Foveal drusen increase fixation instability: fixational eye movements as biomarkers for pre-symptomatic AMD.

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Abstract

Purpose: We conducted a clinical study to characterize fine spatiotemporal alterations of FEM in controls and patients with pre-clinical signs of age-related macular degeneration (AMD).

Methods: Twenty-seven participants were recruited from the SilverSight cohort of the Vision Institute - Quinze-Vingts National Vision Hospital, Paris. Subjects were included in three groups: healthy young, healthy aging controls, and aging subjects with drusen. Detection of microdrusen was done using gaze-dependent adaptive optics ophthalmology. Monocular retinal tracking was based on adaptive optics flood illumination ophthalmoscopy (AO-FIO). The system allows for sub-arcminute resolution, high-speed, distortion-free imaging of the foveal area. Local stimulation through digital micromirror (DMD) projection from a near infrared source enabled projection of optotypes. Eye movements were estimated using a phase-correlation registration algorithm.

Results: Compared to controls, patients with foveal drusen showed higher microsaccade amplitude, higher drift diffusion coefficient, and worse fixation stability -i.e. higher isoline area. Among the drusen group, the increase of microsaccade amplitude and ISOA was correlated with drusen eccentricity; the closer the drusen were to the center of the fovea, the more unstable were FEM.

Conclusions: We demonstrate the clinical interest of a novel high-precision retinal tracking technique to characterize FEM changes at the microscopic level. We found significant differences between the drusen group and both healthy young and older adult groups. Central drusen altered

fixation stability characteristics, resulting in compensatory FEM changes. High speed, high resolution FEM analysis may help to define the functional signatures of early age-related changes.

Introduction:

Age-related Macular Disease (AMD) is the main cause of vision loss in developed countries, affecting 180M people in 2020 with an expected prevalence of 288M by 2040 (Pennington 2016, Saunier 2022[1], [2]). AMD can cause geographic atrophies in later stages by being drusen-related (dry-AMD) or neovascularization-related (wet-AMD) (Danis 2015[3]). Wet-AMD, caused by irregular blood vessel growth at the macular level, accounts for 10-20% of the cases (Pugadenzi 2021[4]) and is usually treated using anti-VEGF before it becomes a threat to the patient's vision. Dry-AMD, about 80 to 90% of the cases (Pugadenzi 2021[4]), starts with the accumulation of extracellular deposits called drusen in-between the bruch's membrane and the RPE (Danis 2015[3]). Dry-AMD has become a major public health concern as no effective treatment currently exists and little is known about the drusen development in preclinical stages. Today's challenge is to be able to detect dry-AMD at its earliest stage in order to better track and understand its progression, testing new treatments, as well as preparing patients for possible vision loss.

The development of AMD has already been associated with decreased visual acuity, reduced visual field, slower reading speed and lower contrast sensitivity (Verghese 2021, Pondorfer 2020, Calabrese, Chung[5]–[8]). More specifically, macular disease has been shown to affect oculomotor control and increase fixation instability (Macedo 2011, Kumar 2014[9], [10]). In 2021, Preeti Verghese made an extensive review of eye movements in macular degeneration (Verghese 2021[5]). She specifically describes the consequences of AMD on eye movements to understand the effects the disease can have on daily activities. Reading, visual search and smooth pursuit have been analyzed in depth in multiple papers while the displacement of the preferred retinal locus (PRL) in patients with central vision loss (CVL) is an active field of research.

Understanding how the brain and the retina adapt to scotomas is important to help patients accustom to their condition as well as understand the mechanisms of vision.

Fixation stability has been studied in early to late AMD and has been shown to worsen with the stage of the progression (Macedo 2011, Kumar 2014[9], [10]). However, it is only in 2014 that fixation stability in macular degeneration was studied through the analysis of fixational eye movements with Kumar and Chung (Kumar 2014[10]). During periods of visual fixation, the gaze does not remain completely still. Unaware of the motion, people still produce micro eye motion that has been named fixational eye movements. Fast microsaccades occur 1-2 times per second with amplitudes below 1° (Martinez-Conde 2013[11, p. 201]) are interrupted by slower and longer ocular drift moving the eye according to a random brownian walk (Intoy Rucci 2020, Hermann 2022[12]). Ocular tremor are high frequency sub-arcminute oscillations taking place during the drift (Bolger 1992[13]). Kumar showed that microsaccade amplitude is significantly larger in patients with lesions due to macular degeneration, therefore increasing fixation instability measured with BCEA and ISOA (Kumar 2014[10]). Using the same set of participants, Ağaoğlu focused on ocular drift and observed less spectral whitening of natural scenes in patients with geographic atrophies (Agaoglu 2020[14]). While these two papers show clear signs of changes in fixational eye movements on patients with advanced forms of AMD, they remain exploratory. Authors did not seek a biomarker for early cases, nor characterize the links between the characteristics of retinal structure changes and FEM.

While to the best of our knowledge alterations of oculomotor control with small changes of retinal structure have never been studied, the application of eye movements to identify biomarkers of any kind of disease is not new. The main findings have been summarized by Maradini and Gazerani (Marandini 2019[15]). Eye movements can reveal information about neurological disorders or retinal integrity and could provide non-invasive biomarkers. However, FEM have shown to be trickier to study because of the high end high speed eye-tracker requirement. They have mostly been studied in neurological disorders such as amblyopia (Shaikh 2016[16]),

schizophrenia (Egana 2013[17]), Parkinson's disease (Kaski 2017[18]) and few more (Alexander 2018[19]). Recent attempts to reveal new biomarkers from FEM have relied on microsaccadic intrusions to predict multiple sclerosis and follow its development (Sheehy 2020[20]) or fixation abnormalities in amblyopia to follow treatment efficiency (Ghasia 2022[21]).

In this study, we propose to study FEM in patients with small foveal drusen at a pre-clinical stage of AMD. Pathological participants to this study were not diagnosed with AMD but small drusen were detected within the foveal region. The first objective was to link changes in FEM with subtle retinal structure changes induced by the drusen. This required to estimate FEM with high resolution and to precisely characterize drusen position and size. We believe this level of precision in drusen characterization combined with eye movement exploration has never been done and could help better understand how patients adapt their oculomotor behavior to the appearance and development of drusen. Then, if patients with drusen are confirmed with higher fixation instability at this stage, we aimed at identifying which FEM components modification carry the potential to become biomarkers for the presence of drusen in the foveal region.

Methods

Participants

In total, 39 participants were recruited to the study as part of one of three groups: "young controls" (n=7 [2 females], age=31±7 yo), "older controls" (n=21, [13 females], age=75±2 yo) and "pre-symptomatic AMD" (n=11, [6 females], age=79±2 yo). The latest group was renamed "drusen group" as the only certainty was the presence of foveal drusen, first revealed by OCT imaging. Before the beginning of the experiment, each participant from the older controls and the drusen group were imaged using a commercial flood-illumination adaptive optics ophthalmoscope (rtx1, Imaging Eyes) and following the gaze-dependent protocol described by Rossi et al. (Rossi 2021[22]). This allowed for a more precise visualization of the drusen in the foveal region.

Three participants from the drusen group had too blurry images for the gaze-dependent protocol when using the rtx1, mostly due to strong lens opacity. Three participants had to stop the experiment due to poor signal-to-noise ratio during the recordings. Two participant's ocular traces were discarded because they were not fixating at the right target during the task. Three participants were not included in the data analysis because of an excessive blinking rate. Eleven older controls with no detected foveal drusen on OCT were found to have several of them using the gaze-dependent imaging protocol and had to be switched to the drusen group.

Overall, 7 young controls([2 females], age=31±7 yo), 4 older controls ([1 female], age=72±2 yo) and 16 drusen patients ([10 females], age=74±4 yo) were kept for the study and their visual characteristics are given in table 1.

Ethics Committee approval (CPP) was obtained and adhered to the tenets of the Declaration of Helsinki. All participants were part of the SilverSight cohort of the Vision Institute (Paris, France) and a signed consent was obtained to participate to the study (ID_RCB 2015-A01094-45, n. CPP: 16122 MSB) as well as for the inclusion to the Multimodal Ophthalmologic Imaging protocol (IMA-MODE, ID_RCB 2019-A00942-55 CPP: Sud Est III 2019-021-B) of the Quinze-Vingts National Ophthalmology Hospital.

