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Real-time optical stabilization of retinal motion at micrometer precision using Adaptive Optics Flood-Illumination Ophthalmoscope

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Abstract: Eye motion poses a significant challenge in efficiently capturing high-resolution retinal images and efficiently delivering cell-targeted photostimulation using adaptive optics (AO). In this manuscript, we present a solution to optically correct retinal movements in real-time by integrating galvanometer mirrors, pupil-conjugated, in an AO Flood-illumination ophthalmoscope (AO-FIO). Our approach involved using AO-FIO images to measure retinal motion with sub-pixel precision and correct it in a closed-loop fashion and at 200Hz. Through the proposed closed-loop optical stabilization, we achieved a notable reduction in retinal motion from 25 μm rms to 1.2 μm rms. This performance was achieved 88% of the time over more than 40s without any decay of precision. Moreover, optical stabilization at this high precision was possible using images acquired in both photoreceptor and nerve fiber layers. To our knowledge, this level of precision and efficiency surpasses any previously reported method.

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1. Introduction

Adaptive optics (AO) has become an important tool in ophthalmology due to its ability to correct ocular aberrations in real time [1]. AO technology has opened new avenues for earlier diagnosis and better treatment of retinal diseases, as well as a better understanding of the normal and diseased retina [2]. Indeed, applications such as cellular imaging of retinal neurons, super-resolution structured illumination [3], functional assessment by cell-targeted photostimulation [4], cellular psychophysical testing [5, 6], and high-precision laser photocoagulation surgery [7] benefit from AO's ability to achieve micrometer resolution. However, the improved spatial resolution afforded by the use of AO also makes these applications more sensitive to the effect of constant, involuntary eye movements, even during careful fixation [8, 9]. The main consequences of these fixational eye movements are 1) intra- and inter-frame distortion in point-scanning modalities such as scanning light ophthalmoscopy (SLO) and optical coherence tomography (OCT); 2) longer image acquisition sessions because some of the data is lost due to motion blur/distortion; and 3) inaccurate photostimulation, psychophysical testing, and photocoagulation. The consequences are even more critical in patients with retinal diseases or poor vision, as fixation eye motion is magnified, which severely limits the widespread use of AO technology in clinical settings. Therefore, to successfully achieve high-resolution imaging and precise delivery of visual stimuli to the retina in a clinical setting, it is critical to efficiently track and optically correct this motion in real time.

To date, closed-loop AO eye tracking systems have been developed primarily for AO-SLO. The first one used a tracking beam reflectometer to measure the displacement of the optic disc in real time and tip and tilt mirrors to optically stabilize the retinal motion [10]. Using this

45 method, the authors demonstrated a tracking accuracy of 10-15 μm . However, this system has
46 important limitations in terms of stability and robustness, as it requires significant tuning of
47 parameters and settings for each eye. Furthermore, the achieved accuracy was not sufficient
48 to eliminate intra- and inter-frame motion distortions in AO-SLO images. Later, Yang *et al.*
49 showed that closed-loop image-based optical stabilization can be achieved in an AO-SLO by
50 replacing the slow scanner mirror with a 2-axis tip/tilt mirror and using AO-SLO images for
51 motion measurement [11]. Their method achieved very good efficiency (85%) and accuracy
52 of $\sim 1.66\mu\text{m} - 2.56\mu\text{m}$ (an order of magnitude better than the previous work). Nevertheless,
53 their optical stabilization technique suffers from the inherent limitations of an AO SLO, the most
54 important of which are 1) intra-frame distortion in the reference frame encoded in the optical
55 stabilization; 2) tracking failure due to large amplitude motion and eye torsion; 3) decrease in
56 tracking efficiency with increasing imaging duration (20-30 seconds in duration) due to subject
57 fatigue and eye torsion; 4) and difficulty in resetting the position of the tracking mirror after
58 micro-saccades, blinks, frame-outs, and torsions occur. All of these limitations are a major
59 problem for clinical imaging, where motion is amplified, subjects tend to fatigue more quickly,
60 and longer acquisition sessions are typically required.

61 In contrast to point-scanning modalities, there are full-field modalities, such as AO flood-
62 illumination ophthalmoscope (FIO) [12] and full-field OCT [13]. These full-field modalities
63 are distortion-free due to their high pixel rate, which combines a short exposure time with
64 full-field illumination and detection. AO-FIO images are often used as ground truth to evaluate
65 the ability of image registration methods to minimize distortion in AO-SLO, and also to validate
66 photoreceptor-based biomarkers extracted from AO-SLO images [14]. In addition, full-field
67 modalities allow combining a high acquisition rate with a large field of view [12], parameters
68 that together can play an important role in achieving high-precision and high-efficiency optical
69 stabilization of retinal motion in real time.

