ABSTRACT

**Purpose.** Recent technological advances in fundus autofluorescence (FAF) are providing new opportunities for insight into retinal physiology and pathophysiology. FAF provides distinctly different imaging information than standard photography or color separation. A review of the basis for this imaging technology is included to help the clinician understand how to interpret FAF images. Cases are presented to illustrate image interpretation.

**Methods.** Optos, which manufactures equipment for simultaneous panoramic imaging, has recently outfitted several units with AF capabilities. Six cases are presented in which panoramic autofluorescent (PAF) images highlight retinal pathology, using Optos' Ultra-Widefield technology. Supportive imaging technologies, such as Optomap® images and spectral domain optical coherence tomography (SD-OCT), are used to assist in the clinical interpretation of retinal pathology detected on PAF.

**Results.** Hypofluorescent regions on FAF are identified to occur along with a disruption in the photoreceptors and/or retinal pigment epithelium, as borne out on SD-OCT. Hyperfluorescent regions on FAF occur at the advancing zones of retinal degeneration, indicating impending damage. PAF enables such inferences to be made in retinal areas which lie beyond the reach of SD-OCT imaging. PAF also enhances clinical pattern recognition over a large area and in comparison with the fellow eye. Symmetric retinal degenerations often occur with genetic conditions, such as retinitis pigmentosa, and may impel the clinician to recommend genetic testing.

**Conclusions.** Autofluorescent ophthalmoscopy is a non-invasive procedure that can detect changes in metabolic activity at the retinal pigment epithelium before clinical ophthalmoscopy. Already, AF is being used as an adjunct technology to fluorescein angiography in cases of age-related macular degeneration. Both hyper- and hypoautofluorescent changes are indicative of pathology. Peripheral retinal abnormalities may precede central retinal impacts, potentially providing early signs for intervention before impacting visual acuity. The panoramic image enhances clinical pattern recognition over a large area and in comparison between eyes. Optos' Ultra-Widefield technology is capable of capturing high-resolution images of the peripheral retina without requiring dilation.

Key Words: fundus autofluorescence, Optos, panoramic, retinal imaging technology, retinal pathology, retinitis pigmentosa, spectral domain optical coherence tomography, SD-OCT, ultra-widefield

**BACKGROUND**

Recent technological advances in fundus autofluorescence (FAF) are providing new opportunities for insight into retinal physiology and pathophysiology. Optos, which manufactures equipment for simultaneous panoramic imaging, has recently outfitted several units with AF capabilities. Several cases are presented in which panoramic autofluorescence (PAF) provides new diagnostic and/or pathophysiological data.
(RP), Cone- and Cone-rod Dystrophies, X-linked Retinoschisis, Best Disease, Stargardt Disease, Diabetic Retinopathy, Wilson Disease, and Choroidal Tumors. In recent years, AF technology has been used in retinal imaging to provide supplementary information on the disease process and progression timeline.

FAF uses the excitation of fluorophores inherent in the retina. Fluorophores release a packet (quantum) of light as they change from their excited state to their resting state. The light emitted by the fluorophores is then recorded to create a brightness map, which is the image. To capture this light specifically, a barrier filter is inserted along the return path of the light. This transmits light over a specific range of wavelengths, including the wavelengths which match the emissions of retinal fluorophores. The barrier filter blocks most of the light which would reflect off the retina in a typical photo.

Different AF systems may display slightly different patterns of AF. This is due to variations in the excitation wavelengths used and in the fluorescent wavelengths which are detected, depending on the transmission spectrum of the barrier filter being used to screen for the fluorescing light. Different excitation wavelengths/barrier filters for emission can be paired to probe for specific autofluorescent elements. For example, excitation at 470 nm with detection ≥510 nm preferentially details LF. However, this typically results in a lack of AF at the fovea, masked by the absorption of the excitation wavelength by the macular pigments (see Fig. 1d). Excitation at 550 nm with detection >600 nm preferentially details Bruch’s membrane deposits, as found in drusen.