Experimental design and setup

The imaging setup is located at the Quinze-Vingts National Ophthalmology Hospital in Paris, France. Subjects had their dominant eye dilated with a drop of 1% tropicamide (*Mydriaticum*, tropicamide 0.5%). Then, in a dark room with minimal stray light, they placed their head on a chinrest where video recordings of their fovea focused at the photoreceptors layer were made using an *Adaptive Optics Flood-Illumination Ophthalmoscope* (Paris AO-FIO) developed by ONERA (Gofas 2018, Krafft 2021[23], [24]) and described in the following section. First, a full field sequence of images covering a 4° diameter field of subjects' retina was recorded at 100Hz (2048 x 2048 pixel frames) over 5 sec. This served to generate a reference image of the center of the

retina. Then, for the fixation task, only a subband (2°x0.5° with the fixation target at the center) was recorded at 800 Hz. Participants were asked to fixate a black circle over a red background (outer diameter: 17 arcmin, inner diameter: 11 arcmin) for 30 seconds. Presentation and recordings were repeated 5 times. The instructions were *"Fixate the circle for 30 seconds. Do not speak and try not moving your head. Do not hesitate to blink if you need to."* and a vocal indication about time progression was provided after 15 seconds. The stimuli projection with the Digital Micromirror Device (DMD) were controlled using a custom-made Python code and the ALP4Lib python library wrapper. The adaptive optics loop was controlled with a LabVIEW program and image acquisition was monitored via Holovibes software.

Table 1: detailed visual characteristics of participants from the drusen group and overall information from young and older adults.

Table 1

Tag	Age (yo)	Tested eye	Visual acuity	Number of drusen	Mean area ± std (arcmin²)	Mean eccentricity ± std (arcmin)
ALG	78	OD	1	7	153 ± 60	28 ± 23
ATA	74	OD	1	2	96 ± 26	45 ± 14
BEH	70	OS	1	6	162 ± 36	56 ± 32
BEY	81	OD	1.2	8	112 ± 58	69 ± 42
ATM	75	OD	1.2	20	167 ± 35	68 ± 34
BLJ	76	OD	1.2	28	172 ± 68	92 ± 34
DEB	73	OD	0.8	4	120 ± 33	62 ± 22
DEC	80	OD	1	5	68 ± 16	30 ± 25
LED	79	OD	1.2	7	72 ± 13	27 ± 22
LES	76	OS	1	9	119 ± 66	45 ± 27
MAC	80	OD	0.7	6	116 ± 45	68 ± 19
NAM	74	OS	0.8	14	53 ± 14	76 ± 25
OLD	84	OS	1	13	161 ± 44	63 ± 25
PEF	80	OD	1.2	32	307 ± 134	49 ± 31
PEN	83	OS	1	12	160 ± 46	42 ± 26
ROM	84	OS	1.2	7	129 ± 29	37 ± 29
Total (Drusen group, n=16)	78 ± 4	/	1 ± 0.2	11 ± 8	133 ± 59	53 ± 27
Older adults (n=4)	72 ± 2	/	>1	/	/	/
Young adults (n=7)	31 ± 7	/	>1	/	/	/

Image acquisition

The Paris AO-FIO has been described in detail in [Gofas 2018[23]], and enhanced to permit DMD-based retinal projection in NIR for structural imaging (Krafft 2021[25]) and partial-field illumination [Krafft 2021[24]]. The system consists of two optical channels: i) the Wavefront correction channel and ii) the Illumination and imaging channel, combined with the Stimulation channel. A fibered SuperLuminescent Diode (SLD) (Exalos, Switzerland) with nominal center wavelength of 750 nm is used to measure the aberrations and an 850nm LED source (Thorlabs, USA) coupled with a liquid fiber homogeneously illuminates the retina over a 4° diameter field of view. Importantly, the aberrations affecting the illumination patterns were pre-compensated before being projected into the retina: the light coming from the illumination path reaches the deformable mirror of the AO sub-system before reaching the eye. Then the backscattered light coming from the eye is reflected back on the deformable mirror before reaching the imaging camera (Orca Fusion, Hamamatsu, Japan).

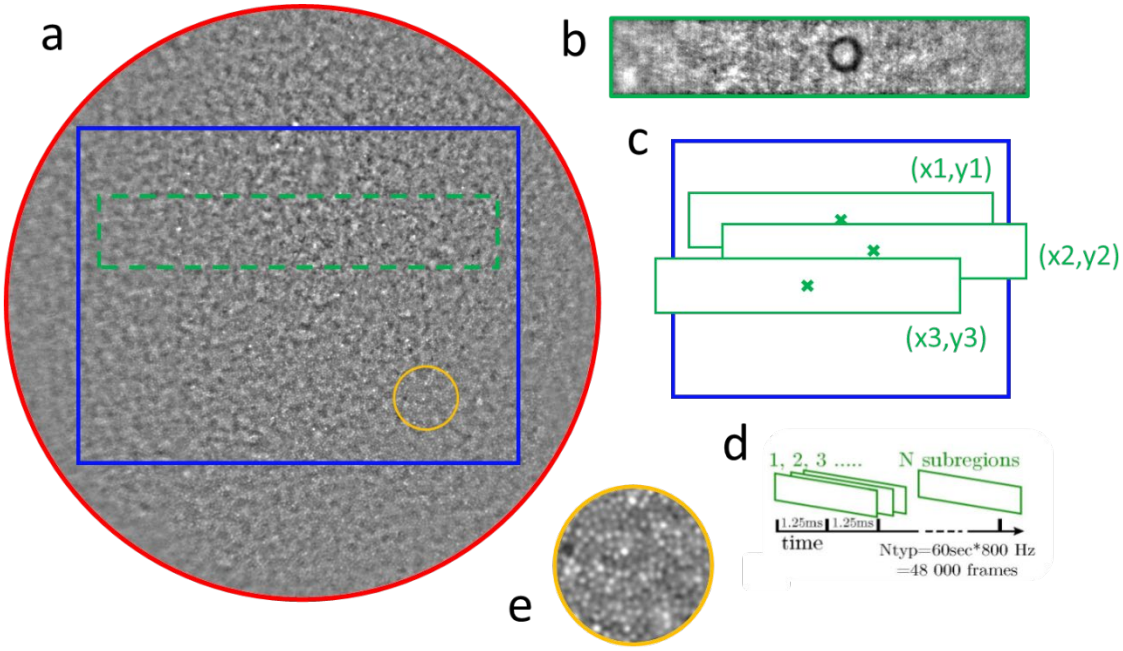


Figure 1. Principle of the AOFIO retinal tracking. a. The 4° full field was obtained from 5 sec. recording at 100 Hz after registration (red). A rectangular sub-part (blue) is enough to track and monitor eye movements on task requiring central fixation (typ. <2°). The 2 x 0.5° subregion (green) is tracked at very high speed . b. An actual frame recorded at 800 Hz on 16 bits. c. The displacement is obtained by search (phase-correlation) over the rectangular full-field. d. An adhoc eye position extraction software was necessary to process large sequences of up to 5 Mbits/frames. e. A 3x zoom on an area 1° away from the fovea.

Tracking eye position

Subjects were required to fixate a central cross during an initial step of 5 sec. The sequence of full field retinal images obtained at 100 Hz during these 5 sec were first stabilized and averaged into a unique full field reference frame. This reference frame was used to track the retinal motion of each frame of the high speed image sequences of the experiment. These recordings were performed at 800 Hz on a 2° x 0.5° subregion (1400 x 256 pixel). Even if the maximum sampling frequency of the camera was only limited by vertical pixel resolution, we found that 1400-pixel horizontally offered a good tradeoff between field coverage and video bandwidth. The resulting 0.684 MB/frame (16 bits) leads to a very large 547 MB/s video bandwidth that could only be recorded with solid state hard drives and ad-hoc recording software (Holovibes). Videos were recorded on a separate computer than the one used for stimuli presentation. Retinal motion was tracked using a phase-correlation registration method based on Mecê[26] after bandpass filtering the image sequences.