70 However, the main challenge of using an AO-FIO to track and correct for fixation eye
71 movements lies in the fact that the AO-FIO does not have optical sectioning capability [15].
72 Multiple scattered light and light backscattered by out-of-focus retinal layers are all detected
73 in AO-FIO, making its image contrast and SNR worse than AO-SLO. Due to the low SNR
74 and poor contrast, the eye motion estimation methods currently used in AO-SLO, which are
75 based on simple cross-correlations, tend to fail in AO-FIO [11, 16]. To overcome this inherent
76 drawback of AO-FIO images, several methods have been proposed, the most successful being
77 Maximum-Likelihood estimation [17] and Peak Tracking [18]. However, although these methods
78 allow for subpixel motion estimation, they are time-consuming and not compatible with the
79 application of real-time optical stabilization of eye movements.

80 To explore the advantage of full-field modalities, i.e., high-throughput, distortion-free, and
81 large FOV images, we present here, for the first time, an AO-FIO designed to provide real-time
82 optical stabilization of retinal motion. To mitigate the low image contrast and SNR, and to
83 estimate the eye motion with precision at the millisecond scale, we propose a method based
84 on phase correlation of high spatial frequencies. Although AO-FIO has no optical sectioning,
85 high spatial frequencies are generated only by the retinal layer within the depth of focus [15].
86 By relying on the cross-correlation of these frequencies and filtering out low and noisy spatial
87 frequencies, one can minimize the noise error propagation in motion estimation. With this
88 method, and without the use of special integrated circuits such as field-programmable gate
89 arrays (FPGAs) and graphics processing units (GPUs) previously required in AO-SLO methods,
90 we can measure the lateral retinal displacement in the millisecond range and with sub-pixel
91 (sub-micrometer) precision, thus allowing robust correction of eye movements during the imaging
92 session with micrometer precision.

93 **2. Methods**

94 *2.1. Experimental set-up*

95 The AO-FIO system with robust closed-loop optical stabilization can be decomposed in three
96 subsystems: the imaging, the wavefront sensing/correction, and the eye motion tracking/correction
97 subsystems (see Fig. 1).

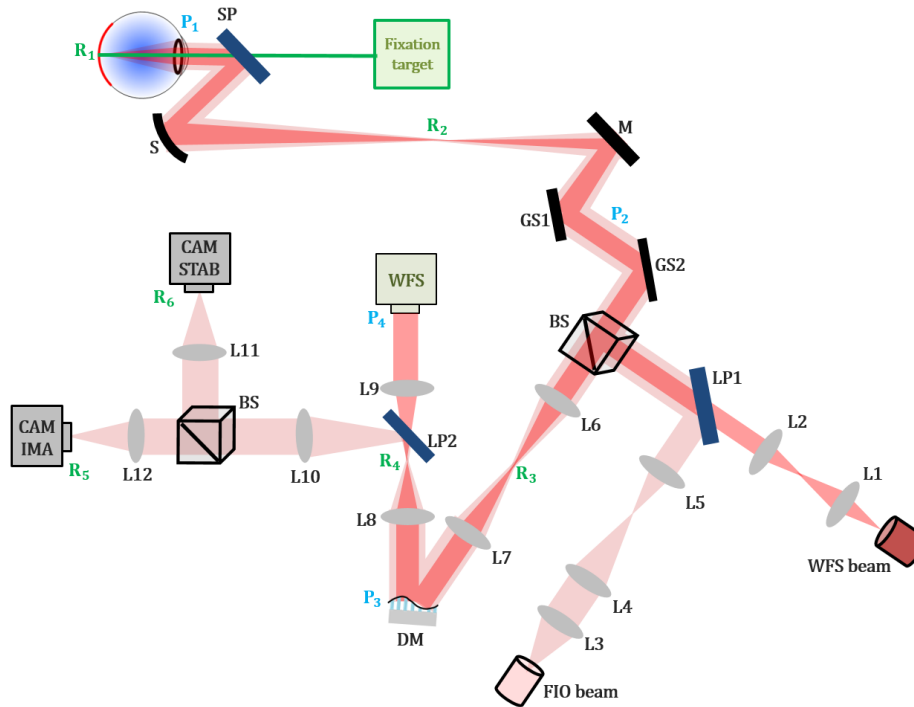


Fig. 1. Synoptic schema of the Adaptive Optics-Flood Illumination Ophthalmoscope set-up. The system can be divided into three main systems: wavefront sensing and correction part at 750 nm (dark red beam path), the imaging part with CAM IMA and the eye motion tracking and correction subsystem. (L stands for lenses and doublets, BS for beam splitters, R for focal/retinal planes, P for pupil planes).

