Non-invasive methods are increasingly used to diagnose and treat various retinal diseases. Besides optical coherence tomography (OCT), one of the major methods of such analysis is through confocal laser scanning ophthalmoscope (cSLO), which can record in the autofluorescence mode a complex signal predominantly derived from autofluorescent lipofuscin granules in the retinal pigment epithelium (RPE). This method allows one to record fundus autofluorescence intensity and distribution in vivo.

RPE is known to play an important role in the pathogenesis of different macular diseases such as age-related macular degeneration (AMD). These multifunctional cells consist of a single layer of hexagonal cells, the apices of which contain villous processes that envelop the outer segments of the photoreceptors. In the fovea RPE cells are taller and thinner, contain more and larger melanosomes than elsewhere in the fundus. The RPE has important functions in maintaining the integrity of the subretinal space, the space between photoreceptors and RPE cells. It is part of the outer blood-retinal barrier and pumps ions and water out of the subretinal space. Photoreceptor function is critically dependent on healthy RPE layer while RPE cells remove ‘spent’ photoreceptors tips in the diurnal process of photoreceptor renewal and participates in 11-cis retinol recycling. These phagocytosed outer segment tips are digested in the RPE phagolysosomal system throughout life. With time, there is an accumulation of substances, like lipofuscin pigment, which may reflect the declining ability of the RPE to handle large amount of relatively indigestible material. RPE lipofuscin normally accumulates with age, but increasing lipofuscin represents also a common pathogenetical pathway in several retinal degenerations and dystrophies.

Lipofuscin is mainly derived from the chemically modified residues of incompletely digested photoreceptor outer segment discs. The retinoid A2E is the dominant fluorophore in the lipofuscin granules. Controversy exists with regard to effects of lipofuscin on RPE cell function and its relevance to retinal diseases, including Best disease, Stargardt disease, and age-related macular degeneration (AMD). Recent experimental findings imply that lipofuscin and certain compounds such as A2E interfere with normal RPE cell functions including lysosomal degradation and thereby release of free radicals finally leading to RPE cell death.

There are other fluorophores, which may also contribute to the fundus autofluorescent, present in various anatomic layers of the posterior pole. The basemembrane has also been shown to possess autofluorescent properties. However, the excitation and emission spectra are different, although with slight overlap, from those of lipofuscin in RPE cells. The same is the case for fluorescence appearing from choroidal components and from the sclera. Furthermore, sub-pigment-epithelial

Figure 1: Fundus autofluorescence mean image of a 66-year-old male patient with normal topographic distribution of autofluorescence intensity. Absorption by retinal vessels and by macular pigment results in decreased autofluorescence signal intensity.
fluid in the presence of pigment epithelial detachments or longstanding subretinal haemorrhages may contain fluorophores that induce an increased autofluorescent signal in the wavelength ranges relevant to the method applied herein. In spite of the various retinal cells and fluids that may fluorescence, it has been shown by Delori et al. that autofluorescence is mainly derived from RPE lipofuscin.

The cSLO system allows to visualize fundus autofluorescence. Images of the fundus are recorded through a small pinhole aperture which suppresses light originating from the outside of the focal plane to enhance the image contrast compared with the nonconfocal images. For excitation at 488 nm an argon blue or optically pumped solid-state laser is used, and emission is recorded above 500 nm with a barrier filter. The images encompass the entire macular area. To enhance the quality of the image, single images can be aligned using an image analysing software leading to an amplification of the autofluorescent signal.

Absorbing structures anterior to the RPE, like macular pigment, retinal vessels, blood, hard and soft exudates are associated with a decreased autofluorescent signal. Lack of lipofuscin due to RPE atrophy leads also to decreased autofluorescence. Therefore, normal fundus autofluorescence shows a decreased amount of autofluorescence in the area of the fovea, the retinal vessels and the optic nerve head. The autofluorescence decreases also toward the periphery. The relative decrease of autofluorescence in the fovea is partly due to absorption by intraretinal yellow pigments like lutein and zeaxanthin.

Age-related macular degeneration has become the most common cause of legal blindness in industrialized countries. Strong evidences indicate an important role of RPE in the pathogenesis of the disease. It has been shown that excessive lipofuscin accumulation precedes geographic atrophy, one of the major forms of AMD. The pattern of abnormal autofluorescence in the junctional zone of a geographic atrophy has been shown to have an impact on the progression of the disease. Drusen, a hallmark of the disease, can be associated with increased, decreased or normal autofluorescence. Whether this finding is of prognostic relevance needs to be elucidated. In a large study fundus autofluorescence changes have been classified in eyes with early and dry AMD into eight phenotypic patterns including normal, minimal changes, local increased, patchy, linear, lace-like, reticular and speckled. Therefore, visualization of RPE changes appears to be particularly attractive to further elucidate AMD.

In macular and retinal dystrophies different changes in fundus autofluorescence have been described for further characterization of these diseases. Markedly increased autofluorescence intensity has been shown in Best disease, Stargardt macular dystrophy and adult vitelliform macular dystrophy. Fundus autofluorescence seems to be a more suitable diagnostic tool for following patients with macular and retinal dystrophies when compared with normal fundus photography.

In summary, fundus autofluorescence imaging represents one of various methods to investigate the ocular fundus, beside biomicroscopic examination, fundus photography, fluorescence and indocyanin angiography, and optical coherence tomography (OCT). This non-invasive method visualises changes of the RPE layer in vivo, while the signal is mainly derived from lipofuscin. Fundus autofluorescence imaging has and will contribute to our understanding of pathophysiology and treatment in several retinal diseases.

Reference:
www.oftalmolog.com
Reference List: Autofluorescence of the Macula, Pascal W. Hasler