Eye motion processing and event detection

Processing of the raw pixel displacement of each frame relative to the reference images to ocular movement in arcminutes with annotated events was made with a MATLAB (Mathworks, USA) program. Microsaccades were detected using an acceleration threshold inspired by Engbert's detection method (Engbert 2006[27]). Here, acceleration rather than velocity threshold (factor N=3 standard deviation of the median) was used in order to differentiate fast ocular drift epochs from small and slower microsaccades which can have similar velocities but distinct accelerations. The acceleration was computed for the 30s fixation, and smoothed over 70 ms windows. This adaptive algorithm allowed for thresholds that fitted both participants with small microsaccades and stable ocular drift, and participants with larger microsaccades and drift amplitudes. However, to make sure microsaccades were all entirely detected or that some parts of ocular drift were not

mislabelled as microsaccades, the traces were manually verified after the automatic annotation. Blinks were detected automatically as sudden drops in mean frame pixel intensity, which corresponded to the closed eye reflecting less light back to the camera. Rejected data were epochs of ocular coordinates marked by the user as false data due to failures in the imaging or the tracking (to the exclusion of blinks). They accounted for 0-3% of the fixation data for each participant. Everything in-between two events –microsaccades, blinks or rejected data– was automatically marked as drift.

Root mean square sample to sample (RMS-S2S) deviation was calculated as a way to measure the precision of the tracking method. A given RMS-S2S of an eye-tracker would mean the average movement due to noise between successive data is its squared value (Holomqvist & Nystrom 2011[28]). Since the ocular traces used in this tracking method were not filtered, which can have a strong effect of RMS-S2S and skew the results (Niehorster 2020[29]), it appeared to be the right metric to measure precision. The average RMS-S2S across all participants was 0.001° (σ=0.0005°), and 0.0001° on a non-moving model eye (OEMI-7 Ocular Imaging Eye Model). This allowed the explicit visualization and detection of microsaccades as small as one or two arcminutes (see figure 2).

Computing fixation stability and PRL position

The Bivariate Contour Ellipse Area (BCEA) and more recently the ISOline Area (ISOA) have been used in clinics to measure fixation stability of patients with Central Vision Loss (CVL), including AMD [Whittaker *et al,* 1988[30]]. ISOA provides additional benefits as it appears more robust to delineate non-ellipsoidal fixation patterns i.e. multiple fixation loci (Castet and Crossland 2012). As suggested by these authors, the existence of multiple loci might not necessarily represent multiple PRLs, providing that the subject might voluntarily aim at different locations of the fixation target. In this study, using precise instructions, a small fixation cross and numerous trials obtained over relatively long durations, we aim at answering whether ISOA might serve as a method to

identify multiple PRLs on patients with early form of AMD. The choice of the bandwidth and the extent- number of standard deviations- to compute the BCEA and ISOA appear relatively arbitrary and open to criticism [Ref Castet, ref Martinez-Conde, 2006; Otero-Millan *et al,* 2008[30]–[32]]. The choice of using a 1° window for Kernel Density Estimation (KDE) has been justified (Martinez-Conde, 2006; Otero-Millan *et al,* 2008[31], [32]) and robust to a classical definition of the 1° foveola. We report the 68% ISOA (encompassing 68% of the data points) as done in the literature. Additionally we report the 86% ISOA, i.e. computed over 2 standard deviations in the 2D space defined from the center of the fixation distribution (Wang et al. 2015[33]). We think adding 86% (2 STD) ISOA contour provides complementary insights on the global fixation pattern that might differentiate healthy people from patients with drusen.

Drift diffusion coefficient and amplitude spectrum computation

In the framework of Brownian motion [Engbert 2004, Intoy 2020[12], [34]], the movement during the drift epochs can be modeled by a random walk and the general motion can be measured by the diffusion coefficient. A similar method to that used by Kuang et al. [Kuang 2012[35]] was applied.

The motion amplitude spectra of these drift segments were then further analyzed using a multitaper spectral analysis. This was carried separately on the horizontal and vertical component of the eye motion, as proposed by Thomson [Thomson 1982[36]], and Babadi and Brown [Babadi Brown 2014[37]]. As in [Bowers et al. 2019[38]], the analysis was run on overlapping segments of 1sec. The time half-bandwidth product was fixed at 2.5 and the last taper was dropped to maximize spectral concentration ratios in the Slepian sequences.

Gaze-Dependent imaging and drusen visualization

Foveal drusen were first revealed using an SD-OCT (SPECTRALIS OCT, Heidelberg Engineering, Germany). However, in order to get more precise information about the position and

size of the drusen in the fovea, gaze-dependent imaging was performed on every participant from the older adults and the drusen groups using the same commercial FIAO device (rtx1, Imagine Eyes, Orsay, France). It is an imaging protocol which uses FIAO to reveal drusen with high contrast and resolution (Rossi 2021[22]). The method allows for the visualization of small drusen (<63µm) in the fovea (zone covering 2° radius around the center of the fovea) better than previously known methods. The mean drusen diameters across the 16 participants of the drusen group was 65 µm (σ=20). The protocol described by Rossi et al. consists in taking a 4° fovea-centered image of the retina, focused on the photoreceptors, and eight ±2° horizontally and vertically displaced images. Then, image registration was made to overlay the nine images and the standard deviation was computed to make gaze-varying structures visible. Two protocols exist for the image acquisition and for this study the “5-image protocol” was used: participants had a 4° fovea centered image taken and four others ±1° horizontally and vertically displaced from the center image. Image filtering, registration and processing were made using a MATLAB software. Annotations of the drusen on the resulting images were performed manually using another MATLAB software to draw ellipses on the structures believed to be drusen.

Results:

Characterisation of healthy and pathological fixational eye movements

Participants from the drusen group were split into two sub-groups after information was extracted from gaze-dependent imaging. Taking the approximate size of the foveola (0.4-0.6°), the stimulus diameter (0.28°) and the mean foveal drusen diameter in this study (0.25°), the two subgroups were decided as such: participants with drusen in their foveola (mean drusen eccentricity less than 1° away from the center of the fovea) and participants with drusen outside their foveola.

Microsaccade amplitude, drift diffusion coefficient and isoline area were analyzed to characterize the fixational eye movements of the different groups. Individual distributions of microsaccade amplitudes can be found in Figure 2, sorted from lowest mean amplitude to highest and color-coded according to their groups. Healthy participants were overall shifted to the lowest amplitudes and pathological participants to the highest ones, but mean amplitudes seemed distributed on a continuous function with no clear separation. On the microsaccade amplitude and ISOA scatter plot (Figure 4), participants with drusen inside their foveola are clustered around higher values.

T-tests and linear mixed effect statistical model analysis revealed significant differences between the microsaccade amplitudes of the drusen inside the foveola group and the three other groups ($|t|>5$, $p<10^{-5}$). The drusen outside the foveola group had overall larger microsaccade than young and older adults but no significant difference (respectively $|t|=1.7$, $p=0.09$ and $|t|=1.6$, $p=0.06$). There was no major difference between young and older adults ($|t|=0.4$, $p=0.66$).

The same tendency was found when comparing ISOA 86% values, which is consistent with microsaccade amplitude and ISOA being highly correlated (Figure 4 $R^2=0.85$). All t-values and p-values can be found in table 2. The isoline contours 68% and 86% are shown in figure 3. The ISOA approach offered an interesting topology of the shape of the fixation instability. This shape appeared specific to each participant, especially for those with higher instabilities. However, the participants with the highest ISOA -from the drusen group- had contours with very different patterns and island and not a uniform larger area shape. While some fixations expanded more on the horizontal axis, a few participants had their fixation area grow on the vertical axis, and a couple of them started creating multiple loci of fixation. Overall, these specificities were more visible using contours encompassing 86% of the fixated locations.

Drift diffusion coefficient has more inter- and intra- individual variability, as shown in Figure 2D and 4B, which is consistent with literature saying that drift reveals more than just fixation stability, being an indicator of visual acuity, fatigue, comfort and other types of neurological

diseases (ref). No significant difference was found between the groups but the drusen groups had higher average drift coefficients than healthy groups.

High frequency of microsaccade is a common cause for higher fixation instability. Those changes can be explained by changes in visibility (Cui et al 2009), visual or auditory stimuli detection (Rolfs Engbert 2008[39]), signs of covert attention (Engbert 2003[40]), or discomfort and fatigue (Di Stasi 2013[41]). They can also reflect neurological disorders or medical treatment (Alexander 2018[19]). However in this study there were no significant differences in microsaccade rate between groups that could explain the differences observed in fixation stability and almost every participant had a normal microsaccade rate of 1-2 Hz (figure 5).

