



HAL
open science

Single-shot quantitative aberration and scattering length measurements in mouse brain tissues using an extended-source Shack-Hartmann wavefront sensor

Sophia Imperato, Fabrice Harms, Antoine Hubert, Mathias Mercier, Laurent Bourdieu, Alexandra Fragola

► To cite this version:

Sophia Imperato, Fabrice Harms, Antoine Hubert, Mathias Mercier, Laurent Bourdieu, et al.. Single-shot quantitative aberration and scattering length measurements in mouse brain tissues using an extended-source Shack-Hartmann wavefront sensor. *Optics Express*, 2022, 10.1364/OE.456651 . hal-03608265v2

HAL Id: hal-03608265

<https://hal.science/hal-03608265v2>

Submitted on 24 Jun 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Single-shot quantitative aberration and scattering length measurements in mouse brain tissues using an extended-source Shack-Hartmann wavefront sensor

SOPHIA IMPERATO,^{1,2,*} FABRICE HARMS,³ ANTOINE HUBERT,^{1,3}
MATHIAS MERCIER,¹ LAURENT BOURDIEU,² AND ALEXANDRA
FRAGOLA¹

¹Laboratoire de Physique et Etude des Matériaux, ESPCI Paris, Université PSL, CNRS, Sorbonne Université, 10 rue Vauquelin, 75005 Paris, France

²Institut de Biologie de l'ENS (IBENS), Département de biologie, École normale supérieure, CNRS, INSERM, Université PSL, 46 rue d'Ulm, 75005 Paris, France

³Imagine Optic, 18 rue Charles de Gaulle, 91400 Orsay, France

*sophia.imperato@espci.fr

Abstract: Deep fluorescence imaging in mammalian brain tissues remains challenging due to scattering and optical aberration-induced loss in signal and resolution. Correction of aberrations using adaptive optics (AO) requires their reliable measurement in the tissues. Here, we show that an extended-source Shack-Hartmann wavefront sensor (ESSH) allows quantitative aberration measurements through fixed brain slices with a thickness up to four times their scattering length. We demonstrate in particular that this wavefront measurement method based on image correlation is more robust to scattering compared to the standard centroid-based approach. Finally, we obtain a measurement of the tissue scattering length taking advantage of the geometry of a Shack-Hartmann sensor.

© 2022 Optica Publishing Group under the terms of the [Optica Publishing Group Publishing Agreement](#)

1. Introduction

Optical microscopy has emerged as a major tool in neuroscience as it allows to image the architecture of neuronal networks and to record their functional activity in several animal models as zebrafish, rodents, ferrets or primates through different methods including widefield fluorescence microscopy [1], confocal [2], one-photon [3, 4] or two-photon light-sheet microscopy [5] and non-linear scanning microscopy [6–8]. Modern optical sectioning methods such as confocal or light-sheet can image a large part of the entire brain of relatively transparent animal models such as zebrafish, drosophila or C-Elegans. In mammalian brains, depth penetration is limited even at low depth by scattering and imaging can only be achieved using non-linear microscopy. However, at large depths, optical aberrations linked to the refractive index inhomogeneity of biological tissues still limit resolution and signal intensity in all microscopy modalities [9, 10]. To overcome this difficulty, adaptive optics (AO) has been implemented on several linear and non-linear optical sectioning microscopy setups and currently provides a reliable live correction of the aberrations [11], enabling functional imaging of synaptic boutons [12], axons and spines [13] and soma [14] in infragranular layers of the mouse cortex in 2-photon microscopy, or enabling subcellular imaging of organelle dynamics in the early zebrafish brain in light-sheet microscopy [15], and deeper imaging of multicellular tumor spheroids [16, 17]. The first strategy to implement AO in microscopy is based on a sensorless configuration [18–21], that does not make use of a wavefront sensor but analyses the variation of the fluorescence signal induced by a wavefront modulator to drive the correction of aberrations. This method minimizes instrumental complexity, and provides a good resilience to scattering. However, it relies on a time-consuming

