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Longitudinal foveal fluorescence lifetime characteristics in geographic atrophy using fluorescence lifetime imaging ophthalmoscopy (FLIO)

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Running head:

Foveal sparing and FLIO

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Summary Statement: (Wordcount: 40)

Short foveal fluorescence lifetimes are associated with foveal sparing (FS) and correlate with visual function. However, they are not an exclusive feature of FS and can occur in geographic atrophy without FS, presumably due to macular pigment retention.

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Abstract (Wordcount: 267)

Purpose: Short foveal fluorescence lifetimes (fFLT) in geographic atrophy are typically found in eyes with foveal sparing (FS) but may also occur in eyes without FS. We investigated whether short fFLT could serve as a functional biomarker for disease progression in geographic atrophy (GA).

Methods: Thirty three eyes were followed over the course of 4-6 years. FS was assessed using fluorescence lifetime imaging ophthalmoscopy, OCT, FAF and macular pigment optical density.

Results: Eyes with FS exhibited shorter fFLT compared to eyes without FS. Short fFLT (<600ps) were measured in all eyes with FS and half of the eyes without FS. Eyes with FS showed a bigger increase in fFLT per year (+39/+30 ps (SSC/LSC) in FS vs. +29/+22 ps (SSC/LSC) in non FS). BCVA correlated significantly with fFLT (p=0.018 and p=0.005 for

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SSC/LSC). MPOD measurements correlated significantly with fFLT but not in all spectral channels (p ranging from 0.018 to 0.077).

Conclusion: In GA, shorter fFLT are associated with foveal sparing but they can also be observed in eyes without FS. Our longitudinal data suggests that shorter fFLT features in eyes with loss of FS represent an earlier stage of disease and may be more prone to loss of visualacuity.

Keywords:

AMD, fluorescence lifetimes, fundus autofluorescence, FLIO, foveal sparing, geographic atrophy, retinal imaging

Manuscript (Wordcount: 4038)

Introduction:

Geographic atrophy (GA) due to age-related macular degeneration (AMD) is a progressive retinal disease, in which the retinal pigment epithelium and its over- and underlying structures, the outer retinal layers and the choroid, are mainly effected.[1, 2] It can lead to a severe loss in visual acuity, especially in cases in which the fovea is affected.[3] Various patterns of progression have been described. The causes leading to a specific pattern are unclear but they might have a prognostic value.[4, 5] In some patients, the central part of the macula including the fovea are spared from atrophy for a long time compared to other patients in which this central region is affected at early stages of the disease.[6] Involvement of the fovea seems to be dependent on the number of lesions, with eyes with multifocal lesions being at a higher risk.[6] Fundus Autofluorescence (FAF) is currently the main imaging modality to image the extent of the GA.[7, 8] FAF images enable us to clearly see the borders of

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atrophy and to document growth over time.[9] However FAF is not the best choice for delineating foveal sparing (FS) in patients with GA, mainly because of interference of the macular pigment which attenuates the FAF signal. Therefore, functional assessment and spectral-domain ocular coherence tomography (SD-OCT) is better suited to identify FS.[10] Fluorescence lifetime imaging ophthalmoscopy (FLIO) enables a two-dimensional topographical mapping of fluorescence lifetimes of the retina in-vivo.[11, 12] With this technique, metabolic or pathologic processes of the retina can be better portrayed.[13] FLIO is able to detect early changes in various retinal diseases, such as Stargardt disease[14], hydroxychloroquine-retinopathy[15] or macular-telangiectasia type 2.[16] The FLIO signal has been shown to be strongly influenced by macular pigment[17], which leads to shortening of fluorescence lifetimes.[18] GA was specifically investigated with FLIO in 2016 by Dysli et al.[14, 19]and in 2018 by Sauer et al.[20] In these studies a better BCVA correlated significantly with foveal fluorescence lifetimes (fFLT). Furthermore, foveal sparing (FS) was associated with shorter fFLT. Therefore, declining visual function and/or loss of foveal sparing due to atrophy extending towards the fovea should be accompanied by increasing fFLT. However, it was observed that short fFLT are not an exclusive feature of eyes with FS[19]. It can be hypothesized, that these eyes might be at the turnover point of losing their FS status, which is already showing through diminishing visual performance, while still displaying shorter fFLT. Another possibility is that these eyes represent a subcategory where short fluorescence lifetimes are preserved even in presence of atrophy. The goal of this study was to evaluate longitudinal changes of fFLT in patients with and without FS in GA due to dry AMD, to describe temporal changes in fFLT and to investigate cases of persisting short fFLT in eyes without foveal sparing.