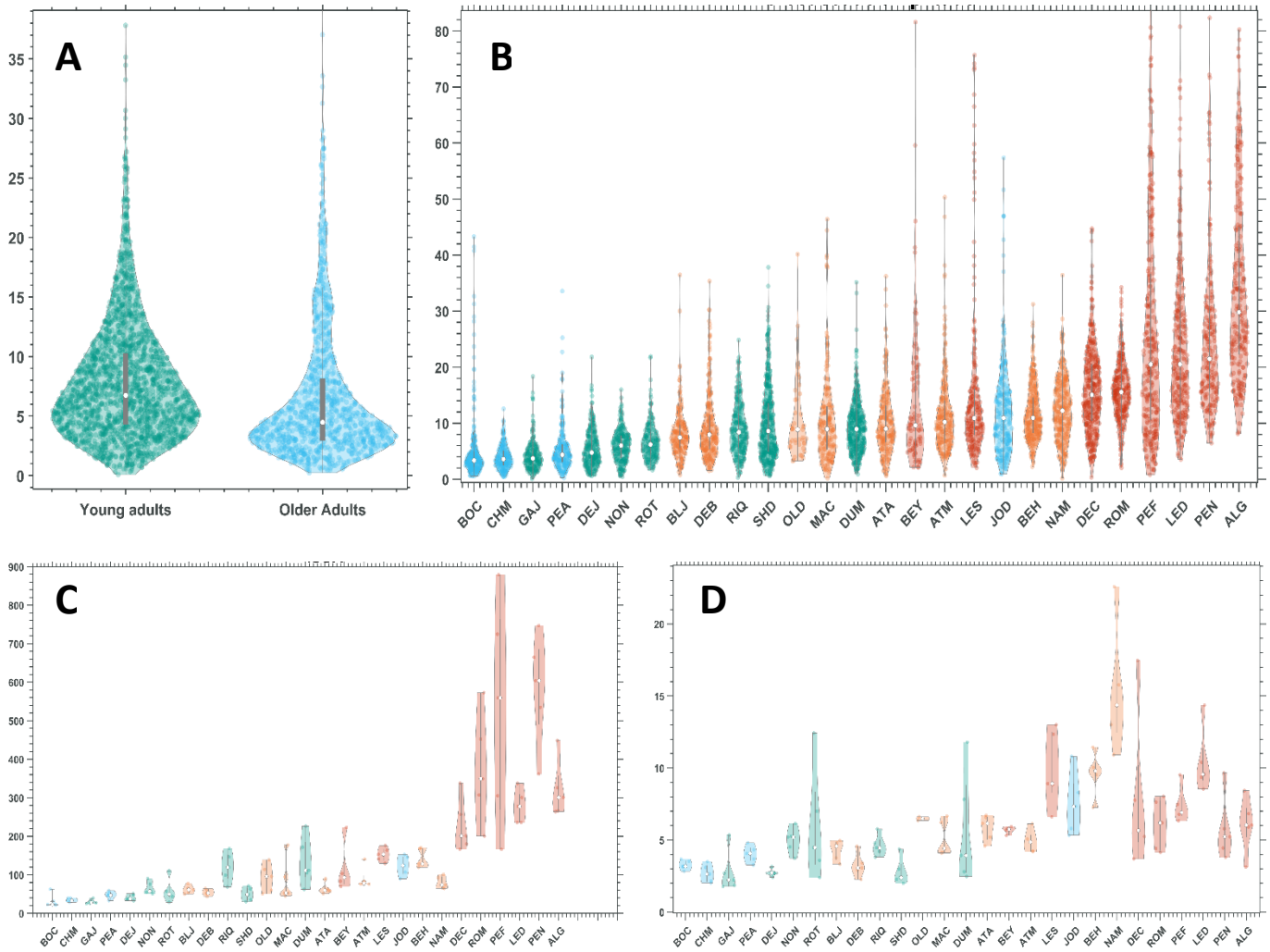


Figure 2. Fixation characteristics. (A) Microsaccade amplitudes distribution for healthy young and older controls. Each dot is one microsaccade during one of the trials. (B) Individual microsaccade amplitudes distribution for each participant to the study, color-coded by group and sorted by mean microsaccade amplitude. Green is for young controls, blue for older controls, orange for the drusen outside of the foveola group and red for drusen inside of the foveola group. (C-D) Respectively ISOA 86% and drift diffusion coefficient distribution for each participant, sorted by mean microsaccade amplitude. Each dot is one trial.

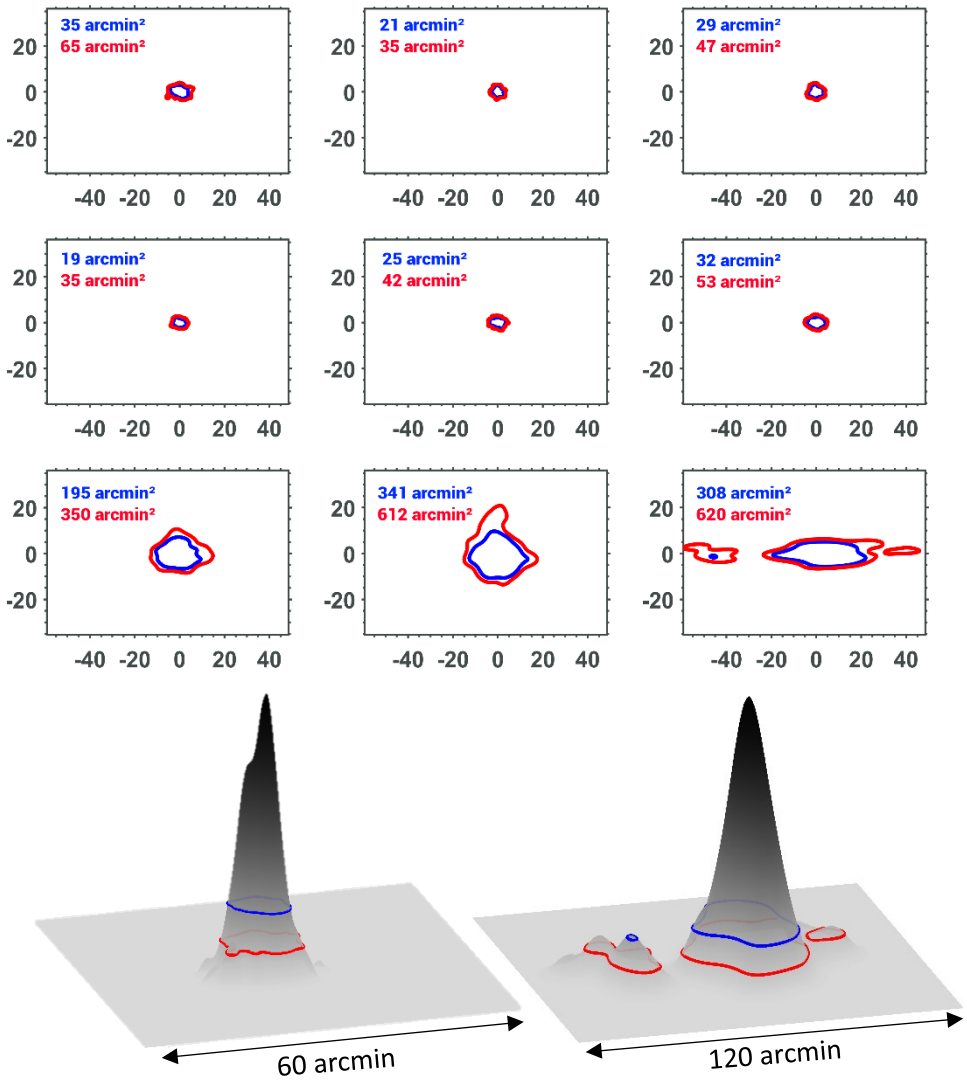
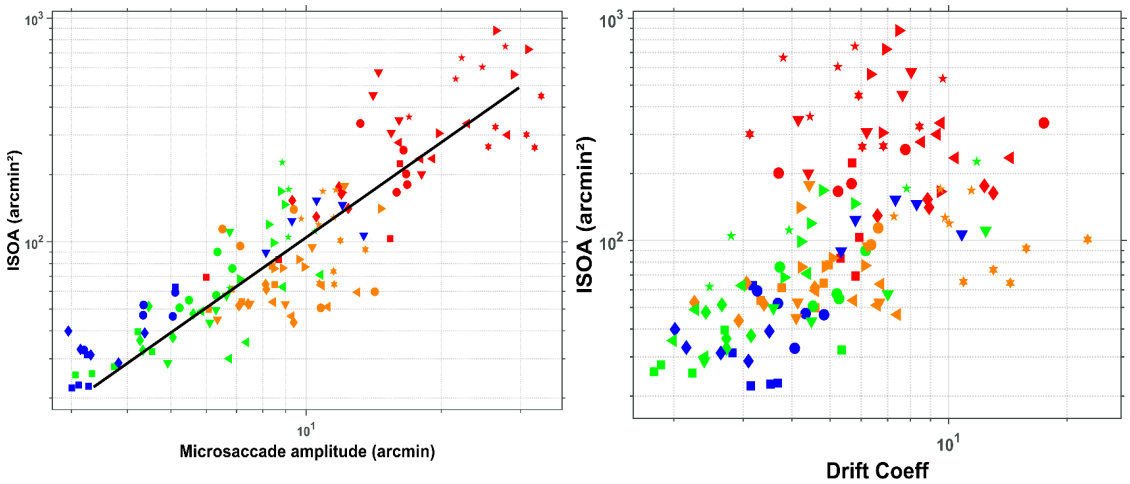


