THE LOWDOWN ON FLUORESCENCE LIFETIME IMAGING OPHTHALMOSCOPY

A novel mode of diagnostic imaging provides new information about retinal diseases.

BY CHANTAL DYSLI, MD, PHD; SEBASTIAN WOLF, MD, PHD; AND MARTIN S. ZINKERNAGEL, MD, PHD

Fluorescence lifetime imaging ophthalmoscopy (FLIO) measures the lifetime of fluorophores originating from the retina. The lifetime of a fluorophore is defined as the time it remains in the excited state before emitting a photon to return to the ground state. Fluorescence lifetimes are specific for individual fluorophores and depend on the metabolic environment.

In contrast to fundus autofluorescence intensity, which is mainly dominated by lipofuscin as the predominant fluorophore in terms of fluorescence intensity in the retina, specific fluorescence lifetime data can also be detected from other fluorophores within the retina. In this way, FLIO is able to provide information about the integrity of retinal tissue, such as the photoreceptors.[1]

AT A GLANCE

- Fluorescence lifetime imaging ophthalmoscopy (FLIO) is a noninvasive imaging technique based on fundus autofluorescence imaging; it measures the lifetimes of retinal fluorophores.
- Unlike fundus autofluorescence, in which the RPE provides most of the contrast, there is additional contrast from the photoreceptor layer with FLIO.
- Disease-specific fluorescence lifetime patterns can be characterized for various retinal diseases.
- FLIO might be used for early detection and follow-up of subtle retinal changes.

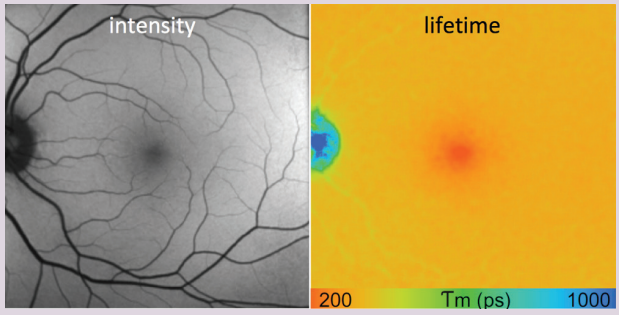

Figure 1. In healthy retinas, the shortest fluorescence lifetimes are located in the macular center and probably originate from macular pigment.[4,12] Toward the retinal periphery, lifetimes are prolonged. Retinal vessels and the optic nerve head feature the longest fluorescence lifetimes, probably due to high collagen content.

WHAT IT IS

The basic principle of FLIO originates from fluorescence lifetime imaging microscopy (FLIM). This technique is commonly used to detect metabolic and structural changes in cell cultures and tissues, such as hypoxic conditions, aging processes, and accumulation of metabolic byproducts. FLIO has been adapted for use in ophthalmology with the use of a fluorescence lifetime imaging ophthalmoscope based on a Spectralis HRA (Heidelberg Engineering) device.

In this procedure, retinal fluorophores are excited by a 473-nm pulsed laser. Emitted fluorescence is simultaneously detected in two distinct wavelength channels: a short spectral channel (SSC) between 498 and 560 nm and a long spectral channel (LSC) between 560 and 720 nm. An infrared eye tracking system corrects for eye movements. The acquisition time is about 2 minutes per eye with dilated pupils. Detection of emitted single fluorophores over time results in a fluorescence decay curve for every pixel within the field of registration. From this data, a mean fluorescence

lifetime, in the range of 200 to 1000 picoseconds (ps), is calculated. The lifetime is color-coded, and a topographic lifetime map can be displayed (Figure 1).[1]

Fluorescence lifetime data can be analyzed either qualitatively, by investigating the lifetime distribution maps, or quantitatively, by numerical analysis and comparison of lifetime values of individual pixels, specific regions of interest, or mean values for subfields from the standard ETDRS grid.

In a study of 32 healthy individuals, we found high reproducibility and repeatability of the lifetime values.[2] In addition, we found a correlation of fluorescence lifetimes with age. Older individuals exhibited longer fluorescence lifetimes, possibly due to progressive accumulation of visual cycle end products within the retina. Age-matched healthy individuals or the person's own unaffected contralateral eye are generally used as controls for FLIO.