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Methods:

The registration for this study can be found at ClinicalTrials.gov under NCT01981148. Written informed consent was obtained from all participants before study enrollment. All procedures used in this study adhered to the tenets of the Declaration of Helsinki and the International Ethical Guidelines for Biomedical Research involving Human Subjects (Council for International Organizations of Medical Sciences). They were approved by the local Ethics Committee of the University of Bern, Switzerland.

Patients

Patients were recruited from the outpatient department of ophthalmology at the Inselspital, University Hospital of Bern in Switzerland. Patients with GA secondary to dry AMD were included. Patients with signs of neovascular AMD and progressing cataract were excluded. None of the included patients exhibited further ocular pathologies, which could have a significant influence on measured BCVA. For further analysis a pseudophakic subgroup was formed. The clinical characteristics of the complete cohort and the pseudophakic subgroup can be found in Table 1. None of the included participants took carotenoid supplementations. Geographic atrophy was defined by areas of decreased retinal pigmentation in fundoscopy and decreased autofluorescence with sharply demarcated borders in FAF images with complete retinal pigment epithelium (RPE) and outer retinal atrophy (cRORA) depicted by spectral-domain OCT (as defined by the consensus definition for atrophy associated with AMD on OCT[21]). Foveal sparing was defined by isles of intact outer retinal layers and retinal pigment epithelium in the central foveal area in spectral-domain OCT images. Eyes were divided into a group with foveal sparing and a group without according to the OCT findings. The group without foveal sparing was further subdivided into two subgroups: one with shorter foveal fluorescence lifetimes (fFLT) and one with longer fFLT. The cut-off point

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separating the two subgroups was set at a fFLT of 600 picoseconds (ps) measured in the short spectral channel (SSC).

Examinations

All patients were examined at a baseline and a follow-up visit by an ophthalmologist. Examinations consisted of best corrected distance visual acuity (BCVA, with Early Treatment Diabetic Retinopathy Study (ETDRS[22]) letters) testing and a slit lamp and dilated fundus examination. Multimodal imaging consisted of spectral-domain OCT and blue-light FAF using a Heidelberg Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany) and Fluorescence Lifetime Imaging Ophthalmoscopy (FLIO). In addition, Macular Pigment Optical Density (MPOD) measurements were obtained for all patients at the follow-up visit using dual wavelength autofluorescence (Heidelberg Engineering MultiColor Spectralis Module, Heidelberg, Germany). Additionally the optical density of macular pigment was acquired at 0.5° and 2° eccentricity at the follow-up visit. More peripheral eccentricities were not considered as this would lead to inclusion of atrophic areas with zero-line or negative optical density readings. The outer reference point was set at 9° eccentricity, which allowed the measurement of reliable macular pigment data in previous studies.[23, 24]

FLIO Measurement

Fluorescence lifetimes (FLT) of the retina were acquired using a prototype fluorescence lifetime imaging ophthalmoscope (FLIO) device from Heidelberg Engineering.

The FLIO device radiates with a 473 nm pulsed blue laser at 80 MHz repetition rate for excitation of retinal autofluorescence. The emitted fluorescence is registered by time-correlated single-photon counting (TPSPC) modules using two highly sensitive hybrid photon-counting detectors (Becker&Hickl, Berlin, Germany). Following detection channels

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with distinct wavelengths ranges were used. A short spectral channel (SSC, 498–560 nm) and a long spectral channel (LSC, 560–720 nm) as displayed in Figure 1. A confocal high-contrast infrared image was recorded simultaneously to ensure that each recorded photon is localized at the correct spatial location. A photon count of 1000 photons per pixel was acquired at least for every location within the image. FLIO image acquisition took approximately 1.5 to 3 minutes per eye.