Figure 3. Fixation stability (A) Isoline contours and areas 68% and 86%. The first line shows data from the young controls, the second line from older controls and the third line from participants with drusen. (B) probability density 3D plot of the top left participant of (A) on the left and the bottom right participant of (A) on the right.

Figure 4. scatter plot of microsaccade amplitude vs ISOA on the left and drift coefficient vs ISOA on the right. One symbol represents one trial from a participant from its colored group. The black line is the linear regression of the data. R^2=0.83, p<0.001.



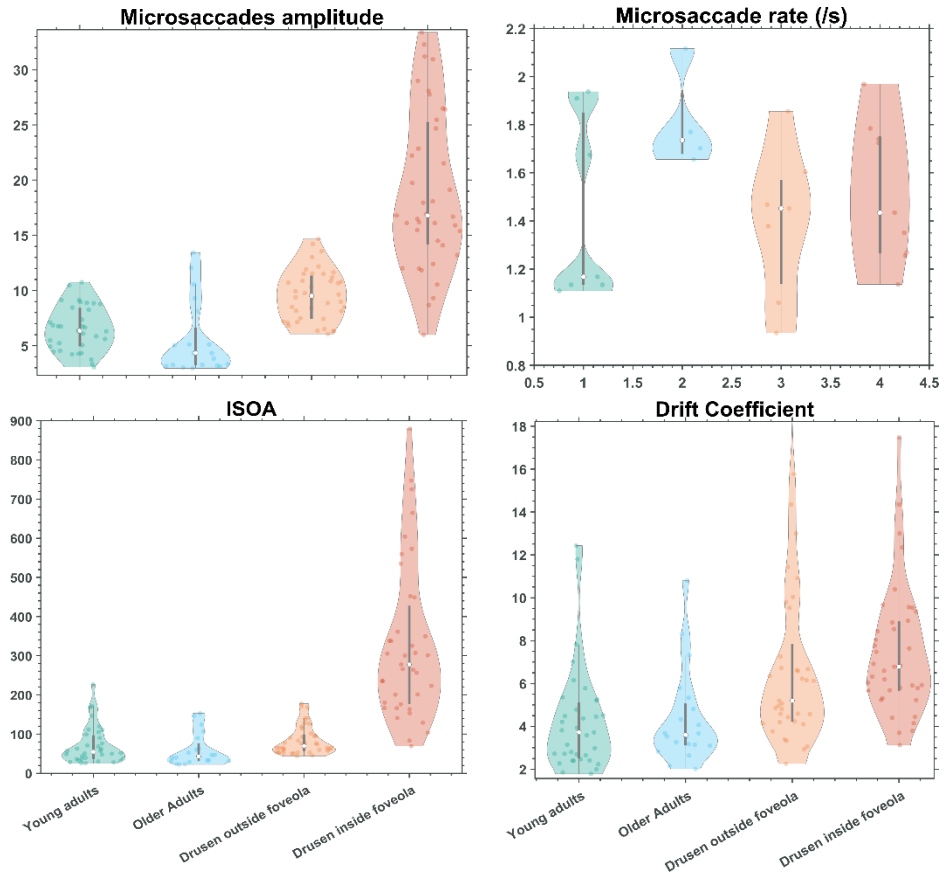


Figure 5. Fixation characteristics comparison by groups. All statistical analysis can be found in Table 2

		Microsaccade amplitude		ISOA		Drift Coefficient	
Groups compared		|t-value|	p-value	|t-value|	p-value	|t-value|	p-value
Young controls	Older controls	0.4	0.66	0.2	0.83	0.1	0.93
Young controls	Drusen outside foveola	1.7	0.09	0.24	0.81	**2**	**0.04**
Older controls	Drusen outside foveola	1.8	0.06	0.4	0.67	1.7	0.1
Young controls	Drusen inside foveola	**6.5**	**10-9**	**5.5**	**10-7**	**2.5**	**0.01**
Older controls	Drusen inside foveola	**6**	**10-9**	**4.9**	**10-6**	**2**	**0.04**
Drusen outside foveola	Drusen inside foveola	**5**	**10-7**	**5.4**	**10-7**	0.4	0.67

Table 2. t-values and p-values of the linear mixed-effect statistical model to compare microsaccade amplitude, ISOA and drift coefficient values between groups. P-values below 0.05 are marked in bold.

Characterization of drusen and their effects on fixation

Foveal drusen information was extracted from gaze-dependent images by manually drawing ellipses around them (Figure 6C). The number of drusen, the eccentricity from the center of the drusen to the center of the fovea, the areas, the diameters of the ellipses (average of small and large diameter) and total area covered by all drusen were computed. Individual information about drusen are given in table 1 as well as average number, size and eccentricity. Overall distribution of drusen diameters is given in Figure 6B.

As a first step to find a correlation between drusen and fixation, a simple linear correlation map was computed using different drusen and fixational eye movements characteristics. The most significant correlations were found for the mean drusen eccentricities and both microsaccade amplitudes and ISOA ($r^2 = 0.52$, $p=0.001$). Since ISOA and microsaccade amplitude are highly correlated, only the results of microsaccade amplitude will be shown from now on. The negative correlation means microsaccades get larger the closer drusen are to the center of the fovea. However, as made in evidence in Figure 6, the best correlation was not using a linear regression but by fitting a rational function to the scatter plot ($r^2=0.6$, $p=0.002$). The closer drusen are to the center of the fovea, the larger microsaccades are, but the further away drusen are, the more subjects tend to produce healthy microsaccades.

Finally we hypothesize that more than drusen eccentricities, or their local density, it's their density related to their eccentricity that might best reflect their influence on fixation stability. The rapid drop-off of both cone density and acuity in the fovea suggest that both drusen size and eccentricity can have tremendous effect on foveal vision. We then reported drusen areas divided by their eccentricities. The mean drusen density was also even further correlated to microsaccade amplitude ($p=0.0002$, $r^2=0.66$) but with higher standard deviations for each participant.