USE IN RETINAL DISEASES

Although FLIO is relatively new, a broad range of retinal diseases has already been investigated using this technique.

In central and branch retinal artery occlusions, prolonged fluorescence lifetimes were measured during acute ischemia.[3] However, lifetimes returned to normal values over time, even though there was atrophy of the inner retinal layers due to ischemia; this suggests that the inner layers contribute relatively little fluorescence lifetime signal.

In macular holes, short fluorescence lifetimes were shown to be disrupted in the area of the macular hole corresponding to the defect of the retinal layers containing macular pigment on optical coherence tomography (OCT).[4] Normalization of the lifetime distribution pattern was observed after surgical intervention with anatomic recovery of the retinal layer structure.

In intermediate age-related macular degeneration (AMD), generally prolonged fluorescence lifetimes were observed, compared with control eyes in the same age range.[5] Whereas individual soft drusen did not display specific fluorescence lifetimes, hyperreflective subretinal deposits on OCT featured short fluorescence lifetimes and intraretinal deposits featured long lifetimes.

In geographic atrophy (GA) in AMD, areas of total retinal atrophy with hypoautofluorescence in intensity measurements exhibited long lifetimes, possibly due to the contribution of fluorophores from the underlying choroid and connective tissue components.[6] However, areas of remaining photoreceptor fragments still displayed short lifetimes, also within the area of hypofluorescence in the intensity image (Figure 2). FLIO additionally allows specific analysis of the margins of GA, which are of particular interest in analyzing the progression of disease.

In central serous chorioretinopathy (CSCR), FLIO showed shortened fluorescence lifetimes in the areas of active

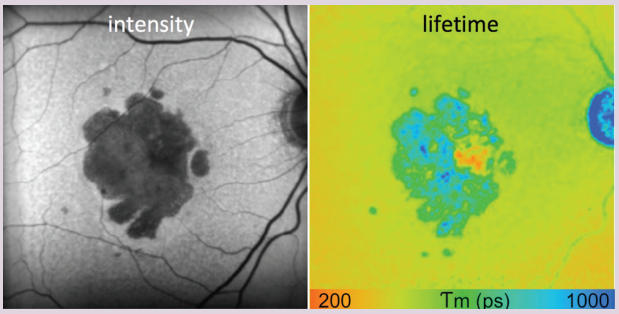

Figure 2. GA in AMD typically appears as hypoautofluorescence in the intensity image. Total atrophy of the RPE and the photoreceptors shows long fluorescence lifetimes, whereas areas with remaining photoreceptor segments show short lifetimes.

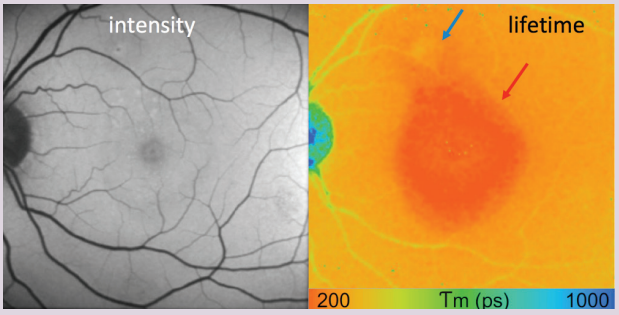

Figure 3. In CSCR, the area of current disease activity and prolonged photoreceptor outer segments features short fluorescence lifetimes (red arrow). Previous sites of CSCR lesions show prolonged lifetimes (blue arrow).

lesions, correlating with prolonged outer photoreceptor segments and possibly indicating accumulation of intermediate products from the visual cycle (Figure 3).[7] In the chronic disease stage, secondary retinal changes such as scar formation or atrophy exhibited prolonged lifetimes consistent with findings from FLIO in AMD.