<u>Analysis of Fluorescence Lifetime Data</u>

The Becker&Hickl software (SPCImage 4.4.2) was used for the analysis of recorded lifetime data. A biexponential decay model was applied using a binning factor of one. This procedure resulted in a short (*T1*) and a long (*T2*) lifetime component with corresponding amplitudes *a1* and *a2*.

For topographical mapping and quantitative analysis of measured fluorescence lifetimes, the software "FLIO Reader" (ARTORG Center for Biomedical Engineering Research, University of Bern, Bern, Switzerland) was used. Foveal fluorescence lifetimes (fFLT) were measured, using a circular measurement area with a diameter of 100 µm within the central foveal area (defined by a circular area with the central point of the foveola and a diameter of 300 µm). Mean fluorescence lifetime (*Tm)* as well as the individual components *T1* and *T2* and amplitudes *a1* and *a2* were recorded for each region of interest. *Tm* represents an amplitude-weighted average of the decay parameters *T1* and *T2*.

<u>Statistical analysis</u>

For statistical analysis the software Prism 8 (GraphPad Software Inc., La Jolla, CA, USA) was utilized. Data was verified for a Gaussian distribution using the d'Agostino-Pearson

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omnibus K2 test. Pearson correlation and one-way ANOVA with a multiple-comparison test was used. P-values of ≤0.05 were considered as statistically significant.

Results:

Thirty three eyes of 19 patients were included. 11 patients were female, 8 were male. The mean age of all patients was 77.9 years at baseline and 82.8 years at follow-up. Ten eyes (30%) exhibited foveal sparing (FS) at baseline. Of these 10 eyes, 6 exhibited persisting FS at follow-up. In the pseudophakic subgroup 19 eyes of 12 patients were analyzed. An overview of the complete cohort and the pseudophakic subgroup is listed in Table 1.

Best Corrected Visual Acuity (BCVA)

The decrease in BCVA per year (mean ± Standard Error of the Mean "SEM") of the entire cohort was -1.64 ±0.38 letters. The yearly decrease of BCVA in the pseudophakic cohort was similar with -1.46 ±0.42 letters (Figure 2). All BCVA results for the the complete and pseudophakic cohort and their subgroups are summarized in Table 1, 2 and 3.

Foveal Fluorescence Lifetimes (fFLT)

The fFLT increased yearly by an average (±SEM) of 47 ±17 ps in the SSC and 29 ±10 ps in the LSC (in the complete cohort) and by 32 ±11 ps in the SSC and 24 ±9 ps in the LSC in the pseudophakic subgroup (Figure 2). In pseudophakic eyes with FS, the fFLT prolonged yearly by (mean ±SEM) 39 ±11 ps in the SSC and by 30 ±13 ps in the LSC (Figure 3, left column). In the group with persisting FS at follow-up, the fFLT prolonged yearly by 32 ±7 ps in the SSC and by 16 ±7 ps in the LSC.

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In comparison, in the group without FS the fFLT prolonged each year by 29 ±15 ps in the SSC and by 22 ±13 ps in the LSC (Figure 3, right column). FLT data for each group is displayed in Table 1, 2 and 3.

Correlating the BCVA of all eyes with the corresponding fFLT showed a significant correlation for both spectral channels at baseline (SSC p=0.018 and LSC p=0.005) and at follow-up (SSC p=0.0355 and LSC p=0.0066). In the pseudophakic group no significant correlations at baseline and follow-up were noted (SSC p=0.11 and LSC p=0.17 at baseline and SSC p=0.13 and LSC p=0.17 at follow-up). The correlation between the mean prolongation in fFLT and mean decrease in BCVA between baseline and follow-up was significant for the complete cohort in the LSC with p=0.02 (SSC p=0.7).