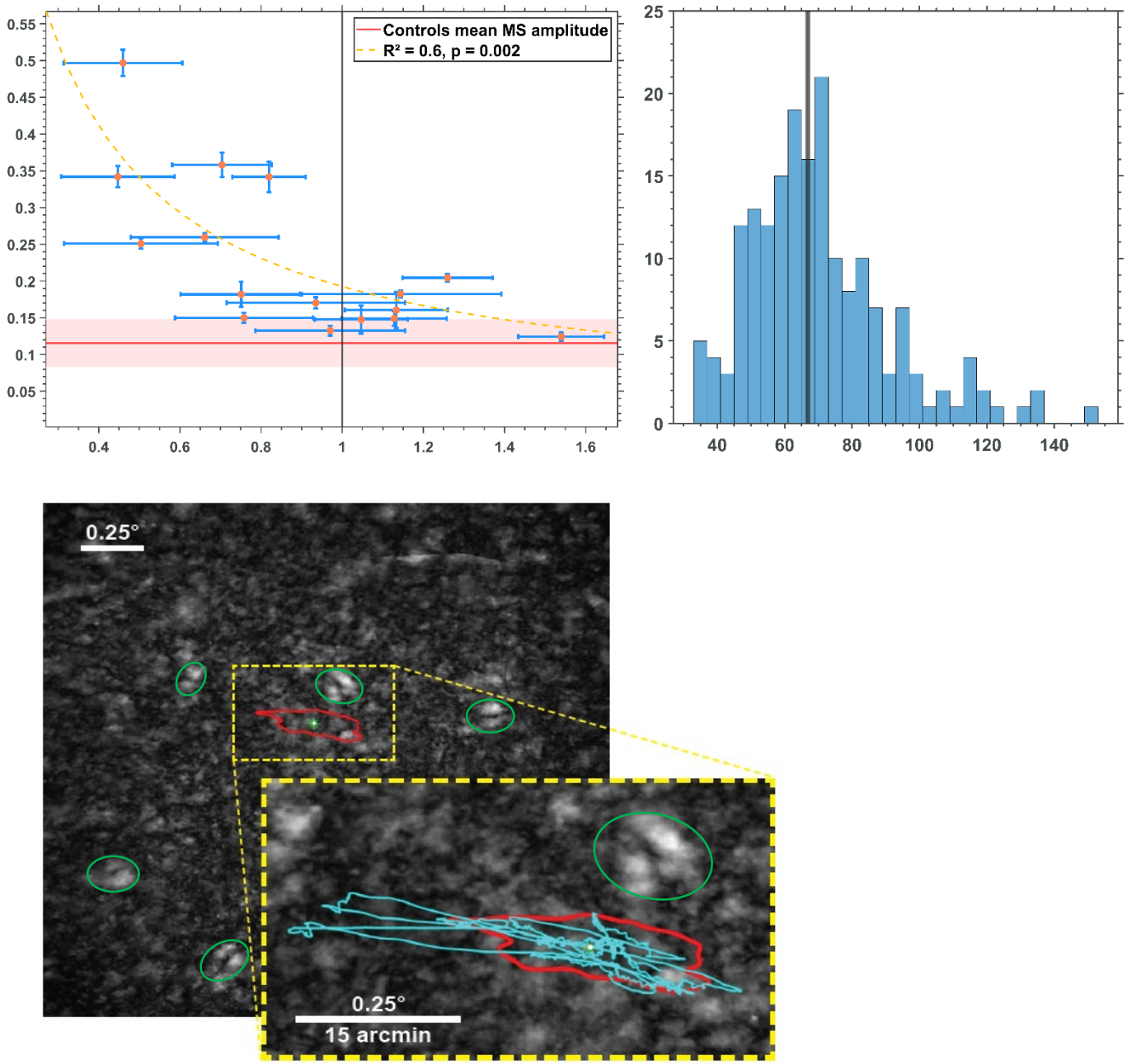


Figure 6. (A) Microsaccade amplitude as a function of drusen eccentricity for the 16 participants from the drusen group. (B) Drusen diameter distribution from all participants. The mean value is 63µm as the black vertical line. (C) Gaze-dependent image with isoline area 86% (red line), PRL (white dot) and ellipses around the drusen (green lines). 3x zoom is shown in the yellow dotted rectangle with 5 seconds of ocular traces in blue.

Discussion

In this study, the role of foveal drusen on fixational eye movements in patients with early AMD is investigated. Patients with drusen exhibited significantly larger microsaccades and fixation stability (ISOA) while maintaining similar microsaccade rates compared to healthy age-control subjects. The ocular drifts possessed higher diffusion coefficients. However, it drove less the fixation instability than microsaccade did (Fig.9A, B, C or D), markedly in the drusen group. In order to understand and quantify further the role of these structural markers on functional vision, we mapped these drusen onto the retina. To do so, we used a recently developed enface drusen identification method (Rossi 2021[22]).

Drusen eccentricity to the center of the fovea was the main source of the larger microsaccade amplitude and ISOA. The closer the drusen were, the more unstable the fixation was. The further away the drusen were, the more the participants tended to behave like control participants, with minimal to no changes in fixation stability. The figure 6A plots the drusen eccentricity to the microsaccade amplitudes and reveals a beginning of continuum following the eccentricity axis, but we nonetheless tried to understand if there was a limit at which point drusen had less influence on fixation. We observed that MS amplitude starts increasing much faster when drusen are closer than around 0.8° to 1°. Patients with drusen further than that had only a minimal increase compared to controls and had less variability in-between them. Considering the size of the drusen and the fixation target, we hypothesized that the limit at which drusen start to disturb fixation is at the foveola, the highest acuity area of the fovea. The function of microsaccades is still a matter of debate but many researchers agree on saying that they mainly serve a purpose inside of the foveola, and taking its size as a reference to define microsaccades (Martinez-Conde 2013, Poletti 2023[11], [42]). As discussed in Poletti's review on attention and eye movements at the foveal scale (Poletti 2023[42]), any stimuli in the foveola can be attention-grabbing and whether it is voluntary or not, it is possible to have microsaccade directed towards different stimuli

as close as 20 arcminutes from each other. Drusen might not be direct stimuli but they change retinal structure in distinct areas. It is possible that they influence perception at those spots and therefore affect fixation if they are located in the foveola. If so, a fixation test could be suitable to discriminate healthy participants to ones having retinal anomalies in the foveola, such as drusen, and it would focus on microsaccade amplitude and ISOA.

Another interesting observation made in this study is about how we defined healthy older adults. Many of them were recruited as healthy controls because no visible drusen were found on the fovea using SD-OCT imaging analyzed by eye doctors. However, using gaze-dependent imaging, a lot of them were found to have drusen in their fovea. Dry-AMD always starts with the development of small drusen but it has been established that the sole presence of small hard drusen does not mean the patient will advance into the different stages of AMD (Ferris et al 2013[43]). Our observation aligns with findings that suggest drusen appearing under the retina is part of normal aging and very prevalent (Ronald Klein 1992, Neil Bresseler 1989[44], [45]), and it would seem that older participants without drusen were actually the outliers but no recent studies with more advanced imaging techniques have been made to confirm those findings. That would also partly explain why some studies reported differences in fixation stability between young and older adults (Cutini 2014, Agathos 2015, Morales 2016[46]–[48]), and some studies do not (Kosnik 1986, Gonzales 2019, Becu 2018[49]–[51]) such as ours. Since having macular drusen is a common trait and that they affect fixation, some results could be distorted if some studies excluded because of signs of drusen and others did not. It is currently impossible to know whether or not drusen will grow into bigger and more dangerous ones. The important aspect today is to detect them at the earliest possible moment to track its progression and understand its development. That is why the emergence of recent imaging methods that allow the precise visualization of small drusen is crucial, such as retromode imaging (Cozzi et al 2023[52]) or gaze-dependent imaging (Rossi et al 2021[22]) that can both detect smaller drusen before a classic SD-OCT analysis. Our

findings show that those small drusen could also be detected by looking at the changes in fixational eye movements during a fixation task.