45 iterative approach requiring many acquisitions before reaching a good correction: typically, 30
46 seconds are necessary for a single iteration of a sensorless process, and a couple of iterations are
47 required to reach an optimal correction. The approach is thus hardly compatible with time-varying
48 aberrations and with photobleaching issues, in particular when multiple corrections in a volume
49 are required, e.g. for a small isoplanetic patch which is often the case in depth [15]. The second
50 approach to enable AO in microscopy is based on direct wavefront sensing, and makes use
51 of a wavefront sensor to allow a fast and accurate convergence of the AO loop, with a better
52 photon budget: direct wavefront sensing is thus a key method to evaluate and correct aberrations
53 *in vivo* over large scales. Direct wavefront sensing for AO-enhanced optical microscopy has
54 been reported over the last years through several approaches, using either Shack-Hartmann
55 (SH) sensors [13–16, 22, 23], or partitioned aperture wavefront (PAW) sensors [24] as a variant
56 of pyramid wavefront sensors applicable to microscopy. The use of PAW in fluorescence
57 microscopy is severely limited, since it requires only a moderate spatial incoherence of the
58 excitation source, so that its demonstration in fluorescence microscopy was restricted to widefield
59 using a specific illumination geometry (Oblique Back Illumination) [24, 25]. Direct wavefront
60 sensing in microscopy is thus currently mostly based on SH sensors. Early AO demonstrations
61 based on SH [16, 22] used extrinsic fluorescent beads injected into the sample to provide a guide
62 star for centroid computation, which is usually unwanted. More recently, Wang et al. used
63 two-photon excitation, scanned over a given field of view, to create a guide star outside the sample
64 by descanning the fluorescence signal, the guide star being then used by a SH sensor based on a
65 conventional centroid computation [14]. The approach was successfully used to drive AO in
66 Lattice Light-Sheet [15], as well as in two-photon [14] and in Structured Illumination Microscopy
67 (SIM) [26, 27]. Recently, we demonstrated the use of an Extended-Source SH wavefront sensor
68 (ESSH) to enable AO in light-sheet microscopy [23], by adapting pioneer work from astronomy
69 to the constraints of fluorescence microscopy. The method relies on the cross-correlation of
70 images of an extended source obtained through a microlens array. In fluorescence microscopy,
71 when coupled to an optical sectioning method such as two-photon microscopy or light-sheet
72 fluorescence microscopy, the ESSH uses the fluorescence signal as a guide plane, providing lower
73 instrumental complexity and cost than the previous approach based on the scan and descanning of a
74 non-linear signal. Its efficiency has been proven when coupled to light-sheet for AO-enhanced
75 neuroimaging in the adult drosophila brain in weakly scattering conditions [23].

76 However, direct wavefront sensing in scattering tissue is limited in depth penetration. Indeed,
77 in pioneering SH wavefront measurements based on centroid estimation in the rodent brain, the
78 strong scattering of the emitted fluorescence reduces significantly the capacity to measure the
79 centroid position due to the decreasing ballistic signal and increasing background on images
80 below each lenslet [13, 14]. A method for direct wavefront sensing more resilient to scattering of
81 the fluorescence emission would therefore improve the use of AO in optical microscopy. To this
82 aim, we analyse here the performances of an ESSH in scattering tissues and show that it provides
83 quantitative aberration measurements in highly scattering fixed mouse brain tissues, together
84 with a fast and precise estimation of its scattering length. We compare our extended-source
85 wavefront measurement approach to the Shack-Hartmann method based on centroid calculation,
86 and show the benefit of the former in the case of scattering samples, and its improved accuracy at
87 large depths in low Signal to Background Ratio conditions. In a proof of principle experiment,
88 the ESSH method is finally used with a model sample consisting of neurons in culture placed
89 under a fixed brain slice to illustrate the image quality improvement that AO, driven by ESSH,
90 can provide in microscopy experiments in brain tissue at large depths, and in particular in the
91 mouse cortex.

92 **2. Material and methods**

93 **2.1. Set-up**

94 To perform aberration measurements within scattering conditions, an ESSH wavefront sensor was
 95 implemented on a custom-made epifluorescence set-up represented in Fig. 1a, composed of a laser
 96 light source at 488 nm (Cobolt 06-MLD) focused in the back focal plane of the water immersion
 97 objective (Olympus 20X, NA = 1, XLUMPFLN20XW) by L1 (Thorlabs, AC254-100-A, $f' = 100$
 98 mm). The fluorescence emitted was collected through the objective, its back focal plane being
 99 conjugated with the ESSH microlens array by a pair of achromatic lenses L2-L3 (L2, Thorlabs,
 100 AC508-300-A, $f' = 300$ mm and L3, Thorlabs, AC254-80-A, $f' = 80$ mm). The ESSH was similar
 101 to the device reported in our previous publication [23], and was composed of an array of 17 x 23
 102 microlenses, with a focal length of 5.1 mm, the CMOS sensor being in the focal plane of the
 103 microlenses. A squared field diaphragm was placed in front of the ESSH, leading to a field of
 104 view of $120 \times 120 \mu\text{m}^2$ in the object plane, for each microlens, thus selecting the correlation area
 105 and avoiding crosstalk between adjacent thumbnails, defined as the undersampled images behind
 106 each microlens. A 50:50 beamsplitter (BS) separated the beam between a full-field camera
 107 (Thorlabs) and the ESSH path. The observation wavelength was selected by either two bandpass
 108 filters (525/50, 625/40 from Chroma) or a long-pass filter (LP715 from Semrock) allowing us to
 109 measure the wavefront at three different wavelengths using three types of emitters (see details in
 110 ‘Sample preparation’ section) being deposited on a single coverslip. A white-light transmission
 111 imaging path was added in order to check sample positioning, in particular to localize precisely
 112 the cortex area of the coronal slices for wavefront measurement. This path was composed of a
 113 white light source, followed by L4 (Thorlabs, AC254-150-A, $f' = 150\text{mm}$), and L5 (Thorlabs,
 114 AC508-250-A, $f' = 250$ mm), and an objective (Olympus 5X, NA = 0.15, MPLFLN5X)