Macular Pigment Optical Density (MPOD)

The MPOD value for the follow-up visit at 0.5° eccentricity was 93 ±36 auc (mean ±SEM; auc: area under the curve) and at 2° it was 814 ±396 auc. In the pseudophakic subgroup it was 136 ±59 auc at 0.5° and 1272 ±627 auc at 2° respectively.

Correlating the MPOD values of all eyes with the respective fFLT showed a significant correlation at 0.5° (SSC p=0.046 and LSC p=0.018) and at 2° (SSC p=0.077 and LSC p=0.034)

In the pseudophakic group no significant correlation was noted (SSC p=0.081 and LSC p=0.084 at 0.5° and SSC p=0.015 and LSC p=0.16 at 2°).

Discussion:

A previous study, which examined foveal sparing with FLIO, showed that large FLT differences between the fovea and the surrounding atrophic areas correlate with better visual acuity[20]. Atrophic areas usually exhibit very long lifetimes (>1000 ps), whereas healthy retina

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exhibits shorter lifetimes with the shortest lifetimes measured in the fovea. This points towards a strong association of short retinal lifetimes with good visual acuity.

However, a big difference in FLT between the foveal area and surrounding atrophic areas or even simply short fFLT does not seem to reliably predict cases of FS. In our cohort about half of the patients without FS exhibited persisting, relatively short fFLT features (<600 ps in the SSC). The mean fFLT of these eyes in the pseudophakic subgroup was 354 ps (SSC), which may not be very short in comparison to the mean fFLT of 181 ps (SSC) of the eyes with FS, though there were individual cases in the No FS/short fFLT subgroup with a very short fFLT comparable to cases with FS, the shortest being 105 ps (see Figure 4, in which the eye with FS and the eye without FS but short lifetimes exhibits roughly the same fFLT). The yearly decrease in BCVA of -2.1 letters of these eyes with shorter fFLT features was more pronounced compared to eyes with fFLT >600ps where the decrease was -0.9 letters (Table 3). This is an indication that non FS eyes with shorter fFLT might be in an intermediary state of foveal atrophy, in which the functionality (BCVA) is already severely diminished but structural components responsible for the short fFLT are not yet totally degraded and that these two subgroups do not represent two different disease entities. Furthermore this confirms that short fFLT should not be used as a surrogate marker for visual acuity in these cases.

While various retinal components display short fluorescence decay lifetimes, specifically macular pigment (MP) correlated favorably with good visual function in AMD.[25] It is presumed that MP contributes a large part of the short fFLT in FLIO measurements. The typical distribution of lifetimes in FLIO, with the shortest FLT observed in the foveal region, is presumed to be highly correlated with the distribution of macular pigment.[17] MP can be measured using dual wavelength autofluorescence.[23] It has to be noted, that the measurement of MP in areas affected by GA is very unreliable, resulting in having unfeasibly large

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standard deviations or partly resulting in negative readings. However, we decided to include this measurement to have an additional reference point available and to support the hypothesis that macular pigment can be preserved in eyes with non FS in geographic atrophy. We measured MPOD in the central 0.5° and 2° area to compare it to our measured fFLT. MP and FLT parameters showed to correlate significantly in a previous study[18]. This is in keeping with our data.

We verified all cases of FS by analyzing the respective OCT images. Patients with foveal sparing exhibited central isles of intact RPE and outer retinal layers, whereas in eyes without sparing RPE was absent and the photoreceptor layers were completely eradicated or severely disorganized (Figure 4). When comparing the OCTs of the individual subgroups, no significant differences were noticed, which could explain the observed differences in fFLT. One theoretically possible explanation would be that cells storing macular pigment are still present in these cases, though their function might be severely impaired or their remains are not yet cleared from the tissue. However, this hypothesis could not be objectively evaluated on basis of the OCT scans.