98 **Imaging subsystem.** A SLD (LedMOD.850.800.V2, Omicron, Germany with a central wave-
99 length of 860nm (100nm bandwidth) is used as illumination source. A squared field diaphragm
100 is positioned at the retinal plane R_7 , which defines an illumination field of view in the retina
101 of $4^\circ \times 4^\circ$. The light backscattered by the retina is split in half by a beam splitter and then
102 captured by two scientific CMOS cameras (ORCA flash4-v2, Hamamatsu, Japan), each one with
103 a different purpose. The first one, the imaging camera, enables a large field-of-view imaging at
104 diffraction-limited resolution. It can reach 200Hz while acquiring 1024×1024 pixel per frame,
105 corresponding to approximately $4^\circ \times 4^\circ$ in the retina. This camera is mounted on a motorized
106 stage, enabling focusing on different retinal layers. The second camera, the stabilization camera,
107 provides a small field-of-view and undersampled resolution, for faster computation of eye motion.
108 Images of 512×512 pixel ($4^\circ \times 4^\circ$ field of view) are acquired at 200Hz. Although a faster
109 acquisition rate would be possible for the stabilization camera, a 5ms exposure time was necessary

110 to precisely measure eye motion and beat the noise level, which is high in an AO-FIO system.

111 **Wavefront sensing/correction:** A fibered SLD (EXS210017-01, Exalos, Swiss) with a central
 112 wavelength of 750nm is used as a wavefront sensor beacon. The size of the beacon at the eye pupil
 113 is $277\mu\text{m}$, which is defined by a mask positioned at a pupil plane of the wavefront sensor beacon.
 114 The beacon is offset by two 2cm in the eye's pupil to prevent stray light. Wavefront sensing is
 115 performed using a custom-built Shack-Hartmann sensor consisting of a 18×18 microlens array
 116 (MLA300-14AR-M, Thorlabs, USA). Relay optics conjugate the focal plane of the microlenses
 117 onto a CMOS camera (UI-3360CP-NIR-GL Rev.2, IDS, Germany), where 16×16 pixels are
 118 allocated to each lenslet. The pitch of the microlens array corresponds to $278\mu\text{m}$ in the eye pupil
 119 plane. Wavefront correction is performed by a deformable mirror with 97 actuators (ALPAO,
 120 France). The deformable mirror diameter limits the pupil size of the system to 5mm. The
 121 deformable mirror can also be used to change the focal plane of both detectors during imaging
 122 and optical stabilization of eye motion. To control the adaptative optics, we used our custom-built
 123 real-time calculator (RTC), allowing us to achieve a 50Hz loop rate without jitter. We applied an
 124 integrator control law with a 0.5 gain, assuming a two-frame delay.

125 **Eye motion tracking/correction:** Two galvanometer mirrors are positioned very close to the
 126 pupil plane P_2 and responsible for correcting eye motion in real-time. Both illumination and
 127 wavefront sensor beacon beams pass by the galvanometers, on the way towards the retina and
 128 from the retina towards the detectors. An independent custom-built RTC, written in Matlab
 129 (MathWorks, Natick, MA), was used to measure eye motion through the images provided by the
 130 stabilization camera. No particular integrated circuit, such as field-programmable gate array
 131 (FPGA) or graphics processing units (GPU), was used, but rather a CPU (Core i7 - 4790K). Eye
 132 motion correction is performed at a 200Hz loop rate without jitter. As for AO, the control law is
 133 an integrator with a 0.5 gain, with a two-frame delay chronogram (see Fig.2).

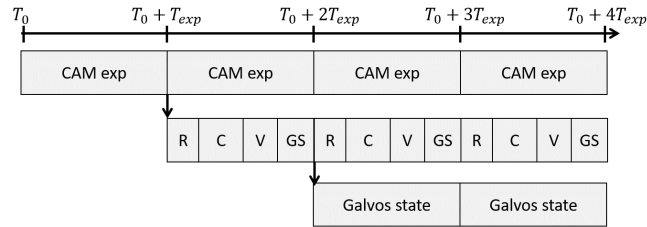


Fig. 2. Breakdown of the eye motion optical stabilization loop chronogram. After acquiring an image during the camera exposure time T_{exp} , the image is read by the computer (R), the eye motion is computed (C) the corresponding voltage is estimated (V) and sent to the galvanometer scanners with a given response time (GS). Correction is thus applied in the galvanometer scanners after two frames from the start of the image acquisition.

134 2.2. Eye motion tracking algorithm and control loop

135 The tracking algorithm is image-based and relies on the rapid matching of an acquired reference
 136 frame and an acquired frame. The algorithm can be divided into two main parts: selection of
 137 the appropriate reference frame and estimation of eye movement by comparing the reference
 138 and acquired frames. In this study, the reference frame was automatically chosen as the first
 139 acquired frame of the sequence without any blink and micro-saccade occurrence (see the blink

140 and micro-saccade detection method below). We then compute the Fast Fourier Transform
141 (FFT) of the reference frame and apply a bandpass filter to filter out the low spatial frequency
142 (dominated by photons collected from out-of-focus retinal layers and multiply scattering) and
143 the high spatial frequency (dominated by photon noise) of the image. For the bandpass filter,
144 we used a Butterworth of order 2 with normalized cut-off frequencies of 0.01 and 0.5. The
145 normalized cut-off frequencies were chosen based on a parametric study described in Supplement
146 1. After this step, the algorithm is ready to start the real-time optical stabilization. First, the same
147 reference frame procedure is applied to the acquired frames, *i.e.* FFT computation and bandpass
148 filtering with the same parameters. Then a phase correlation is performed by multiplying the
149 reference filtered FFT with the acquired filtered FFT. By taking the real part, one can obtain a
150 correlation map where the 2D position of the maximum value provides the shift between the
151 two images. Finally, sub-pixel estimation is performed by computing a parabolic fit of pixels
152 surrounding the maximum position in the horizontal and vertical directions. Here, 5 pixels were
153 used for each direction. From the parabolic fit, a new maximum can be found with sub-pixel
154 precision.