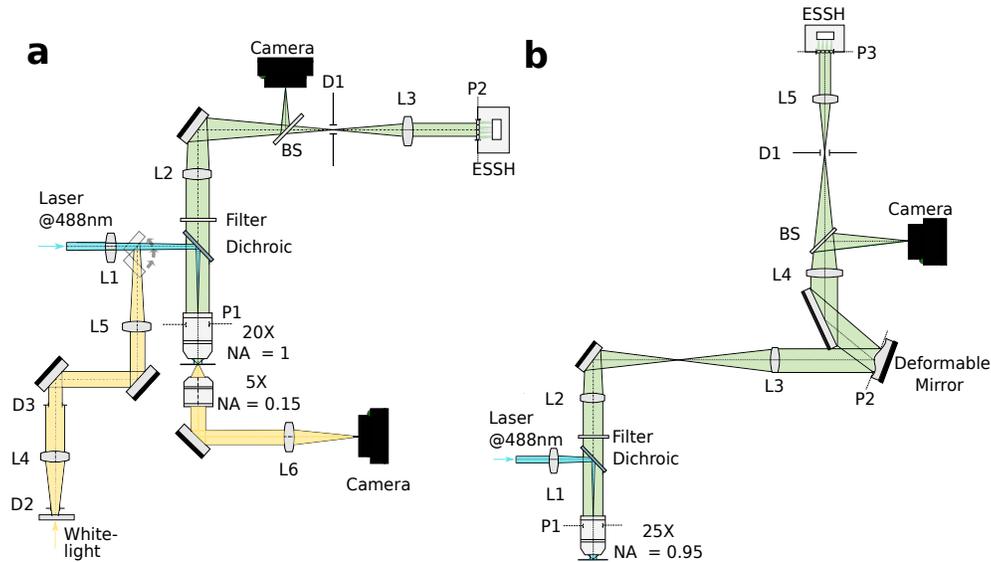


Fig. 1. Schematic of the set up. (a) Aberration measurement set up. L1-6 lenses, D1 squared field diaphragm, D2 aperture diaphragm, D3 field diaphragm, BS 50:50 beamsplitter, P1 back focal plane of the objective, conjugated to P2 located on the microlens array of the ESSH. (b) Closed-loop AO set-up. L1-5 lenses, D1 squared field diaphragm, BS 50:50 beamsplitter, P1 back focal plane of the objective, conjugated to P2 located on the Deformable Mirror, and to P3 on the microlens array of the ESSH.

115 The set-up used for closed-loop AO, shown in Fig.1b and corresponding to the results presented

116 on Fig.6, was composed of a water immersion objective (Leica 25X, NA = 0.95), a first pair of
 117 achromatic relay lenses L2-L3, (L2, Thorlabs, AC508-150-A, $f' = 150$ mm, and L3, Thorlabs,
 118 AC254-125-A, $f' = 125$ mm) to conjugate the back focal plane of the objective on a deformable
 119 mirror (Mirao52e, Imagine Eyes, fully characterized by Fernandez et al. [28]). A second pair of
 120 relay lenses L4-L5 (L4, Thorlabs, AC508-250-A, $f' = 250$ mm, L5, Thorlabs, AC254-80-A, f'
 121 = 80 mm) was added to conjugate the back focal plane on the ESSH. The scientific image was
 122 realised on an ORCA Flash V2 (Hamamatsu). The correlation computation was performed over
 123 the $130 \times 130 \mu\text{m}^2$ central zone of the full-field image.