Limitations

One of the main limitations of our study is the number of patients, especially of those with foveal sparing. This can be largely explained by the longitudinal nature of the study, the relative new and scarcely deployed imaging technique used as well as the advanced age of the typical patient with GA. A confounding bias might have been introduced by partly using both eyes for the measurements and analysis. However, disease progression shows a low concordance between the two eyes of the same patient concerning BCVA; and only concerning GA size and progression a moderate to good concordance was found in a

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previous study.[26] Another limitation represents the inclusion of MPOD measurement in eyes affected by geographic atrophy. In cases of significant GA, measurements can return zero line or even negative optical density reading with a big standard deviation and are generally unreliable. Nevertheless, we decided to include these measurements as we only measured the central 0.5° and 2°, which were often free from gross atrophy. Furthermore, this measurement represents the only method for measuring abundance of macular pigment, which was shown to correlate with visual acuity.

Conclusion:

Foveal sparing in eyes with GA due to dry AMD is associated with shorter foveal fluorescence lifetimes (fFLT) compared to eyes with GA and no foveal sparing. A major finding is that short fFLT may be retained even in eyes with no foveal sparing. While higher BCVA was reported to correlate with shorter fFLT and thereby indirectly indicating a functioning central retinal metabolism, this correlation is not valid for these cases. They seem to represent eyes with a slower degradation of short FLT emitting components, e.g. retained macular pigment. Furthermore, they do not seem to represent a different disease entity, but more so an earlier stage of disease, as they show a faster decline in BCVA and more pronounced increase in fFLT per year.

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Figure Legends

Fig 1 Representative images of Fluorescence Lifetime Imaging Ophthalmoscopy (FLIO) and corresponding Fundus Autofluorescence (FAF) measurements as well as Optical Coherence Tomography (OCT) with a horizontal cut through the fovea (white dotted line in the FAF image) for the baseline and follow-up visit for one eye. In this individual patient foveal sparing persisted and foveal fluorescence lifetimes stayed short. SSC: Short Spectral Channel, LSC: Long Spectral Channel

Fig 2 Two graphs representing the decrease of Best Corrected Visual Acuity (BCVA, top row) and the increase of foveal fluorescence lifetimes (foveal FLT, bottom row) in the complete cohort (left column) and the pseudophakic group (right column). Eyes with and without foveal sparing were included. SSC: Short Spectral Channel, LSC: Long Spectral Channel, ps: picoseconds.

Fig 3 Representative Fluorescence Lifetime (FLIO) images of an eye with foveal sparing and one without at baseline and follow-up. On the graphs the Best Corrected Visual Acuity (BCVA) measured in ETDRS letters and the foveal Fluorescence Lifetimes for the short and long spectral channel (SSC and LSC respectively) of the pseudophakic subgroup are drawn.

Fig 4 Representative images of three eyes with different foveal fluorescence lifetimes (FLT) presentation. In the left column an eye with foveal sparing is shown. In the middle column, an example with persisting short foveal FLT but no clear foveal sparing in OCT and FAF, and greatly decreased BCVA. In the right column, a third example with no foveal sparing and

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prolonged foveal FLT is depicted. FLIO: Fluorescence Lifetime Imaging Ophthalmoscopy, FAF: Fundus Autofluorescence, OCT: Optical Coherence Tomography, ps: picoseconds.

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Table 1 Overview of characteristics* of the complete cohort and the pseudophakic subgroup.

Complete Cohort	Baseline	Follow-up
age	77.9 (66 – 90) years	82.4 (70 – 95) years
gender	11 female / 8 male (n=19)	
BCVA	45.5 Letters (≈20/125)	36.9 Letters (≈20/200)
BCVA change	-1.64 ±0.38 Letters / year	
fFLT (SSC)	620 ±72 ps	841 ±100 ps
fFLT (LSC)	697 ±57 ps	839 ±69 ps
fFLT change (SSC)	+47 ±17 ps / year	
fFLT change (LSC)	+29 ±10 ps / year	
Foveal Sparing	10 eyes	6 eyes