Lipofuscin as a Marker for Disease

The value of FAF lies in the use of LF as a marker for disease processes. LF accumulates in the RPE as a result of three primary processes: lysosomal dysfunction, autophagy, and cellular stress. When lysosomes in the RPE fail to completely degrade the LF, the pH of the intracellular environment is altered, leading to cellular dysfunction. Such cellular dysfunction contributes to photoreceptor dysfunction and degeneration. LF is accumulated in cellular dysfunction via these three main mechanisms.

Lysosomes are the cellular components responsible for degrading and recycling cell materials. With age and with neuronal dysfunction, lysosomes fail to work as efficiently, with the result that LF and its components are not fully broken down, left to accumulate in the cell.

Autophagy is the process by which cellular components are transported to the lysosome for degradation. When the rate of autophagy decreases, again, LF accumulates in the cell. In turn, the excess accumulation of LF may interfere with the autophagy process, further hastening LF accumulation.

Cellular stress results when excess reactive oxygen species (free radicals) alter the intracellular environment and alter the pH to

FIGURE 1.

Case 1: Normal 25-year-old patient, Optos images OS. (a) Color Optomap; (b) red separation; (c) green separation; and (d) PAF. Note that granular appearance is normal, representing variable levels of LF in the RPE. Note that there is reduced AF at the fovea due to absorption of the excitation wavelength by macular photopigments. A color version of this figure is available online at www.optvissci.com.
become more acidic. Again, lysosomal dysfunction is largely responsible for these environmental changes, resulting from the inefficient break-down of cellular components. Senescent (aging) mitochondria which fail to be broken down in the lysosome contribute to the build-up of reactive oxygen species in the cell.¹

Each cell acts independently within its own environment. In this way, a cell which is functioning efficiently tends to continue to function, without excess LF accumulation. Conversely, a cell which is under stress begins to accumulate additional LF, which interferes with cellular processes, hastening LF accumulation. For this reason, LF accumulation in the RPE does not appear uniformly across the retina.

Secondary Fluorophores

In addition to the lipopigments, there are several other fluorophores found in the eye which may contribute to the AF signal.³ These include:

- Bruch’s membrane deposits (i.e., drusen)
- Macular pigment⁶
- Flavins in the inner retina
- Collagen⁷
- Hyaluronic acid (in the vitreous)⁸
- Choroid (with contributions from elastin, collagen, porphyrins, melanocytic melanofuscin, and macrophages)⁹
- Sclera⁹
- RPE melanin³

Applications of Fundus Autofluorescence

Increases in AF indicate an increase in the presence of LF. Reduced AF indicates loss of photoreceptors, or their outer segments, or of the underlying RPE.¹⁰ LF has been associated with retinal degeneration. It can be observed in early drusen.² In fact, the presence of LF may serve as an immunogenic stimulus for localized inflammation.¹¹ As addressed here, excess accumulation of LF is a marker of RPE dysfunction, whether by natural aging or any of the lysosomal dysfunction-related mechanisms. Zones of RPE have been observed to exhibit increased FAF (hyperfluorescence) preceding atrophy.¹²⁻¹⁴

FAF has been studied in the central retina, revealing certain patterns. For example, macular dystrophies such as Stargardt disease present with focal rather than continuous lesions.¹⁰ Outside of the macula, FAF appears normal in these cases.¹⁵⁻¹⁶ Disease progression is marked by the presence of new sites of increased AF followed by atrophy of these regions (dark spots on imaging). Bull’s-eye macular dystrophies tend to present as a continuous ring of increased AF, followed by a non-fluorescing ring of photoreceptor cell loss.¹⁵,¹⁷

AMD has certain expected patterns of FAF. When present in AMD, drusen do not fluoresce brightly. Focal increases in AF in

FIGURE 2.