124 The sample was imaged by the ESSH microlenses onto the camera sensor, leading to
 125 undersampled thumbnails. A reference thumbnail was chosen centrally, and correlations were
 126 computed between each thumbnail and the reference one in order to retrieve the relative
 127 displacement of each thumbnail to the reference. A pre-processing step was required to avoid
 128 correlation errors induced by noise. Considering the low SBR on our thumbnails (see SBR
 129 definition below), a Laplacian of Gaussian filter was selected. A pyramidal fit was used to
 130 find the correlation peak with sub-pixelic displacement for each thumbnail, leading to the local
 131 displacement of the wavefront; this choice results in a trade-off between accuracy and time
 132 calculation. These steps followed the procedure and pseudo-code described in Anugu et al [29].
 133 Finally, the wavefront reconstruction was achieved with a conventional zonal method. This
 134 process is summed up in Fig 2. The closed-loop can reach a maximum rate of 10Hz, with the
 135 following computer configuration: processor IntelCore i5-8400 CPU@2.8GHz, 32Go RAM,
 136 Windows 64bits, and to which must be added the exposure time imposed by the sample labelling.

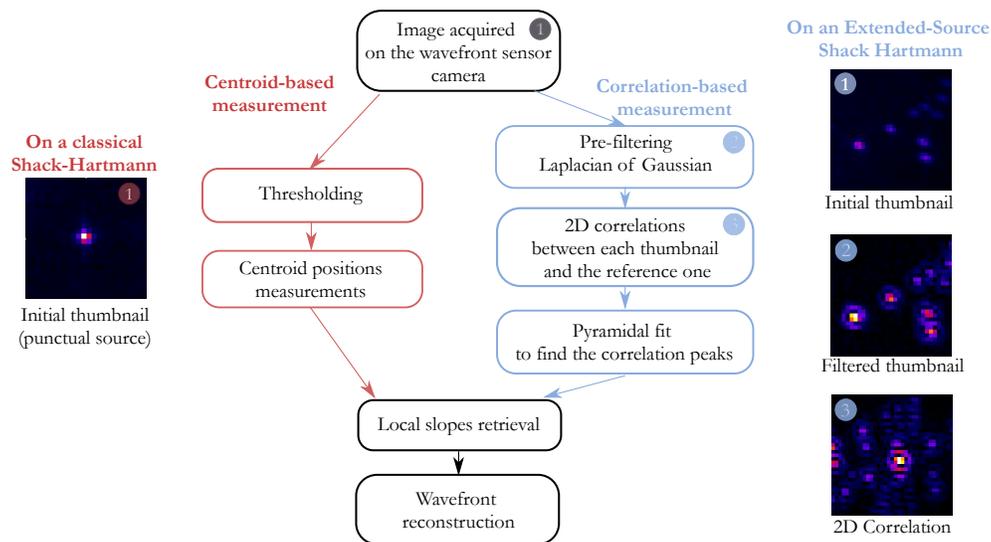


Fig. 2. Flowchart representing the steps of the wavefront reconstruction, for the centroid based measurement and the correlation based-measurement used with our ESSH.

137 2.2. Sample preparation

138 Because of the low numerical aperture (NA) of the microlenses (NA = 0.03), the ESSH required
 139 to be coupled with an optical sectioning method to provide quantitative wavefront measurements
 140 in thick 3D samples. To demonstrate the performances of the ESSH in the case of a scattering
 141 sample, we used a simple configuration, where the sectioning was inherent to the samples: we
 142 used 2D fluorescent samples placed below unlabelled 3D scattering samples (fixed mouse brain
 143 slices) and achieved by this mean wavefront measurement on an epifluorescence microscope

144 lacking optical sectioning. This approach allowed to easily control the scattering properties of
145 the sample using slices of known thicknesses, which would be extremely challenging using intact
146 biological samples, and relaxed the need for implementing optical sectioning in the instrument.
147 Such a model has already been used in other AO-based experiments [30].

148 Experimental procedures were conducted in accordance with the institutional guidelines and in
149 compliance with French and European laws and policies and with the ARRIVE guidelines [31].
150 All procedures were approved by the ‘Charles Darwin’ local institutional ethical committee
151 registered at the French National Committee of Ethical Reflection on Animal Experimentation
152 under the number 05 (authorization number: APAFIS 26667).

153 Three 6-months old C57BL6 male mice were sacrificed by an overdose of Euthasol, after being
154 placed under deep sedation by overdose of isoflurane (5 min at 5% isoflurane in an induction
155 box). The extracted brain was then stored overnight in a solution of 4% paraformaldehyde and
156 finally rinsed in phosphate buffer solution (PBS). Coronal slices of different thicknesses ranging
157 from 50 μm to 300 μm were then cut and stored in PBS.