Pseudophakic Subgroup	Baseline	Follow-up
age	79.6 (71 – 90) years	84.5 (75 – 95) years
gender	7 female / 5 male (n=11)	
BCVA	46.7 Letters (≈20/125)	39.6 Letters (≈20/160)
BCVA change	-1.46 ± 0.42 Letters / year	
fFLT (SSC)	456 ±78 ps	613 ±87 ps
fFLT (LSC)	578 ±69 ps	699 ±72 ps
fFLT change (SSC)	+32 ±11 ps / year	
fFLT change (LSC)	+24 ±9 ps / year	
Foveal Sparing	6 eyes	4 eyes

* Expressed in mean values ±Standard Error of the Mean (SEM) for fFLT and range for age.
BCVA: Best Corrected Visual Acuity in ETDRS letters, fFLT: foveal Fluorescence Lifetimes, SSC: Short Spectral Channel, LSC: Long Spectral Channel, PS: Picoseconds

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Table 2 Overview of the foveal sparing and non foveal sparing subgroup of the complete cohort and the pseudophakic subgroup.

Complete Cohort	Foveal sparing (n=10)	No foveal sparing (n=23)
Age at baseline (range)	76.6 (70 – 84) years	78.3 (66 – 90) years
BCVA at baseline	74.6 ±2.9 letters	32.8 ±3.2 letters
BCVA at follow-up	62.6 ±5.6 letters	25.7 ±3.5 letters
BCVA change	-2.1 ±1 letters / year	-1.4 ±0.4 letters /year
fFLT at baseline (SSC)	334 ±90 ps	744 ±85 ps
fFLT at follow-up (SSC)	630 ±196 ps	933 ±113 ps
fFLT at baseline (LSC)	432 ±43 ps	812 ±67 ps
fFLT at follow-up (LSC)	620 ±94 ps	934 ±83 ps
fFLT change (SSC)	+62 ±35 ps / year	+40 ±20 ps / year
fFLT change (LSC)	+36 ±13 ps / year	+26 ±13 ps / year

Pseudophakic Subgroup	Foveal sparing (n=6)	No foveal sparing (n=13)
Age at baseline (range)	76.5 (72 – 83) years	81.1 (71 – 90) years
BCVA at baseline	73.5 ±4.7 letters	34.3 ±3.7 letters
BCVA at follow-up	67 ±4.9 letters	27 ±5.3 letters
BCVA change	-1.35 ±1 letters / year	-1.5 ±0.5 letters / year
fFLT at baseline (SSC)	181 ±24 ps	583 ±95 ps
fFLT at follow-up (SSC)	368 ±64 ps	726 ±112 ps
fFLT at baseline (LSC)	264 ±38 ps	681 ±81 ps
fFLT at follow-up (LSC)	496 ±55 ps	793 ±92 ps
fFLT change (SSC)	+39 ±11 ps / year	+29 ±15 ps / year
fFLT change (LSC)	+30 ±13 ps / year	+22 ±13 ps / year

Values expressed in mean ±Standard Error of the Mean (SEM) for BCVA, fFLT and range for age. BCVA: Best Corrected Visual Acuity in ETDRS letters, fFLT: foveal Fluorescence Lifetimes, SSC: Short Spectral Channel, LSC: Long Spectral Channel, PS: Picoseconds

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Table 3 Functional and fluorescence lifetime data for the non foveal sparing subgroups

Complete Cohort	No foveal sparing / short fFLT (<600 ps) (n=10)	No foveal sparing / long fFLT (>600 ps) (n=13)
Age at baseline (range)	81.9 (71 – 90) years	75.5 (66 – 84)
BCVA change	-2.1 ±0.53 letters / year	-0.9 ±0.45 letters / year
fFLT (SSC) at baseline	371 ±53 ps	1033 ±76 ps
fFLT (SSC) at follow-up	527 ±77 ps (+42%)	1245 ±138 ps (+21%)
fFLT (LSC) at baseline	513 ±59 ps	1042 ±51 ps
fFLT (LSC) at follow-up	654 ±78 ps (+27%)	1150 ±101 ps (+10%)
fFLT change (SSC)	+30 ±9 ps / year	+47 ±35 ps / year
fFLT change (LSC)	+26 ± 8 ps / year	+25 ± 22 ps / year