Case 2: 56-year-old woman. Optos images OS. (a) Color Optomap; (b) red laser separation; (c) green laser separation; and (d) PAF. Pigment clumping visible in (a) and (c) but does not predict AF pattern observed in (d). A color version of this figure is available online at www.optvissci.com.
FIGURE 3.
Case 2: 56-year-old woman. Optomap color fundus and PAF comparison OD (left, a and c) and OS (right, b and d). Note that color fundus images exhibit only a minor difference between the two eyes (a and b) but the PAF differences (c and d) are dramatic and unmistakable with an advancing border of paracentral hyperfluorescence and peripheral hypofluorescence. A color version of this figure is available online at www.optvissci.com.

FIGURE 4.
Case 3: 63-year-old woman. (a, b) Color Optomaps OD and OS. (c, d) FAF OD and OS reveals symmetric arcuate pattern of hypoautofluorescence. Symmetry suggests a retinal degeneration. Hard exudates around the fovea OS (b) are not imaged in FAF (d). A color version of this figure is available online at www.optvissci.com.
AMD are often indicative of a risk of geographic atrophy, rather than choriotiretal neovascularization. Drusen occurring in the young, or as part of a monogenic disorder (such as Doyne Honeycomb Dystrophy), are bright on AF, in contrast with drusen observed in AMD.

Presently, very little is known about AF in the peripheral retina. Optos offers the first technological advancement which is capable of detecting FAF into the peripheral retina (Ultra-widefield AF). The simultaneous imaging of a panoramic retinal area offers the advantage of rapid pattern recognition which may easily be missed on central FAF.

Standard Optos images are obtained through a 2 mm pupil with a scanning laser ophthalmoscope, which uses two laser wavelengths: green (532 nm) and red (633 nm). Optos uses an aspheric mirror design to obtain images of about 180 to 200° of the fundus without pupil dilation. For AF, the same 532 nm laser is used as the excitation wavelength, and the FAF signal emitted from the retina is detected from a raster scan and a bright-band detector for 570 to 780 nm. Optos uses an aspheric mirror design to obtain images of about 200° of retina.

Scanning laser ophthalmoscope optics are much less susceptible to media opacities than standard fundus cameras. Because of the asphericity of the optics, the optical resolution is not linear and cannot be compared directly with standard fundus images. An approximate resolution of 10 to 11 pixels per degree has been cited elsewhere. The Optos images in this case report have a 150 pixel per inch resolution, and an image size of 26 in wide × 20.5 in high, covering about 200° of retina.

The following cases introduce PAF as a promising new imaging technology, capable of early diagnosis and/or prediction of diseases affecting the RPE and/or photoreceptors.

**Case Reports**

**Case 1**

A 25-year-old 5 D myope OU exhibits essentially normal color and AF panoramic images in each eye on dilated fundus examination (DFE). As seen in Fig. 1, the choroidal vasculature is generally best observed using red laser separation.

As a general rule, the green separation laser image highlights tissue anterior to the RPE (such as retinal nerve fiber layer) and the red laser separation reveals tissue posterior to the RPE (large choroidal blood vessels). Both typically display the RPE.

This representative PAF image from a normal fundus reveals a slightly granular glow to the RPE. The disc and blood vessels appear as black in distinct contrast to the RPE glow.

**Case 2**

A 56-year-old, 5 D myopic Hispanic female presented for a second opinion concerning her status as a glaucoma patient under treatment elsewhere with Xalatan QHS OU. Maximum intraocular pressure recorded after washout period was 18 and 19 mm Hg OD, OS. Best-corrected visual acuity (BCVA) is 20/20 in both eyes. BCVA is 20/30 OU. The flash ERG was extinguished (flat) in both eyes. The fundus appearance (obtained with DFE) is typical of RP, and a flat ERG confirms the diagnosis. PAF reveals large, midperipheral round and oval dark zones of various sizes. The hypoautofluorescent zones OU are not predictable from either ophthalmoscopy or Optos color fundus images (see Fig. 5).