155 Based on our parametric study described in Supplement 1, we decided to work with an
156 undersampled stabilization camera where the pixel size in the retinal plane is $2.36\mu\text{m}$ while the
157 diffraction limit is $2.9\mu\text{m}$. Undersampling by a factor of two allows: 1) increase the SNR by
158 a factor of two, 2) reduce the computation time by a factor of about 2.22 (FFT computation
159 complexity equal to $n\log n$, where n is the number of the data size). Undersampling could come
160 at the cost of lower motion estimation accuracy. However, since the motion estimation precision
161 is on the order of a tenth of a pixel (subpixel precision), the loss is not significant compared to
162 the temporal error introduced by the 2-frame delay loop. To further reduce the computation time,
163 we only acquire images of 128×128 pixels (about $1^\circ \times 1^\circ$ FOV), which allows us to achieve
164 a computation time of 2.5ms including image transfer and motion estimation in a CPU (Core
165 i7 4790K). The FOV was chosen to avoid frame-out, which can occur after micro-saccades,
166 based on eye movement data collected without correction (peak-to-valley value in table 1). After
167 estimating the eye movement, we calculate the voltage needed to drive the galvanometers and
168 correct for this movement. Voltage calculation and response time of the galvanometer mirrors is
169 typically 2ms, which allows the use of a two-frame delay control loop chronogram (see Fig. 2)
170 with 5ms exposure time for the stabilization camera, *i.e.* at 200Hz loop rate.

171 Blinks and micro-saccades can be a potential source of control instability during eye movement
172 correction [11]. Here, to avoid instability during and after blinks and micro-saccades occur, we
173 keep the galvanometer state at the same position just before they occur. Blinks are detected by an
174 image intensity threshold. Micro-saccades are detected by computing the integral of the FFT of
175 the filtered image [19], which allows us to evaluate the energy associated with the high spatial
176 frequencies of the in-focus structure of the retina. In fact, during micro-saccades, the AO-FIO
177 images lose high spatial frequencies due to image blur. Once detected, the loop is reactivated
178 only when the difference between two successive motion estimates is less than $2\mu\text{m}$.

179 2.3. Image acquisition

180 We acquired images from three healthy subjects. Participants followed institutional guidelines
181 and adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from the
182 participants after the natural and possible outcomes of the study were explained. The participants
183 were seated in front of the system and stabilized with a chin and forehead rest. The participants
184 were asked to stare at the fixation target, which is an image of a yellow cross not affected by the
185 optical sectioning, enabling guiding the subject's line of sight and exploring various areas of
186 the retina. Image acquisition sessions were performed in standard conditions with neither pupil
187 dilation nor cycloplegia, in a dark room, leading to the largest accessible natural pupil dilation.
188 The total light energy entering the eye from the illumination source and the wavefront sensor

189 beacon source were, respectively, 3mW and $6\mu W$, which is less than the limits established by
190 ISO standards for group 1 devices.

191 3. Results

192 3.1. Closed-loop optical stabilization internal performance

193 The overall system temporal performance of a control loop can be assessed and optimized by
194 computing its rejection transfer function (or sensitivity function) [20,21]. The rejection transfer
195 function describes how different eye motion temporal frequencies are attenuated or amplified
196 by the closed-loop optical stabilizer. From this function, one can also assess the correction
197 cutoff frequency, *i.e.* the highest corrected temporal frequency. To compute the rejection transfer
198 function, we injected in the galvanometer mirrors a pink noise with a power spectrum density
199 following an f^{-1} law (this type of colored noise avoids applying too much energy at high temporal
200 frequencies that could degrade the galvanometer mirrors). Eye motion is then estimated in a
201 model eye with correction (loop on, *i.e.* residual eye motion) and without correction (loop off).
202 The rejection transfer function is the ratio between temporal power spectral densities of the
203 residual eye motion (*i.e.* with correction) and eye motion (*i.e.* without correction). Figure 3
204 shows the experimental computed rejection transfer function (blue: horizontal, green: vertical)
205 and the theoretical one (red line) assuming a two-frame delay and 0.5 gain (which are the actual
206 settings used during the acquisition). The good match between the experimental and theoretical
207 plots demonstrates that no additional delay or jitter was present during the acquisition, meaning
208 that the temporal performance of the loop is as expected. In our case, with a 200Hz loop rate, the
209 cutoff frequency (associated with 0dB) is approximately 14Hz.