Values expressed in mean ±Standard Error of the Mean (SEM) for BCVA, fFLT and range for age. BCVA: Best Corrected Visual Acuity in ETDRS letters, ±: Standard Error of the Mean (SEM); fFLT: foveal Fluorescence Lifetimes, SSC: Short Spectral Channel, LSC: Long Spectral Channel, ps: Picoseconds

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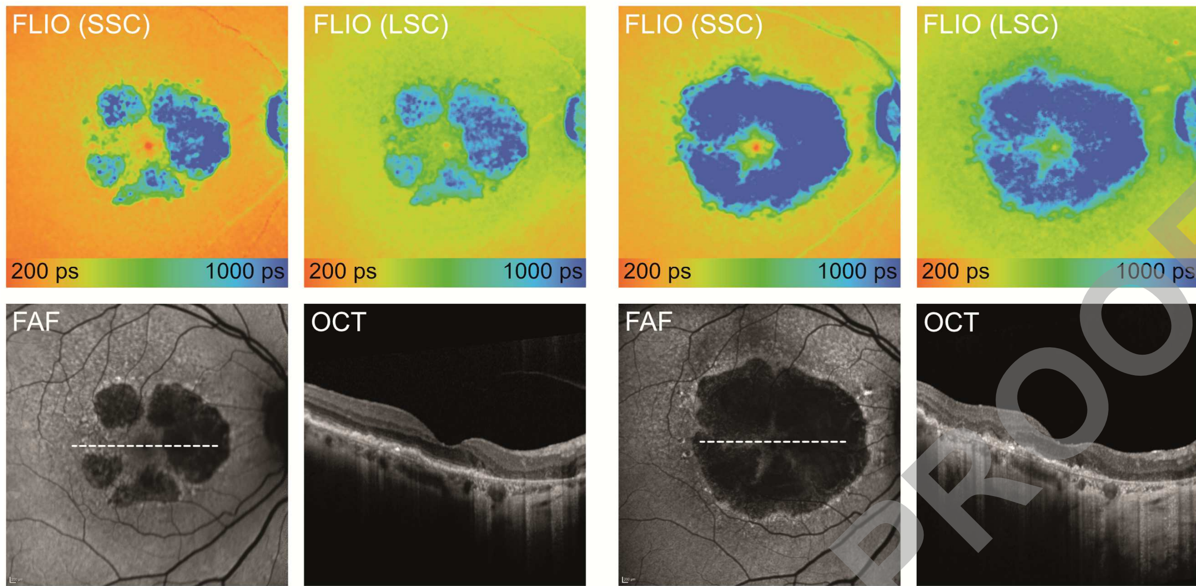

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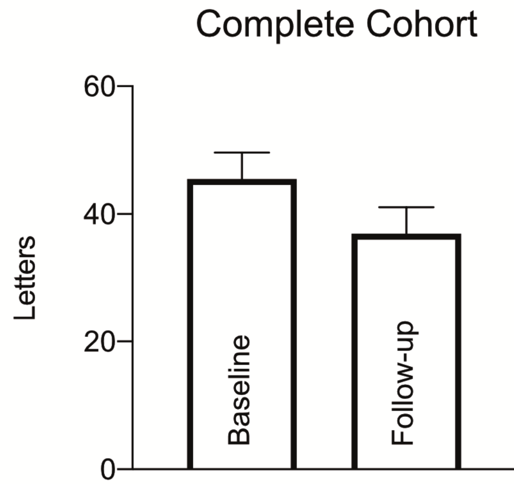