158 Several fluorescent emitters were used; 2- μm fluorescent beads (ThermoFischer, $\lambda_{em} = 515 \text{ nm}$)
159 were selected as emitters within the green range while quantum dots (QD) aggregates were used
160 as emitters in the red and near-infrared ranges in order to keep the same excitation wavelength.
161 ZnCuInSe/ZnS QD emitting at 800 nm were synthesized following the protocol reported by
162 Pons et al [32] whereas those emitting at 614 nm were obtained from protocol described by
163 Yang et al [33]. QD in hexane (4 nmol) were mixed with ethanol (1 mL) to precipitate QD by
164 centrifugation. QD were resuspended in chloroform (250 μL) and CTAB (5 mL, 3 mM) was
165 added. The solution was heated for 20 min at 80°C to evaporate chloroform. The QD aggregates
166 surrounded by hydrophobic ligands were precipitated by centrifugation (5 min, 8500 rpm) and
167 then dispersed into ethanol. A second round of centrifugation was necessary to eliminate all
168 non-aggregated QD. Finally, aggregates were dispersed into ethanol (1 mL). The aggregate
169 solutions were diluted 10 times. 500-nm aggregates were obtained with respectively an emission
170 peak at 614 nm (FWHM = 32 nm) for the visible ones, and 805 nm (FWHM = 17 nm) for the
171 near-IR ones.

172 Samples were prepared with the three types of emitters. 4 μL of the 2- μm commercial beads,
173 diluted 1/5000e, were deposited first on a 170- μm -thick coverslip then dried up for 5 min at 60°C.
174 The two types of aggregates were deposited on the same side of the coverslip (4 μL of each). On
175 the other side of the coverslip a fixed brain slice was placed, with spacers. Another coverslip was
176 added on top to avoid the adhesion of the brain slice to the water-immersion objective during the
177 experiment.

178 Neuronal cultures containing a mVenus fluorescent tag were generated by an ex-utero electro-
179 poration in the sensory cortex of E15 murin embryos. A drop of DNA pH1SCV2_shRNA_control
180 (0.7 $\mu\text{g}/\mu\text{l}$) was injected with a capillary in one hemisphere of the sensory cortex of the embryo
181 and then electroporated. This region was then extracted from the whole brain and dissected
182 in a medium containing 10% HBSS, 5% glucose and 2% HEPES. The piece of remaining
183 cortical hemisphere was digested by chemical and mechanical digestions, first, under the action
184 of papain for 15 min at 37°C and then under pipet up and down movements, to obtain individual
185 neurons. The neurons were plated onto a 18-mm coverslip coated with 80 $\mu\text{g}/\text{ml}$ polyornithine
186 in 12 well plates, at a density of 180,000 cells/well in MEM, 10% HS, 1% glutamax and 1%
187 sodium pyruvate 100 mM and incubated for 2 hours at 37°C, 5% CO₂. When the neurons were
188 completely adherent on the coverslip, the medium was replaced by Neurobasal, 2% B27, 1%
189 glutamax and antibiotics. One third of the medium was changed every 5-6 days.

190 To obtain model scattering samples, fluorescent emitters (2- μm commercial beads diluted
191 1/5000e (4 μL), dried up for 5 min at 60°C) were first deposited on a coverslip. On the other
192 side, 2- μm polystyrene beads (Sigma-Aldrich, 78452) dispersed in water were used as scattering
193 samples and deposited. 1 μL of the commercial solution, containing 10% of beads in mass

194 was diluted 10 times to obtain a solution of 0.002 bead/ μm^3 with a scattering length of 74.9
195 μm . Similarly, a 5-times diluted solution (0.005 bead/ μm^3) with a scattering length of 29.9 μm
196 was realised. The scattering coefficients were calculated with Mie-scattering theory, at 515 nm
197 (online calculator https://omlc.org/calc/mie_calc.html). 10 μL of the diluted
198 solutions were deposited on the coverslip, surrounded by 130- μm -thick spacers, and recovered by
199 another coverslip to retain the scattering droplet.

200 *2.3. Third order Spherical Aberration (SA3) measurement and modelling*

201 SA3 coefficient is described in the Zernike polynomials as $6\rho^4 - 6\rho^2 + 1$. Thus, the focus variation
202 has a strong impact on the spherical aberration value, as a best focus can be found to minimize
203 the later. In order to eliminate this factor, we minimized experimentally the focus aberration to
204 eliminate this bias from our analysis. Only the contribution of the brain slice was considered
205 here: a reference wavefront, corresponding to the aberrations induced by the instrument and
206 the coverslips, was subtracted at each wavelength. For each brain slice thickness, at least seven
207 acquisitions were realised in different areas of the cortex, at the three considered wavelengths
208 simultaneously.