Lastly, this study used a novel method to track FEM by imaging the foveal region of the retina with high speed, high resolution and no distortion thanks to an AO-FIO that could also project stimuli for psychophysical testing. Research on eye movements has existed for more than a century, and the recent video infrared eye trackers carried important promises with applications to the diagnostic, therapeutic and follow-up of neurologic and eye diseases. Pupil-based infrared video eye-trackers remote tracking capabilities, accuracy and accessibility renewed the interest on eye movements to clinicians and psychologists. Research on FEM in particular revealed important insights on neurological diseases. However it is now accepted that such technology suffer of several limitations to study FEM, from the actual main range of microsaccade not acquired, making researchers shift the amplitude-based definition of the microsaccades from limits of 0.2° to 1° (Steinman, Nyström, Poletti and Collewijn, Martinez-conde[42], [53]–[55]), to basic recording of microsaccade dynamic (PSO: Artal, effect of pupil size on MS size, etc). Microsaccades amplitude measurement has been only recently found consistent with early recordings through the use of complex Adaptive Optics (AO) retinal imaging instruments. AOSLO offered a welcomed capability in tracking the landing position of the stimulus onto the retina (see Bowers, Gautier et al. 2021[38]). The AO-FIO in the mode we used in this study does not reach as high image contrast as AO-SLO can. However, it does not suffer from any distortion, unlike any SLO system whose scanning nature does not make it ideal for motion tracking. Using our retinal tracking method, we found an average healthy microsaccade amplitude of 6.3 arcminutes while patients with drusen had an average microsaccade amplitude of 13.8 arcminutes. In the past 20 years, FEM studies using pupil-based eye trackers have defined a microsaccade with amplitudes up to 1-2°, and basic fixation tasks often showed average amplitudes of 20-60 arcminutes. It is not impossible that the differences between controls and drusen participants in this study would not have been found using less precise but more available eye trackers, or a bigger fixation target

since microsaccade amplitude depends on the fixation target (Bowers 2021[38]) and we used a very small one. FEM give unique signatures about a patient's neurological and physical health but the improvement of precision and availability of eye-tracking methods is a key point to being able to use them as biomarkers.

References

[1] K. L. Pennington et M. M. DeAngelis, « Epidemiology of age-related macular degeneration (AMD): associations with cardiovascular disease phenotypes and lipid factors », *Eye Vis (Lond)*, vol. 3, p. 34, déc. 2016, doi: 10.1186/s40662-016-0063-5.

[2] V. Saunier *et al.*, « Incidence of and Risk Factors Associated With Age-Related Macular Degeneration: Four-Year Follow-up From the ALIENOR Study », *JAMA Ophthalmology*, vol. 136, nº 5, p. 473-481, mai 2018, doi: 10.1001/jamaophthalmol.2018.0504.

[3] R. P. Danis, J. A. Lavine, et A. Domalpally, « Geographic atrophy in patients with advanced dry age-related macular degeneration: current challenges and future prospects », *Clin Ophthalmol*, vol. 9, p. 2159-2174, nov. 2015, doi: 10.2147/OPTH.S92359.

[4] A. Pugazhendhi, M. Hubbell, P. Jairam, et B. Ambati, « Neovascular Macular Degeneration: A Review of Etiology, Risk Factors, and Recent Advances in Research and Therapy », *Int J Mol Sci*, vol. 22, nº 3, p. 1170, janv. 2021, doi: 10.3390/ijms22031170.

[5] P. Verghese, C. Vullings, et N. Shanidze, « Eye Movements in Macular Degeneration », *Annual Review of Vision Science*, vol. 7, nº 1, p. 773-791, 2021, doi: 10.1146/annurev-vision-100119-125555.

[6] S. G. Pondorfer *et al.*, « Association of Visual Function Measures with Drusen Volume in Early Stages of Age-Related Macular Degeneration », *Investigative Ophthalmology & Visual Science*, vol. 61, nº 3, p. 55, mars 2020, doi: 10.1167/iovs.61.3.55.

[7] J.-B. Bernard et S. T. L. Chung, « Visual Acuity Is Not the Best at the Preferred Retinal Locus in People with Macular Disease », *Optom Vis Sci*, vol. 95, nº 9, p. 829-836, sept. 2018, doi: 10.1097/OPX.0000000000001229.

[8] A. Calabrese *et al.*, « Wet versus dry age-related macular degeneration in patients with central field loss: different effects on maximum reading speed », *Investigative ophthalmology & visual science*, vol. 52, nº 5, p. 2417-2424, 2011.

[9] A. F. Macedo, M. D. Crossland, et G. S. Rubin, « Investigating Unstable Fixation in Patients with Macular Disease », *Investigative Ophthalmology & Visual Science*, vol. 52, nº 3, p. 1275-1280, mars 2011, doi: 10.1167/iovs.09-4334.

[10] G. Kumar et S. T. L. Chung, « Characteristics of Fixational Eye Movements in People With Macular Disease », *Investigative Ophthalmology & Visual Science*, vol. 55, nº 8, p. 5125-5133, août 2014, doi: 10.1167/iovs.14-14608.

[11] S. Martinez-Conde, J. Otero-Millan, et S. L. Macknik, « The impact of microsaccades on vision: towards a unified theory of saccadic function », *Nat Rev Neurosci*, vol. 14, nº 2, Art. nº 2, févr. 2013, doi: 10.1038/nrn3405.

[12] J. Intoy et M. Rucci, « Finely tuned eye movements enhance visual acuity », *Nat Commun*, vol. 11, nº 1, p. 795, déc. 2020, doi: 10.1038/s41467-020-14616-2.

[13] C. Bolger, N. Sheahan, D. Coakley, et J. Malone, « High frequency eye tremor: reliability of measurement », *Clin. Phys. Physiol. Meas.*, vol. 13, nº 2, p. 151-159, mai 1992, doi: 10.1088/0143-0815/13/2/007.

[14] M. N. Ağaoğlu et S. T. L. Chung, « Exploration of the functional consequences of fixational eye movements in the absence of a fovea », *Journal of Vision*, vol. 20, nº 2, p. 12, févr. 2020, doi: 10.1167/jov.20.2.12.

[15] R. Z. Marandi et P. Gazerani, « Aging and eye tracking: in the quest for objective biomarkers », *Future Neurology*, vol. 14, nº 4, p. FNL33, nov. 2019, doi: 10.2217/fnl-2019-0012.
[16] A. G. Shaikh, J. Otero-Millan, P. Kumar, et F. F. Ghasia, « Abnormal Fixational Eye Movements in Amblyopia », *PLoS ONE*, vol. 11, nº 3, p. e0149953, mars 2016, doi: 10.1371/journal.pone.0149953.
[17] J. Egaña *et al.*, « Small Saccades and Image Complexity during Free Viewing of Natural Images in Schizophrenia », *Frontiers in Psychiatry*, vol. 4, 2013, Consulté le: 2 octobre 2023. [En ligne]. Disponible sur: https://www.frontiersin.org/articles/10.3389/fpsyt.2013.00037
[18] D. Kaski et A. M. Bronstein, « Ocular Tremor in Parkinson's Disease: Discussion, Debate, and Controversy », *Frontiers in Neurology*, vol. 8, 2017, Consulté le: 24 mai 2022. [En ligne]. Disponible sur: https://www.frontiersin.org/article/10.3389/fneur.2017.00134
[19] R. G. Alexander, S. L. Macknik, et S. Martinez-Conde, « Microsaccade Characteristics in Neurological and Ophthalmic Disease », *Frontiers in Neurology*, vol. 9, 2018, Consulté le: 18 juillet 2023. [En ligne]. Disponible sur: https://www.frontiersin.org/articles/10.3389/fneur.2018.00144
[20] C. K. Sheehy *et al.*, « Fixational microsaccades: A quantitative and objective measure of disability in multiple sclerosis », *Mult Scler*, vol. 26, nº 3, p. 343-353, mars 2020, doi: 10.1177/1352458519894712.
[21] F. Ghasia et J. Wang, « Amblyopia and fixation eye movements », *Journal of the Neurological Sciences*, vol. 441, p. 120373, oct. 2022, doi: 10.1016/j.jns.2022.120373.
[22] E. A. Rossi *et al.*, « A New Method for Visualizing Drusen and Their Progression in Flood-Illumination Adaptive Optics Ophthalmoscopy », *Translational Vision Science & Technology*, vol. 10, nº 14, p. 19, déc. 2021, doi: 10.1167/tvst.10.14.19.
[23] E. Gofas-Salas *et al.*, « High loop rate adaptive optics flood illumination ophthalmoscope with structured illumination capability », *Applied optics*, vol. 57, nº 20, p. 5635-5642, 2018.
[24] L. Krafft *et al.*, « Partial-Field Illumination Ophthalmoscope: improving the contrast of a camera-based retinal imager », *Applied optics*, vol. 60, nº 31, p. 9951-9956, 2021.
[25] L. Krafft *et al.*, « Multimodal high-resolution retinal imaging using camera-based DMD-integrated Adaptive Optics Flood-Illumination Ophthalmoscope ». Optica Open, 10 mai 2023. doi: 10.1364/opticaopen.22769519.v1.
[26] P. Mecê, « 4D exploration of the retina for adaptive optics-assisted laser photocoagulation », PhD Thesis, These de doctorat, Sorbonne Paris Cité, 2018.
[27] R. Engbert, « Microsaccades: a microcosm for research on oculomotor control, attention, and visual perception », in *Progress in Brain Research*, vol. 154, S. Martinez-Conde, S. L. Macknik, L. M. Martinez, J.-M. Alonso, et P. U. Tse, Éd., in Visual Perception, vol. 154. , Elsevier, 2006, p. 177-192. doi: 10.1016/S0079-6123(06)54009-9.
[28] K. Holmqvist, M. Nyström, R. Andersson, R. Dewhurst, H. Jarodzka, et J. Van de Weijer, *Eye tracking: A comprehensive guide to methods and measures*. OUP Oxford, 2011.
[29] D. C. Niehorster, R. Zemblys, T. Beelders, et K. Holmqvist, « Characterizing gaze position signals and synthesizing noise during fixations in eye-tracking data », *Behavior Research Methods*, vol. 52, p. 2515-2534, 2020.
[30] E. Castet et M. Crossland, « Quantifying eye stability during a fixation task: a review of definitions and methods », *Seeing Perceiving*, vol. 25, nº 5, p. 449-469, 2012, doi: 10.1163/187847611X620955.
[31] S. Martinez-Conde, S. L. Macknik, X. G. Troncoso, et T. A. Dyar, « Microsaccades Counteract Visual Fading during Fixation », *Neuron*, vol. 49, nº 2, p. 297-305, janv. 2006, doi: 10.1016/j.neuron.2005.11.033.
[32] J. Otero-Millan, X. G. Troncoso, S. L. Macknik, I. Serrano-Pedraza, et S. Martinez-Conde, « Saccades and microsaccades during visual fixation, exploration, and search: Foundations for a common saccadic generator », *Journal of Vision*, vol. 8, nº 14, p. 21, déc. 2008, doi: 10.1167/8.14.21.
[33] B. Wang, W. Shi, et Z. Miao, « Confidence Analysis of Standard Deviational Ellipse and Its Extension into Higher Dimensional Euclidean Space », *PLoS One*, vol. 10, nº 3, p. e0118537, mars 2015, doi: 10.1371/journal.pone.0118537.
[34] « Microsaccades Keep the Eyes' Balance During Fixation - Ralf Engbert, Reinhold Kliegl, 2004 ». Consulté le: 18 juillet 2023. [En ligne]. Disponible sur: https://journals.sagepub.com/doi/abs/10.1111/j.0956-7976.2004.00697.x

