Fundus Autofluorescence Imaging

Subjects: Ophthalmology

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Non-invasive in vivo FAF imaging is based on the visualization of endogenous fluorophores in the ocular fundus. The first scanning laser ophthalmoscope was presented in 1980 by Webb et al. and continuously improved in the following years. FAF imaging using confocal scanning laser ophthalmoscopy (cSLO) was described in 1995. Since then, continuous and further developments have strengthened FAF imaging to become a safe and reproducible imaging method that is essential for the routine clinical examination of various chorioretinal diseases. Nowadays, different commercially available devices are used to record FAF. They may not be absolutely equivalent and comparable. Therefore, it is essential to understand the basic principles of FAF imaging techniques and to know the different imaging systems.

Keywords: fundus autofluorescence ; lipofuscin ; confocal scanning laser ophthalmoscopy ; fundus camera ; wide-field imaging ; choroidal melanoma ; choroidal nevus ; brachytherapy

1. Basic Principles

The term fundus autofluorescence describes the natural transient emission of light by the ocular fundus following excitation by light. More detailed, specific molecules absorb energy and achieve a higher energy level when excited by light of a certain wavelength. These excited molecules then relax to a lower energy state through the emission of light (photons) with a longer wavelength than the excitation light. The different wavelength ranges of the excitation light and the emitted light allow for the detection of the fluorescence signal using appropriate filters.

2. Retinal Pigment Epithelium and Main Fluorophores

In the human eye, the main fluorophores are multiple constituents of intracellular lipofuscin and melanolipofuscin granules at the level of the postmitotic retinal pigment epithelium (RPE) [1][2][3][4][5][6]. The RPE is a monolayer of polygonal-shaped, mitotically quiescent cells between the photoreceptor layer and Bruch's membrane/choriocapillaris complex [7][8]. It possesses various essential physiological functions, such as the absorption of light; the transport of water, ions, and metabolites from the subretinal space to the blood; the transport of nutrients from choroidal circulation to the outer retina, including light-sensitive photoreceptors; and the secretion of growth factors [9]. The lifelong phagocytosis of debris from the daily shedding of photoreceptor outer segments by the RPE [10][11] and the re-isomerization from all-*trans*-retinol to 11-*cis*-retinal as part of the visual cycle play an important role in the lipofuscinogenesis [12][13][14]. Hence, the major fluorophores are by-products of the visual cycle. They consist of a mixture of bisretinoid molecules and accumulate as non-degradable lipofuscin and melanolipofuscin granules in the lysosomal compartment of the RPE with age and in association with various chorioretinal diseases [15][16].

3. Imaging Devices

The FAF signal of a normal ocular fundus is weak. Autofluorescence from other tissues, as well as the absorption of excitation and emitted light, especially by the crystalline lens, may confound the detection of FAF. Therefore, appropriate imaging technology is required to amplify the FAF signal and to record images of adequate quality and contrast [17][18]. Different devices need to be considered, which differ in their applied excitation and emission wavelengths, scanning mode, and image acquisition.

3.1. Scanning Laser Ophthalmoscopy

Confocal scanning laser ophthalmoscopy (cSLO) uses a continuous illumination of the ocular fundus with a low-power, blue-light laser beam that scans the fundus in a raster pattern (excitation wavelength 488 nm, 490 nm, or 450 nm, depending on the manufacturer). An appropriate barrier filter (cut-off filter) ahead of the detector ensures the blockade of reflected excitation light and the detection of the emitted light (e.g., SPECTRALIS HRA2, Heidelberg Engineering, Heidelberg, Germany: >500 nm; F-10 and Mirante Systems, NIDEK, Gamagori, Japan: 510 nm for blue short-wave FAF,

630 nm for green short-wave FAF; EIDON, Centervue, Padova, Italy: 510–560 nm and 560–700 nm). The confocal principle is based on a small pinhole in front of the detector. Thus, back-scattered light originating from structures anterior and posterior to the focal plane is suppressed, and emitted light from the desired plane of focus can be amplified to enhance image contrast [19]. The maximal retinal irradiation is well below the ANSI Z136.1 laser safety standard [20].

3.2. Fundus Camera

A modified fundus camera was used to detect FAF by Delori and co-workers [21]. Later, a modification of a commercially available fundus camera system was applied to FAF imaging [22]. In contrast to confocal scanning laser ophthalmoscopy, non-confocal fundus camera systems use one high-energy flash at maximum intensity. For excitation and the detection of emission, a wide-band filter is used (e.g., TRC-50DX/50IX, Topcon, Tokyo, Japan: excitation wavelength range 500–610 nm, detection of emission 675–715 nm; Visucam 224/524/FF450, Carl Zeiss Meditec AG, Jena, Germany: excitation wavelength range 510–580 nm, detection of emission 650–735 nm; CX-1/CR-2, Canon, Tokyo, Japan: excitation wavelength range 530–580 nm, detection of emission >640 nm).

More recent systems use broad line fundus imaging (BLFI) technology with illumination in two broad wavelength ranges (FAF-Blue: excitation 435–500 nm, FAF-Green: excitation 500–585 nm) and the detection of emission within a band-pass filter range of 532–650 nm (for FAF-Blue) or 630–750 nm (for FAF-Green) (ZEISS CLARUS 500/700, Carl Zeiss Meditec AG, Jena, Germany).

3.3. Wide-Field Imaging

As different pathologies (e.g., choroidal melanoma) can occur on all sides of the ocular fundus, visualization of the central and peripheral retina is of importance. In conventional scanning laser ophthalmoscopy systems, the field of view is restricted to 60° (EIDON), 40° or 60° (F-10/Mirante), or 30° (SPECTRALIS HRA2). The latter may be extended to 55° using a wide-field lens. Composite images may be able to image larger areas.

The fields of view of different fundus camera systems vary between 20° and 50°. Montage images of larger areas can be manually generated. The ZEISS CLARUS 500 and 700 achieve a 133-degree field of view with the possibility of 267-degree montage images. For peripheral FAF images, another non-mydriatic, non-confocal ultra-wide-field scanning laser ophthalmoscope provides up to approximately 180–200° images of the retina in a single capture using a green laser for excitation (532 nm) and a detection of emission in the range of 540–800 nm (Optomap, Optos PLC, Dunfermline, UK) [23]. Ultra-wide-field imaging is of particular importance to visualize lesions anterior to the equator [24][25][26].

4. Interpreting Fundus Autofluorescence Images

The grey value of each pixel depends on the intensity of the FAF signal, with low intensities resulting in low pixel values, which appear dark, and high intensities resulting in high pixel values, which appear bright. A reduced FAF signal arises from absorption effects or from the loss or absence of fluorophores (e.g., RPE atrophy). Absorption phenomena occur in the area of retinal vessels, due to luteal pigment, i.e., lutein and zeaxanthin, an increased RPE melanin content, and migrated melanin-containing cells. Absorption may further be caused by non-fluorescent extracellular material anterior to the RPE (e.g., recent fluid or haemorrhage, media opacities, lens opacifications). In contrast, an increased FAF signal may derive from augmented RPE lipofuscin/melanolipofuscin accumulation, deposited fluorophores (e.g., vitelliform material, orange pigment), a lack of absorbing material, a loss of photopigment, lipofuscin-containing macrophages, devitalized haemorrhages [27], or RPE cell migration. Long-lasting subretinal fluid is associated with a malfunctioning outer segment turnover between photoreceptors and the RPE and can also cause an increased FAF signal [17][28][29][30].

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