209 We simulated the optical system in Zemax in order to obtain an order of magnitude of the
210 refractive index mismatch-induced spherical aberration for mouse brain slices. A simple model
211 consisting in two additional interfaces inserted between the emitters' plane and the objective
212 was chosen. The brain slice was modelled by a layer of refractive index of 1.368, previously
213 experimentally measured on fixed mouse brain slices and reported by Lue et al [34]. The process
214 was repeated for the corresponding experimental thicknesses used.

215 *2.4. Numerical simulation of the measured shift of a thumbnail for several SBR*

216 The SBR of a given thumbnail was defined as the ratio between the average signal value of the
217 1% brightest pixels, and the background mean value, considered as the mean value of the 80%
218 dimmest pixels. A well-sampled point spread function corresponding to a squared aperture, at
219 600 nm, was first generated numerically. Poissonian noise was added with a variance equal
220 to the square root of the signal plus the background value. The generated image was then
221 undersampled on one hand to obtain our reference thumbnail, with dimensions matching to the
222 experimental ones. On the other hand, the equivalent of a 0.5 pixel shift was introduced in the
223 two directions and the shifted image was undersampled afterwards. As discussed in Anugu et
224 al [29] it corresponds to the minimum bias error value in the shift retrieval, for the centroid
225 process as well as for the correlation-based method. The induced shift was then retrieved by the
226 two methods computed here. The centroid position was measured on the two thumbnails, on
227 which a threshold corresponding to the background value plus 10% of the difference between
228 the maximum and the background value was applied. The threshold choice was motivated by
229 the results shown on Fig.4c and Fig.4d of the influence of the threshold choice on the shift
230 retrieval in low SBR conditions. This simulation was run on fifty images for each SBR value. For
231 the subpixelic shift retrieval on the experimental thumbnails, 35 x 35 pixels² images were first
232 oversampled by a factor 100 and then shifted by the equivalent of 0.5 pixel in the two directions.
233 After under-sampling the shifted images, the shift retrieval between the initial thumbnails and the
234 shifted ones was performed as described above.

235 **3. Results**

236 *3.1. Signal to Background Ratio (SBR) across the ESSH wavefront sensor in scattering* 237 *tissues.*

238 The ESSH microlenses are conjugated with the back focal plane of the objective (Fig.2a). Due to
239 the scattering of light in biological samples, the images below each micro-lens are losing contrast

240 (Fig.2b) in depth, as in the case of the centroid-based measurement [13, 14]. This loss in contrast
 241 ultimately limits the possibility to reliably measure a wavefront with Shack-Hartmann sensors at
 242 large depth in scattering tissues. More specifically, the intensity distribution on each thumbnail of
 243 the ESSH shows a decrease of the signal from the central thumbnail to the ones on the edges, with
 244 a rotational symmetry (Fig.3b). At the same time, the background decreases, but slower, from
 245 the centre to the edge of the ESSH (Fig.2b). Accordingly, the signal to background ratio (SBR)
 246 decreases significantly from the centre to the edge with the same rotational symmetry (Fig.3b).
 247 On the contrary, in the absence of a scattering tissue and with a homogeneous fluorescent sample,
 248 the signal on the ESSH is uniform in intensity over all the thumbnails, except the ones on the
 249 most external row where the entrance of light rays starts being limited by the edge of the pupil
 250 (data not shown). This effect can be explained by the geometry of the sensor (Fig.3a): when
 251 a thick scattering sample is positioned on top of the imaged fluorescence emitters, the emitted
 252 signal received by the outer microlenses is propagating through a larger distance in the scattering
 253 sample than the one received by central microlenses. Let us note that this effect is not specific to
 254 the ESSH but is present in SH with centroid-based estimation of shifts too [14]. When measuring
 255 aberrations through scattering media, this Signal to Background (SBR) deterioration across the
 256 sensor limits thus the size of the pupil over which the wavefront can be computed, with the
 257 limiting SBR found at the outer thumbnails.