[35] X. Kuang, M. Poletti, J. D. Victor, et M. Rucci, « Temporal Encoding of Spatial Information during Active Visual Fixation », *Current Biology*, vol. 22, nº 6, p. 510-514, mars 2012, doi: 10.1016/j.cub.2012.01.050.
[36] D. J. Thomson, « Spectrum estimation and harmonic analysis », *Proceedings of the IEEE*, vol. 70, nº 9, p. 1055-1096, 1982.
[37] B. Babadi et E. N. Brown, « A review of multitaper spectral analysis », *IEEE Transactions on Biomedical Engineering*, vol. 61, nº 5, p. 1555-1564, 2014.
[38] N. R. Bowers, J. Gautier, S. Lin, et A. Roorda, « Fixational eye movements in passive versus active sustained fixation tasks », *Journal of Vision*, vol. 21, nº 11, p. 16, oct. 2021, doi: 10.1167/jov.21.11.16.
[39] M. Rolfs, R. Kliegl, et R. Engbert, « Toward a model of microsaccade generation: The case of microsaccadic inhibition », *Journal of Vision*, vol. 8, nº 11, p. 5, août 2008, doi: 10.1167/8.11.5.
[40] R. Engbert et R. Kliegl, « Microsaccades uncover the orientation of covert attention », *Vision Research*, vol. 43, nº 9, p. 1035-1045, avr. 2003, doi: 10.1016/S0042-6989(03)00084-1.
[41] L. L. Di Stasi, M. B. McCamy, A. Catena, S. L. Macknik, J. J. Cañas, et S. Martinez-Conde, « Microsaccade and drift dynamics reflect mental fatigue », *European Journal of Neuroscience*, vol. 38, nº 3, p. 2389-2398, 2013, doi: 10.1111/ejn.12248.
[42] M. Poletti, « An eye for detail: Eye movements and attention at the foveal scale », *Vision Research*, vol. 211, p. 108277, oct. 2023, doi: 10.1016/j.visres.2023.108277.
[43] F. L. Ferris *et al.*, « Clinical Classification of Age-related Macular Degeneration », *Ophthalmology*, vol. 120, nº 4, p. 844-851, avr. 2013, doi: 10.1016/j.ophtha.2012.10.036.
[44] R. Klein, B. E. K. Klein, et K. L. P. Linton, « Prevalence of Age-related Maculopathy: The Beaver Dam Eye Study », *Ophthalmology*, vol. 99, nº 6, p. 933-943, juin 1992, doi: 10.1016/S0161-6420(92)31871-8.
[45] N. M. Bressler, S. B. Bressler, S. K. West, S. L. Fine, et H. R. Taylor, « The Grading and Prevalence of Macular Degeneration in Chesapeake Bay Watermen », *Archives of Ophthalmology*, vol. 107, nº 6, p. 847-852, juin 1989, doi: 10.1001/archopht.1989.01070010869032.
[46] A. Cutini *et al.*, « Normal values and repeatibility of bivariate contour ellipse area (BCEA) with Microperimeter Mp-1. », *Investigative Ophthalmology & Visual Science*, vol. 55, nº 13, p. 169, avr. 2014.
[47] C. P. Agathos, D. Bernardin, D. Huchet, A.-C. Scherlen, C. Assaiante, et B. Isableu, « Sensorimotor and cognitive factors associated with the age-related increase of visual field dependence: a cross-sectional study », *AGE*, vol. 37, nº 4, p. 67, août 2015, doi: 10.1007/s11357-015-9805-x.
[48] M. U. Morales *et al.*, « Reference Clinical Database for Fixation Stability Metrics in Normal Subjects Measured with the MAIA Microperimeter », *Translational Vision Science & Technology*, vol. 5, nº 6, p. 6, nov. 2016, doi: 10.1167/tvst.5.6.6.
[49] W. Kosnik, J. Fikre, et R. Sekulerf, « Visual Fixation Stability in Older Adults », vol. 27, nº 12.
[50] E. G. González, M. S. Mandelcorn, E. D. Mandelcorn, et L. Tarita-Nistor, « Effect of Visual Feedback on the Eye Position Stability of Patients with AMD », *Vision*, vol. 3, nº 4, Art. nº 4, déc. 2019, doi: 10.3390/vision3040059.
[51] M. Bécu, « Impact of healthy aging on spatial cognition. Spatial navigation and gaze dynamics in ecological conditions », phdthesis, Sorbonne Université, 2018. Consulté le: 2 octobre 2023. [En ligne]. Disponible sur: https://theses.hal.science/tel-02862784
[52] M. Cozzi *et al.*, « Retromode Imaging Technology for Detecting Drusen-Like Deposits in Healthy Adults », *Ophthalmology Retina*, p. S2468653023003147, juill. 2023, doi: 10.1016/j.oret.2023.07.012.
[53] R. M. Steinman, R. J. Cunitz, G. T. Timberlake, et M. Herman, « Voluntary Control of Microsaccades during Maintained Monocular Fixation », *Science*, vol. 155, nº 3769, p. 1577-1579, mars 1967, doi: 10.1126/science.155.3769.1577.
[54] M. Nyström, D. W. Hansen, R. Andersson, et I. Hooge, « Why have microsaccades become larger? Investigating eye deformations and detection algorithms », *Vision Research*, vol. 118, p. 17-24, janv. 2016, doi: 10.1016/j.visres.2014.11.007.
[55] H. Collewijn et E. Kowler, « The significance of microsaccades for vision and oculomotor control », *Journal of Vision*, vol. 8, nº 14, p. 20, déc. 2008, doi: 10.1167/8.14.20.