Note also the hyperautofluorescent areas (both eyes) surrounding the fovea, which suggest that this large zone is under metabolic stress. Such zones of hyperautofluorescence most often progress to zones of hypoautofluorescence. Initially, it appears as if the RPE is stressed before cell death. The boundary between central and peripheral retina is hyperautofluorescent, indicative of increased metabolic activity.

**Case 3**

A 63-year-old Afro-American female patient presented for follow-up of diabetic retinopathy previously treated with perimacular focal laser OU, OS>OD. The patient denied having any difficulty seeing at night. BCVA 20/20 OD and 20/30 OS. Images were obtained on DFE. Note the large hypoautofluorescent zones around the disc with an inferior arcuate extension into the temporal retina (Fig. 4c–d). There is also a perimacular ring of hyperautofluorescence. Note very similar findings in the right eye.

In the color fundus image OS (Fig. 4b), note the hard exudates which are typically in the outer plexiform layer. Hard exudates in Henle's fiber layer tend to form a macular star. As expected, hard exudates are virtually invisible with FAF, because FAF is essentially an RPE phenomenon.

Note the remarkable symmetry in the FAF images. Such symmetry suggests a retinal degeneration. This is most likely a case of pericentral RP which often has an autosomal dominant inheritance pattern and typically progresses slowly. Electroretinograms (ERGs) and genetic testing have been recommended.

**Case 4**

A 56-year-old Hispanic male is being followed for primary open-angle glaucoma and RP. He notes severe visual field constriction and on occasion reports that he walks into walls. BCVA is 20/30 OU. The flash ERG was extinguished (flat) in both eyes. The fundus appearance (obtained with DFE) is typical of RP, and a flat ERG confirms the diagnosis. PAF reveals large, midperipheral round and oval dark zones of various sizes. The hypoautofluorescent zones OU are not predictable from either ophthalmoscopy or Optos color fundus images (see Fig. 5).

Note also the hyperautofluorescent areas (both eyes) surrounding the fovea, which suggest that this large zone is under metabolic stress. Such zones of hyperautofluorescence most often progress to zones of hypoautofluorescence. Initially, it appears as if the RPE is stressed before cell death. The symmetry of the PAF images which is quite typical of retinal degenerations.

**Case 5**

A 23-year-old Hispanic female was referred for the evaluation of RP. Her medical history is positive for epilepsy, for which she has...
FIGURE 5.
Case 4: 60-year-old man. Color Optomaps (a, b) and PAF (c, d) OD and OS. The pigment pattern on color imaging (a, b) does not predict the PAF appearance (c, d). Note the symmetric presentation, typical of retinal degenerations. Central hyperautofluorescent zones OU suggest increased metabolic activity which typically precedes disease progression. A color version of this figure is available online at www.optvissci.com.

FIGURE 6.
Case 5: 23-year-old female patient with RP. Asymptomatic for night blindness. Pigmentary changes in midperiphery consistent with hypoautofluorescent areas on PAF. Symmetric, concentric alternating zones of hypo- and hyperfluorescence begin at fovea and alternate in a ring-like pattern out to periphery. A color version of this figure is available online at www.optvissci.com.
been medicated with Lamictal for 9 years. She was asymptomatic for night blindness but showed signs of attenuated arterioles and pigment migration in the midperiphery of both eyes. She has no family history of RP or night blindness.

BCVAs were 20/30/H11002 OD and 20/30/H11001 OS. Visual fields were constricted to 30° in all quadrants, OU. ERG revealed reduced rod and cone response. PAF, obtained on DFE, reveals multiple rings of hyper- and hypofluorescence (see Fig. 6).

PAF helps to identify areas of existing and impending retinal damage with hypo- and hyperfluorescence, respectively. Note that the color, red and green, separations do not identify the zone of activity in the macula (see Fig. 7). The optical coherence tomography

FIGURE 7.
Case 5: 23 year-old woman. (a) Color Optomap; (b) red separation; (c) green separation; and (d) PAF. Although pigmentary changes are apparent in the midperiphery in all images, the Bull’s-eye appearance is most distinct in the PAF image (d). PAF helps to identify areas of damage to the RPE with hypofluorescence indicating tissue loss, as well as areas of impending damage with hyperfluorescent activity. A color version of this figure is available online at www.optvissci.com.