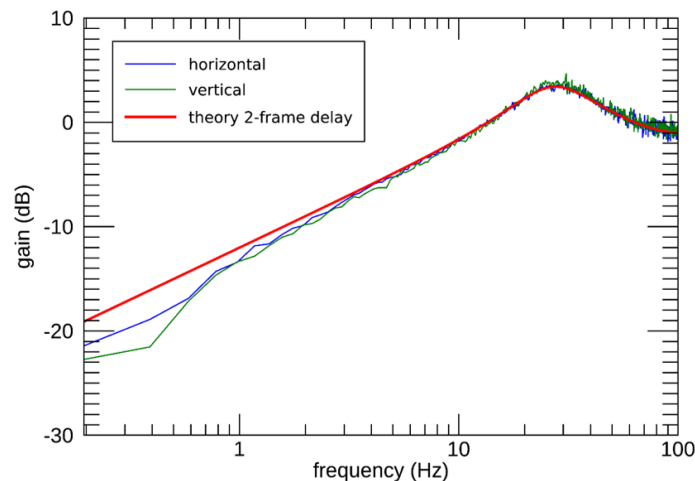


Fig. 3. Rejection Transfer function. Blue and green: experimental rejection transfer function on horizontal and vertical directions respectively. Red: theoretical rejection for a 2-frame delay loop.

210 3.2. Performance of the retinal lateral motion optical stabilization

211 Figure 4(a,b) presents an example of vertical (Y) and horizontal (X) retinal displacement as a
212 function of time. Here, the stabilization camera was conjugated to the photoreceptor layer focal
213 plane, and the loop rate was set to 200Hz. During the first 5 seconds (red line), no correction
214 was applied. Slow drifts, and fast and large amplitude micro-saccades were visible in both

215 X,Y directions. After activating the closed-loop optical stabilization (final 5 seconds), slow
 216 drifts were completely filtered out (blue line). On the other hand, micro-saccades were not fully
 217 corrected (black line). Note that, owing to the real-time detection of micro-saccades and blink
 218 occurrences, the closed-loop correction is barely affected by these spark movements and can
 219 robustly re-activate the closed-loop correction very fast after these occurrences, achieving high
 220 efficiency. Visualization 1 shows the images acquired by the stabilization camera before and after
 221 correction of eye motion in real-time. As a reminder, the images acquired by the stabilization
 camera are those used to measure the retina position in real-time, i.e. undersampled.

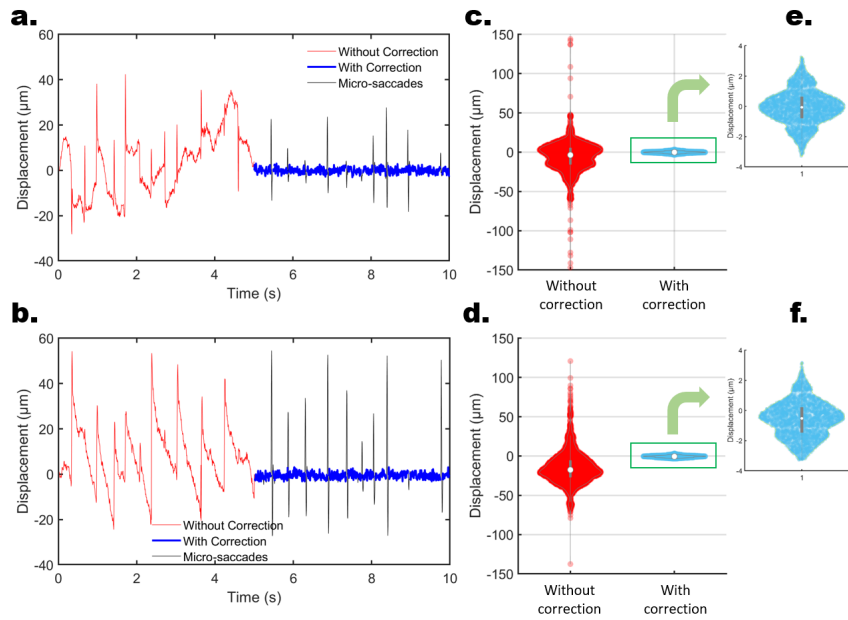


Fig. 4. (a,b) Example of retinal displacement over time in the vertical (Y) and horizontal (X) directions respectively. (c,d) Distribution of displacements without and with correction of retinal motion collected from a 40-s long data set, where the first 20s seconds are without correction, and the final 20s with correction (i.e. 4000 time points for each case). (e,f) is the magnified plot when correction was activated to better visualize its distribution.