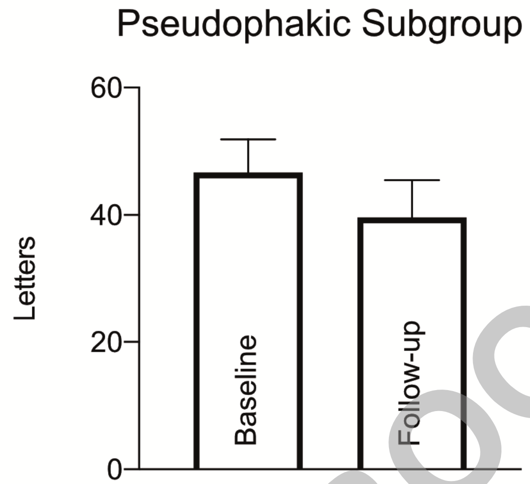

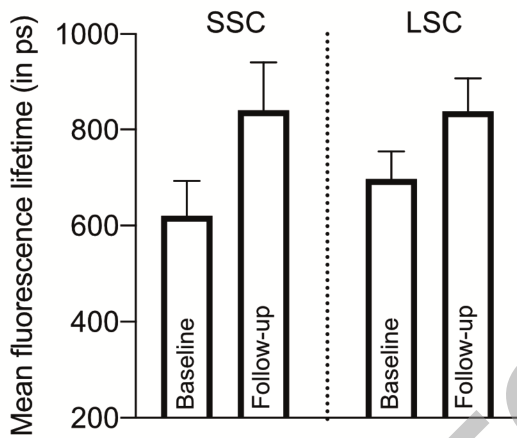

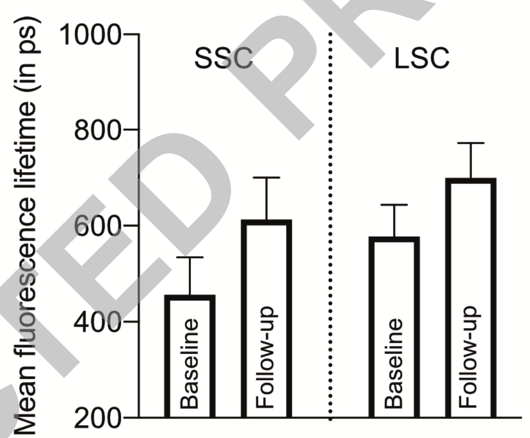

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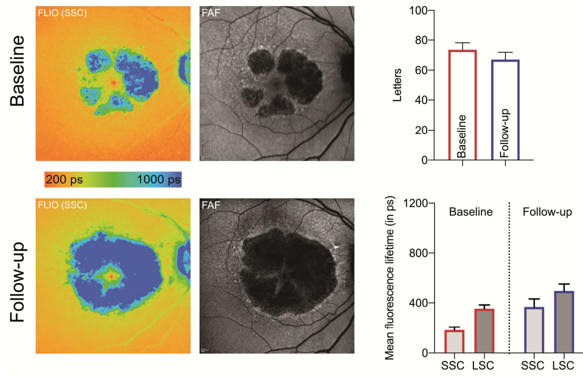

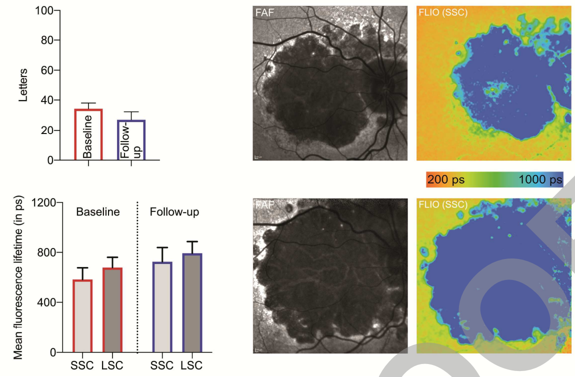

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Foveal Sparing
short foveal FLT
BCVA = 83 letters (≈20/20)

foveal *Tm* = 211 ps

No Foveal Sparing
short foveal FLT
BCVA = 28 letters (≈20/250)

foveal *Tm* = 194 ps

No Foveal Sparing
long foveal FLT
BCVA = 39 letters (≈20/160)

foveal *Tm* = 984 ps

FLIO

200 ps 1000 ps

FAF

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