FIGURE 8.
Case 5: 23-year-old woman. SD-OCT through fovea OD reveals alternating ring pattern of intact and disorganized PIL, consistent with bull’s-eye appearance of FAF in macula. A color version of this figure is available online at www.optvissci.com.
(OCT) confirms the local damage about the fovea which is detectable on FAF. The photoreceptor integrity line\textsuperscript{24} (PIL) is intact directly under the fovea, with a perifoveal ring of disorganized or absent PIL, surrounded by another ring of intact PIL (see Fig. 8).

Case 6

A 46-year-old Hispanic female with very narrow angles presented for examination, complaining of reduced vision in both eyes. She denied night blindness, and her family history is negative for night blindness. Medical history is positive for systemic hypertension (controlled), systemic lupus for 19 years with secondary kidney dysfunction, and deep vein thrombosis. After diagnosis, she was treated with a high dose of steroids but has been maintained at 5 mg daily for over 18 years.

BCVA is reduced to 20/40 right eye, finger counting left eye. Refraction suggests a hyperopic shift OD, with +2.50 sph OD, +1.00 sph OS. Because of risk of angle closure, dilation was not performed. However, Optomap and spectral domain OCT (SD-OCT) imaging were obtained through undilated pupils.

Fig. 9 shows serous retinal detachment on SD-OCT imaging OD, with an area of pigment epithelial detachment identified within that zone. Fig. 10 details the loss of outer retinal structures in the macula OS, as well as an intraretinal fibrovascular scar in the inferior macula OS, likely secondary to choroidal neovascularization.

Mild hypertensive retinopathy is confirmed on color fundus Optomap (Fig. 11). Reduced clarity in macula OD is consistent with appearance of central serous chorioretinopathy (CSCR). PAF images show multiple local hypoaautofluorescent zones OD along the vascular arcades, which is sometimes observed in CSCR.\textsuperscript{25} The hyperautofluorescent macula is indicative of increased metabolic demands on RPE\textsuperscript{12–14} and also consistent with CSCR.\textsuperscript{26,27} Most of the inferior retina beyond the arcades is marked by a coarse
The remarkable amount of information obtained through undilated pupils in this case is a mark of the extraordinary advances in imaging in recent years.

**DISCUSSION AND CONCLUSIONS**

FAF identifies distinct areas of retinal damage which are not predicted by retinal pigmentary changes (cases 2, 4, and 5). Hypofluorescent regions on FAF are identified to occur along with a disruption in the photoreceptors and/or RPE, as borne out on SD-OCT (case 5). Hyperfluorescent regions on FAF occur at the advancing zones of retinal degeneration, indicating impending damage (cases 2, 5, and 6). PAF enables such inferences to be made in retinal areas which lie beyond the reach of SD-OCT imaging (cases 2 and 5). PAF also enhances clinical pattern recognition over a large area and in comparison with the fellow eye (cases 3–5). Symmetric retinal degenerations often occur with genetic conditions, such as RP, and may impel the clinician to recommend genetic testing. Such degenerations may be initially identified on PAF, as exemplified in case 3, an asymptomatic patient who is being monitored for diabetic retinopathy. Optos’ ultra-widefield technology is capable of capturing high-resolution images of the peripheral retina without requiring dilation (case 6).

Autofluorescent ophthalmoscopy is a non-invasive procedure that can detect changes in metabolic activity at the RPE before clinical ophthalmoscopy. Already, AF is being used as an adjunct technology...
to fluorescein angiography in cases of AMD. Both hyper- and hypoautofluorescent changes are indicative of pathology. Peripheral retinal abnormalities may precede central retinal impacts, potentially providing early signs for intervention before impacting visual acuity.

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