222

223 Figure 4(c,d) shows the distribution of displacements without and with correction of retinal
 224 motion collected from a 40-s long data set, where the first 20s seconds are without correction,
 225 and the final 20 s with correction (i.e. 4000 time points for each case). Figure 4(e,f) is the
 226 magnified plot when correction was activated, highlighting the high precision achieved. When
 227 retinal motion is not corrected, we computed a peak-to-valley amplitude X,Y of respectively
 228 $258.35\mu\text{m}$ and $290.98\mu\text{m}$, a mean deviation from zero position X,Y of respectively
 229 $20.6\mu\text{m}$ and $13.13\mu\text{m}$, and a root-mean-square (RMS) error X,Y of respectively
 230 $24.78\mu\text{m}$ and $18.29\mu\text{m}$. On the other hand, the proposed closed-loop optical stabilization system reduced the peak-to-valley
 231 amplitude X,Y to respectively $6.49\mu\text{m}$ and $6.56\mu\text{m}$, the mean deviation from zero position X,Y
 232 to respectively $1.05\mu\text{m}$ and $0.9\mu\text{m}$, and the root-mean-square (RMS) error X,Y to respectively
 233 $1.19\mu\text{m}$ and $1.15\mu\text{m}$. Table 1 summarizes the achieved performance with the proposed method.

234

235 During the eye motion correction in real-time using the images acquired by the stabilization
 236 camera, the imaging camera acquires in parallel large field-of-view images of the retina. Since
 these cameras are independent, it is possible to position the focal plane at different layers. For

	PV (μm)	Mean deviation (μm)	RMS error (μm)
Without correction	X : 258.35, Y : 290.98	X : 20.6, Y : 13.13	X : 24.78, Y : 18.29
With correction	X : 6.49, Y : 6.56	X : 1.05, Y : 0.9	X : 1.19, Y : 1.15

Table 1. Axial motion statistics with and without optical stabilization of retinal motion. Statistics took into account 40-s long data set, where the first 20s seconds are without correction, and the final 20s with correction (i.e. 4000 time points for each case). PV stands for peak-to-valley amplitude.

237 example, the stabilization camera could be focused to the photoreceptor layer while the imaging
 238 camera acquires images from the nerve fiber layer. Visualization 2,3 show the images acquired
 239 without any correction and during the real-time eye motion correction, respectively, in the nerve
 240 fiber layer. Figure 5(a,b) presents the result of averaging 400 images (4 s acquisition) without and
 241 with eye motion correction in real-time. One can noticed that owing to the optical stabilization of
 242 eye movements, the high spatial content in the NFL is maintained while it is totally vanished when
 243 no eye motion correction is applied. Figure 5(c) shows the result of averaging 400 images after a
 244 post-processing digital correction of eye motion, thus without considering the temporal error
 245 added by the real-time control loop. Since the precision obtained with the optical stabilization
 246 method is below the size of a single pixel, the averaged image obtained after post-processing
 247 registration is very similar to the one obtained with real-time correction, without any apparent
 loss of high-spatial frequency content of the nerve fiber layer.

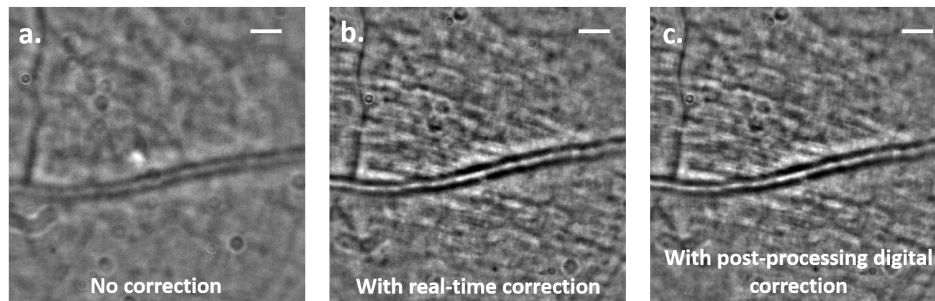


Fig. 5. Result of averaging 400 images acquired by the imaging camera (a) before and (b) during real-time optical stabilization of eye movements.(c) Result of averaging 400 images acquired by the imaging camera during real-time optical stabilization of eye movements and further post-processing image registration at sub-pixel precision. Scale bar $100\mu\text{m}$.

248

249 3.3. Performance for different stabilization camera focal planes

250 To achieve precise optical stabilization on an image-based method, one needs to collect images
 251 with high spatial frequency content to enhance the precision of cross-correlation methods. High-
 252 spatial frequency content can be found when imaging the photoreceptor layer, and it was, so far, the
 253 only retinal layer explored to demonstrate eye motion optical stabilization [11]. However, it is not
 254 rare in patients that the photoreceptor layer is not sharply imaged or partially/completely absent
 255 because of the pathological retinal condition, in particular for age-related macular degeneration
 256 and diabetic retinopathy. For these cases, it is crucial to demonstrate that closed-loop optical

257 stabilization can precisely track eye motion based on images generated in other retinal layers.
 258 Owing to our system design based on two independent cameras, one can conjugate the stabilization
 259 camera in one specific retinal layer while imaging another one. Another retinal layer that presents
 260 relatively high spatial frequency is the nerve fiber layer. Table 2 presents a comparison of
 261 the performance of the proposed method while conjugating the stabilization camera in the
 262 photoreceptor and the nerve fiber layers. Visualization 4 presents a video acquired by the
 263 stabilization camera during real-time eye motion correction while focused on the nerve fiber
 264 layer.