Hereditary retinal diseases also exhibit interesting data when investigated using FLIO. In Stargardt disease, retinal deposits with short fluorescence lifetimes were detected before they were visible in the intensity image (Figure 4).[8] Over time, they appeared as hyperautofluorescent spots and shifted to long fluorescence lifetimes, suggesting a change in the composition of these retinal deposits. In choroideremia, common fundus autofluorescence intensity images clearly depict the borders of the intact retinal pigment epithelium (RPE).[9] However, FLIO identified the margins of preserved photoreceptor layers and remaining choroid even in the absence of the RPE.

Analysis of follow-up examinations and studies of other

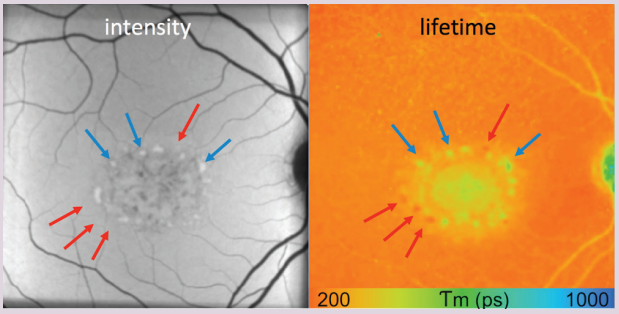

Figure 4. In Stargardt disease, new retinal deposits are first detected in the fluorescence lifetime image with short lifetimes (red arrows). Older deposits are clearly visible as hyperautofluorescent flecks in the intensity image and exhibit long fluorescence lifetimes (blue arrows).

retinal diseases and systemic metabolic changes may provide further insights into retinal fluorescence lifetimes as detected by FLIO.

BASIC RESEARCH

In ex vivo studies, fluorescence lifetimes of different possible retinal fluorophores were measured in order to transfer the findings to in vivo measurements.[1] In vitro, the shortest fluorescence lifetimes of about 40 ps were measured in lutein and zeaxanthin, main components of the macular pigment. Slightly longer values originated from visual cycle compounds such as retinal and retinol. Other fluorophores such as melanin most likely also contribute to the mean lifetime. FLIO measurements in cell cultures may provide additional information about retinal fluorophores in the context of active cell metabolism.

Direct translation of these findings to FLIO measurements in vivo in human eyes must be treated with caution, as in the living eye a mixture of fluorescence lifetimes is expected and there may be substantial interaction of contributing fluorophores.

In a series of experiments, we characterized fluorescence lifetimes in pigmented and nonpigmented mice and in a mouse model of retinal degeneration.[10] Pharmacologic extinction of specific retinal layers such as the photoreceptors in the presence of the intact RPE, and degeneration of the RPE and the photoreceptors, were also performed in order to differentiate the contribution of these layers.[11]

FUTURE DIRECTIONS

By investigating retinal fluorescence lifetimes in vitro, in mouse models, and in healthy and diseased human retinas, we can enhance our knowledge and gain insights into the origin and dynamics of retinal fluorophores. FLIO can provide information that is supplementary to the more commonly used multimodal imaging technologies. It represents a powerful tool for early detection of metabolic changes and retinal deposits, and it can be used to record subtle retinal changes over time.

In the future, FLIO might also be used to monitor patients' responses to therapies, including novel and innovative therapeutic strategies. However, there is still a need for further investigation of the dynamics of different retinal fluorophores in the context of metabolic changes and for further trials investigating mouse models of hereditary, metabolic, and degenerative diseases. ■

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Chantal Dysli, MD, PhD
- resident in ophthalmology, department of ophthalmology, Inselspital, Bern University Hospital, and Department of BioMedical Research, University of Bern, Bern, Switzerland
- financial interest: none acknowledged
- chantal.dysli@insel.ch

Sebastian Wolf, MD, PhD
- head of department and vitreoretinal specialist at the department of ophthalmology, Inselspital, Bern University Hospital, and Department of BioMedical Research, University of Bern, Bern, Switzerland
- financial interest: none acknowledged
- sebastian.wolf@insel.ch

Martin S. Zinkernagel, MD, PhD
- vitreoretinal specialist at the department of ophthalmology, Inselspital, Bern University Hospital, and Department of BioMedical Research, University of Bern, Bern, Switzerland
- financial disclosure: Swiss National Science Foundation grant (#320030_156019)
- martin.zinkernagel@insel.ch