258 3.2. Scattering coefficient measurements

259 Thanks to this intensity profile across the ESSH, the scattering length of the medium can be
 260 measured. In a scattering sample, the intensity of the ballistic fluorescence signal decays with
 261 depth following

$$I(z) \propto \exp\left(-\frac{z}{l_s}\right) \quad (1)$$

262 where l_s is the scattering mean free path (or scattering length) of the sample and z its thickness.
 263 In the geometry of the ESSH, the thickness z_p crossed to reach the thumbnail of index p (p
 264 varies between 0 (central thumbnail) and 7 (most external row of thumbnails) increases as

$$z_p = \frac{z_c}{\cos \theta_p} \quad (2)$$

265 where z_c is the slice thickness and θ_p is the angle over which the thumbnail p is seen, with

$$\theta_p = \arctan\left(\frac{p}{7} \tan \theta_{max}\right) \quad (3)$$

266 and $\theta_{max} = \arcsin(NA_{objective}/n) = 48.8^\circ$, θ_{max} being the maximum angle collected by the
 267 objective.

268 Measurements on a calibrated scattering sample composed of 2- μm polystyrene beads and
 269 130- μm -thick are first performed to confirm the accuracy of the method, at two bead concentrations
 270 chosen to be within the range of l_s values of fixed brain tissues. In these experiments, we position
 271 a single fluorescence emitter at 515 nm in the ESSH field of view. In Fig.3c, the intensity of
 272 the ballistic signal is plotted as a function of the effective thickness after the subtraction of the
 273 background on each thumbnail. This requires the ability to measure both the ballistic signal
 274 and the background level in each thumbnail, and thus to use samples with a relatively sparse
 275 distribution of emitters. From the slope of the semi-log plot, which is equal to $1/l_s$, we measure
 276 for a 0.005 sphere/ μL concentration a scattering length of $26.5 \pm 0.3 \mu\text{m}$, very close to the
 277 expected value of 29.9 μm . Similarly, we measure a $69.5 \pm 1.4 \mu\text{m}$ scattering length at 0.002
 278 sphere/ μL concentration where 74.9 μm was expected. This demonstrates that the scattering
 279 length can be measured using the ESSH sensor with an accuracy of 10%. Measurements on
 280 fixed mouse brain coronal slices, in the cortex, are performed at three different wavelengths

281 (515 nm, 615 nm and 805 nm) to determine the chromatic dependence of the scattering length
 282 of the tissue. To improve the measurement accuracy, three slice thicknesses are used at each
 283 wavelength: at 515 nm and 615 nm, 50-, 100- and 150- μm -thick slices and at 805 nm, 100-
 284 150- and 200- μm -thick slices. The selected slice thicknesses correspond to ranges of l_s to $2.5l_s$.
 285 For each slice, the intensity is normalized to plot and fit the data for all slices on a single figure
 286 (Fig.3d); the ordinate of the individual semi-log plot being subtracted to each acquisition. The
 287 measured scattering coefficients are $38 \pm 2 \mu\text{m}$ at 515 nm, $47 \pm 2 \mu\text{m}$ at 615 nm and $77 \pm 5 \mu\text{m}$
 288 at 805 nm, increasing significantly with the wavelength as expected. The obtained l_s values at the
 289 three wavelengths are compared to previously reported scattering length values in the Discussion.

