified fundus photography technique with Yellow – Green wavelength light, while Optos utilises green wavelength (532nm). Of the Two commercial systems using Blue light RAF, to our knowledge only Heidelberg is currently in production and clinical used. Heidelberg and Optos both are SLO systems though it needs to be recognised that autofluorescent patterns are different with Blue light and Green light; so comparisons cannot be made between these two systems. The most commonly used confocal blue laser autofluorescence system is the Heidelberg Spectralis Blue Peak Retinal Angiograph or Spectralis OCT with Blue peak System (488nm). This article is focussed on the wide spectrum of clinical applications of retinal auto-fluorescence (RAF) using Heidelberg's Blue peak technology with particular emphasis on the principles behind the interpretation of abnormal RAF patterns.

Technique:

Taking retinal images for autofluorescence require the same technique as retinal photography, however one needs to be familiar with the imaging kit to ensure autofluorescence mode is selected for image capture. After positioning the patient on the camera and with the eye aligned for uniform illumination of the retina, a standardised protocol for RAF acquisition should be followed since the autofluorescence pattern differs depending on adaptation of retina, for example bright flash used of retinal photography bleaches retinal pigments. Ideally autofluorescence images should be taken prior to colour photography especially retinal angiography. In situations where, an eye is exposed to bright light/ flash, sufficient interval should be allowed for retina to recover from the bleaching effect. The latter is prolonged in cases of retinal dystrophies especially. It is recommended to defocus the camera by -1 D (from the infrared focus) to get confocal plane of the RPE. The eye should be bleached for 30 seconds with blue light (this takes out masking by rhodopsin). Eye tracking should be used and a minimum of 10 frames of images should be available for averaging to get best possible image. The pre bleaching is useful to reduce masking impact of visual pigments in vertically aligned photoreceptors and providing clearer picture of lipofuscin related autofluorecence from the RPE.

Clinical Patterns of RAF:

Normal RAF

In a healthy eye the short wavelength blue light leads to autofluorescence emitted from the lipofuscin in the RPE that is seen as varying intensity of signal reflected from RPE, where brighter pixel represents more autofluorescence.

Normal RAF pattern has an even glow of low hyperfluorescence from the RPE. The optic disc, which does not have RPE, appears black, the retinal vessels also appear dark as they block the emitted light from the RPE. Around the fovea, there is normally least autofluorescence due to the blockage of emitted light by the lutein pigment that is normally concentrated in Muller cells in healthy foveal zone and parafoveal zone (Figure 1a).

Alterations in RAF patterns are described in various classifications and harmonisation of these terms is needed. The descriptive terms used include focal, diffuse, linear, banded, speckled/ granular, reticular or homogenous[4]. The significance of different patterns of altered (usually high) autofluorescence is emerging.

Table: Causes of altered retinal autofluorescence (RAF)

Decreased RAF	Increased RAF
RPE loss / atrophy	Excessive lipofuscin
Intraretinal fluid	Low visual pigments
Reduced RPE lipofuscin	Drusen
Fibrosis	Thin retina
Luteal pigment	AMD
Blood / exudates	

*Quality of image may be affected by media opacity

Decreased RAF

The normal glow of RAF is reduced in conditions that result in loss of RPE cells as seen in RPE rips (Figure 1b) and Geographic Atrophy GA (Figure 1c). RAF images can clearly outline the area of RPE atrophy/ loss. It is also observed in certain inflammatory condition that has caused RPE damage, multifocal choroiditis, puntcate inner choroidopathy and AZOOR for example. The normal autofluorecence can be masked by any abnormality anterior to the RPE, thus blocking the transmission of emitted light from the underlying healthy RPE- e.g. retinal haemorrhages, lipid exudates, subretinal fluid or fibrosis. Decreased RAF is also useful in identifying specific conditions where there is absent RPE cells such as angioid streaks.

Increased RAF

Increased RAF was described in various retinal dystrophies initially. Increased RAF is also commonly caused by an abnormally high amount of lipofuscin seen in some macular dystrophies such as vitelliform dystrophies and bestinopathies that are characterised by lipofuscin collection (figure 2 a, b,). In RP a mixed pattern of masking (from pigment) and window defect (from thinned retina) can be seen.

Increased lipofuscin collection in the RPE is also recognised as an important feature for AMD pathology. Increased RAF is seen due to excessive accumulation of lipofuscin in RPE and drusen in early AMD[2,5]. In wet AMD, a mixed pattern is seen with masking in area of haemorrhage and fluid, focal loss of RAF with CNV and small area of increased RAF around the CNV is not uncommon. Increased RAF is also seen in areas with metabolically abnormal RPE cells as seen around lesions of geographic atrophy and can evolve into complete atrophy with time. Various patterns of increased RAF seen around GA namely banded, diffuse and focal patterns are subject of further research in progression and treatment of GA.

Increased RAF pattern is seen in conditions where there is reduced blockade due to thinning of the retinal layers[3] e.g. in chronic CSR and AZOOR and especially in macular telangiectasia where there is loss of the natural blockage from the lutein in the foveal area. RAF is particularly useful cases of CSR without subretinal fluid on OCT, where typical gravitational RAF tracks can help to establish the diagnosis of previous CSR (figure 2c) and help the clinician to explain the underlying cause of reduced vision.

White dot syndromes are rare inflammatory disorder that involve choroid, RPE and retina, often presenting a challenge in diagnosis where RAF provides a useful tool as increased RAF is seen MEWDS; while PIC and MFC can start with increased RAF but this can be hypofluorescent too. The latter is associated with poorer visual outcome.

Conclusion:

RAF is a novel non-invasive imaging technique that provides metabolic and functional information of retina (RPE). Availability of RAF in commercial equipment has helped our understanding and diagnosis of a number of retinal disorders. It is increasingly being used in routine retina practice especially for retinal dystrophies, AMD and white dot syndromes, and indications where it is useful are getting broader. Further refinement in technology and introduction of wide field lens will further enhance its use both in clinical research and practice, especially with precision in phenotyping diseases and potential linking with biomarkers of specific diseases.

It is expected that this summary will provide clinicians a practical reference source for clinical practice.

Figures and legends:

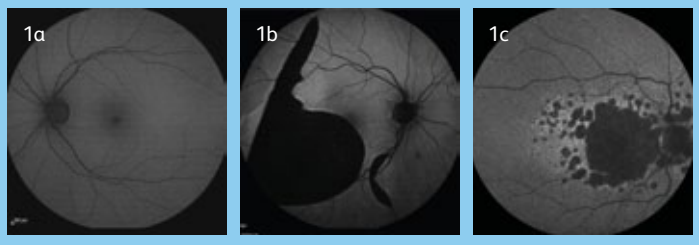

Fig 1. Normal autofluorescence (a), hypo autofluorecence due to no RPE in RPE rip (b) and geographic atrophy (c). Note speckled hyperfluorescence around area of GA.

Fig 2. Hyper autofluorescence: (a) Best's vitelliform dystrophy with characteristic hyperautofluorescence of lipofuscin deposition. (b) Stargardt's disease with hyperfluorescent flecks in macula. (c) a case of CSR note diffuse hyper autofluorescence that tracks inferiorly from the superior temporal arcade. The focal spots of hypoautofluorescence in this case corresponded to IPCV.

References:

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