	Tracked Frames	Failed Frames	Efficiency	RMS error
Photoreceptor layer	19067	2269	88.1%	1.32 μ m
Nerve fiber layer	20197	2444	87.8%	1.48 μ m

Table 2. Axial motion statistics with and without the use of the method to optically stabilize retinal axial motion. Statistics took into account 13 image sequences of 7 to 28s duration from three subjects, for a total of 4700 analyzed A-scans. PV stands for peak-to-valley amplitude.

265 Note that both conditions presented very high and comparable efficiency, around 88%.
 266 Moreover, both scenarios revealed high tracking precision, slightly better for the photoreceptor
 267 layer (1.32 μ m) than the nerve fiber layer (1.48 μ m). This result shows that both retinal layers
 268 could be used to precisely track and correct eye motion in real-time with the proposed method.

269 3.4. Stabilization precision and efficiency over time

270 Using an AO-SLO system to execute a closed-loop image-based optical stabilization, Yang *et*
 271 *al.* noticed in their 20 to 30-second long tracking data that the tracking efficiency decreases as
 272 imaging duration increases [11]. They associated this phenomenon with subject fatigue due to
 273 the long imaging time, increasing eye motion amplitude. To evaluate how our system efficiency
 274 and precision evolved, we acquired 40s long data while the closed-loop optical stabilization was
 275 activated. The stabilization camera was conjugated to the nerve fiber layer focal plane, and the
 276 loop rate was running at 200Hz. Figure 6(a) shows the horizontal retinal displacement over
 277 time, where the blue plot represents the time points where the correction was activated and the
 278 red plot where micro-saccades or blink occurrences were detected. No significant increase of
 279 micro-saccades was detected in this time series. On the other hand, Fig.6 (b) shows an increase
 280 in the mean deviation from zero retinal displacement over time, supporting the hypothesis that
 281 the longer the imaging duration, the larger the retinal motion. However, in contrast to Yang *et*
 282 *al.* results, here both efficiency and precision do not present a decrease over time (see Fig.6(c)).
 283 Efficiency was highly correlated to the occurrences of micro-saccades and blinks. The steady
 284 precision and efficiency in our case, in contrast to the AO-SLO method [11], may be explained
 285 by the use of large FOV and distortion-free images. Another explanation can be the fact that
 286 while image rotation, provoked by eye torsion, does not affect motion estimation in AO-FIO, it
 287 makes AO-SLO image-based registration methods to fail or loose their precision [11].

288 4. Discussion and Conclusion

289 In this manuscript, we have presented an AO-FIO imaging system that is capable of optically
 290 stabilizing the lateral motion of the retina in real-time. We have shown that a loop rate of 200
 291 Hz can be achieved with a temporal bandwidth of 14 Hz, eliminating all slow drifts and being
 292 immune to micro-saccades and blinks. Due to the ability of the AO-FIO to generate distortion-free

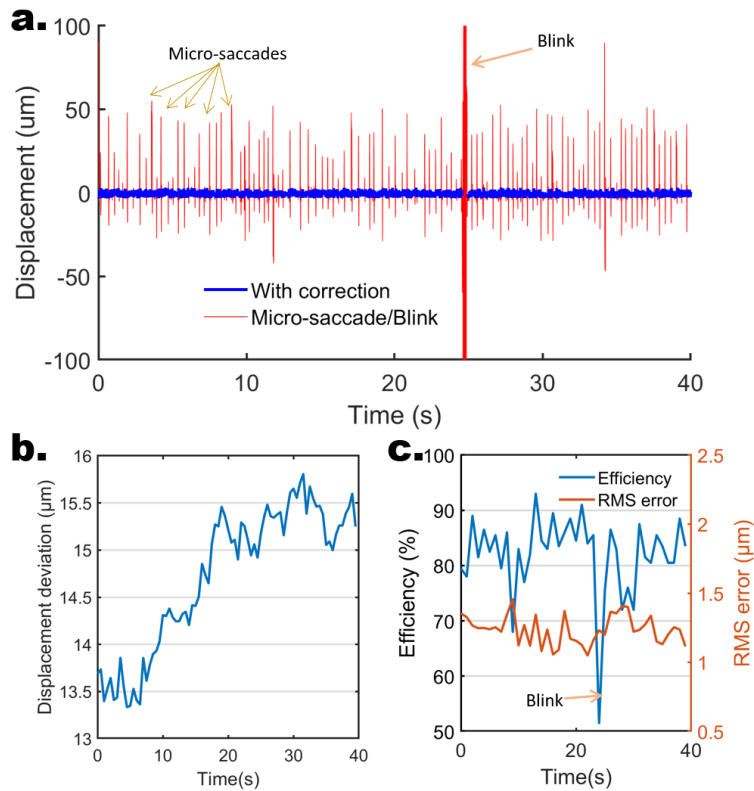


Fig. 6. Evaluation over time of the precision and efficacy of the proposed optical stabilization method to correct for eye motion in real-time. (a) Retinal displacement over time during real-time optical stabilization. Red traces outline high amplitude and spark motion due to micro-saccades and one blink occurrence around 25s. (b) Mean deviation from zero of the retinal displacement over time. (c) Efficiency and RMS error over time.

293 images over a large FOV, optical stabilization was successful in all experiments. Our proposed
 294 optical stabilization method reduced retinal motion from $25\mu\text{mRMS}$ to $1.2\mu\text{mRMS}$, *i.e.* less
 295 than the distance between foveal photoreceptors. This performance was achieved 88% of the
 296 time for a total of 39264 acquired images. The 12% of unsuccessful tracking was mainly due
 297 to the occurrence of micro-saccades and blinks, which can be accurately detected. Due to the
 298 detection of blinks and micro-saccades, the control loop is deactivated to avoid instability, and
 299 reactivated when a normal state is reached again. To the best of our knowledge, the performance
 300 achieved by the proposed method is more precise and efficient than any other method reported in
 301 the literature. Moreover, in contrast to the previous work by Yang et al. [11], such precision and
 302 efficiency was possible without using any special integrated circuit, such as field-programmable
 303 gate arrays (FPGA) or graphics processing units (GPU), but a CPU (7 - 4790K).

304 The use of FPGA and GPU can increase the efficiency/robustness of the proposed method
 305 by increasing the field of view of the stabilization camera (while keeping the loop rate at 200
 306 Hz), especially in patients with large fixational eye movements or poor vision or even nystagmus.
 307 Another advantage of using FPGA and GPU is the possibility to increase the loop rate even more.
 308 In fact, in this study, the loop rate was limited by the image processing time. In Supplement 1, we

309 show that the proposed algorithm could still estimate eye movements with sub-micro precision in
310 images with a SNR 3 times lower than the one used, i.e. an exposure time of 1.67 ms. Another
311 interesting advantage of the proposed method, demonstrated for the first time, is its ability to
312 achieve precise eye motion correction even when the images were acquired in a retinal layer
313 other than the photoreceptors. Here, we obtained equivalent precision and efficiency when using
314 images acquired in both the photoreceptors and the nerve fiber layer. In fact, this advantage can
315 be very helpful when imaging patients for whom it is difficult to obtain a clear and sharp image of
316 the photoreceptors (e.g., patients with age-related macular degeneration or drusen). Contrary to
317 the previous work by Yang et al. using AO-SLO images to track eye movements [11], no decrease
318 in efficiency over time was observed, most likely because all pixels are collected at the same time
319 in a large FOV, avoiding frame-out errors and uncertainties due to motion-induced distortion
320 and eye torsion (provoking image rotation). Finally, the performance in terms of precision and
321 efficiency can be further improved if the reference image has a better SNR than the current images
322 (see Supplement 1). Indeed, this can be achieved as a first step, where acquiring, registering and
323 averaging 25 images and using the averaged image as a new reference could potentially increase
324 the precision of eye motion estimation by a factor of 5 for the same image SNR used in this study
325 (5ms exposure), or by allowing precise estimation in even noisier images with a shorter exposure
326 time, thus potentially allowing a faster loop rate (see Supplement 1).

327 The performance achieved in this study opens a new avenue in terms of applications, especially
328 in subjects with retinal pathology. With the presented performance, one has the tools to image
329 patients who are potentially the most interesting to study with AO ophthalmoscopes, such as
330 nystagmus patients or patients with poor fixation capacity due to poor vision. In addition, precise
331 delivery of visual stimuli for photostimulation or laser strikes for photocoagulation surgery may
332 be possible in patients with poor fixation. Furthermore, the proposed algorithm, which is robust
333 to noisy images, can be used to estimate eye movements during psychophysical testing and
334 may help to find early signs of retinal diseases by characterizing fixational eye movements [6].
335 Finally, other full-field modalities that need to mitigate eye movements to be successful, such as
336 DMD-based partial-field and camera-based multimodal ophthalmoscopes [22, 23], Structured
337 Illumination Ophthalmoscope [3] and Full-Field Optical Coherence Tomography [24], could
338 greatly benefit from the proposed optical stabilization method.

339 5. Backmatter

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343 **Data availability.** Data underlying the results presented in this paper are not publicly available at this
344 time but may be obtained from the authors upon reasonable request.

345 **Supplemental document.** See Supplement 1 for supporting content.

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