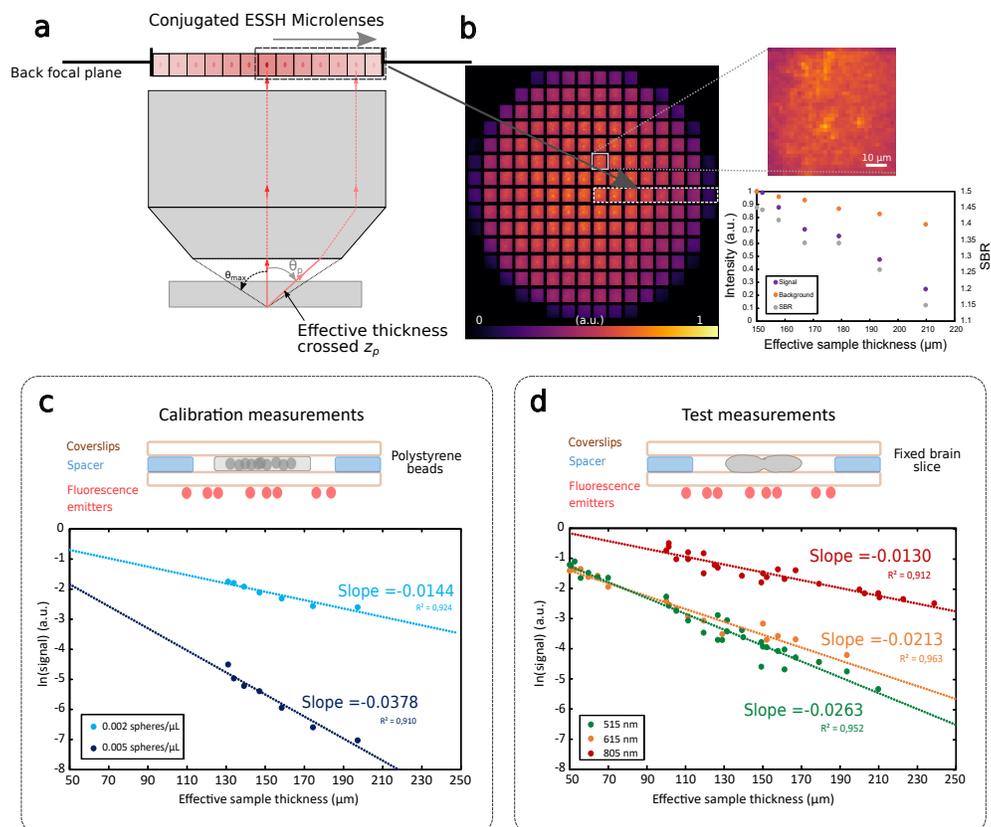


Fig. 3. Scattering length measurement on fixed brain slices. (a) The ESSH microlenses are conjugated with the back focal plane of the objective. Each microlens sees the same field of view but with a different angle. (b) Example of an ESSH image showing the decrease in intensity between the side-by-side thumbnails within the dotted boxes (represented in (a)), with a zoom on one corresponding thumbnail, obtained through a 150- μm -thick brain slice at 515 nm. Corresponding plot of the signal, the background and the signal to background as a function of the effective thickness seen along the direction of the dotted boxes (c) Calibration measurements with the corresponding sample scheme. (d) Measurements on brain slices, with sample scheme.

290 3.3. Spherical aberration measurements with ESSH.

291 In this section, we show that the ESSH sensor provides a reliable wavefront measurement in very
 292 scattering conditions. We consider in particular third-order Spherical Aberration (SA3), which is

293 mainly generated by refractive index mismatch and which depends on the sample thickness, a
294 parameter that we can easily adjust. We use 50- to 150- μm -thick slices within the visible range
295 and up to 250- μm -thick in the near-IR. These thicknesses correspond to $1.3l_s$ to $3.9l_s$ at 515 nm,
296 to $1.0l_s$ to $3.3l_s$ at 615 nm and to $0.6l_s$ to $3.2l_s$ at 805 nm. The increase in thickness corresponds
297 to an increase in Optical Path Difference (OPD), that is the difference of refractive index between
298 the tissue and water times the sample thickness. The variation of the tissue refractive index
299 between the different wavelengths is neglected. Comparisons of the experimental SA3 values
300 to the one obtained by simulations (see Methods), presented in Fig.3a-c, show that our ESSH
301 sensor is providing accurate aberration measurement and confirm quantitatively the increase of
302 the spherical aberration with the brain slice thickness, in agreement with the simulation. At
303 low slice thickness (50 μm), the SA3 induced by the sample is within the measurement error
304 values of the ESSH (8-9 nm is predicted by the Zemax simulation). Our measurement method
305 reaches its limits when the slice thickness became greater than $3.9l_s$ with commercial beads as
306 fluorescent emitters, and $3.3l_s$ with the aggregates. Corresponding images on thumbnails are
307 shown on Fig.3d. These slice thicknesses correspond to effective thicknesses crossed on the outer
308 microlenses of $4.8l_s$ and $5.8l_s$, for which the ballistic signal is considerably reduced at the pupil
309 edges (typ. 0.25%), corresponding to a SBR of 1.3 only. Alongside this loss of ballistic signal,
310 we observe a more important variability of the SA3 values for a single slice, as a consequence of
311 the loss of slope accuracy evaluation at these SBR. Measuring aberrations with thicker slices
312 could be achieved by restricting the pupil over which the wavefront is calculated, but these results
313 could not be compared to the previous ones with thinner slices, performed on the entire pupil.
314 Our ESSH thus allows performing quantitative aberration measurement through scattering slices
315 that are up to $3.9l_s$ -thick.

316 3.4. Comparison of ESSH/centroid computation at low SBR

317 To confirm that ESSH measurements are resilient to strong scattering conditions, in particular
318 in comparison to the centroid-based measurements, we conducted simulations (described in
319 Methods) to assess the accuracy of the displacement retrieval at low SBR for both methods.
320 The chosen SBR corresponds to previous experimental SBR shown on Fig.3d. For the highest
321 SBR, typically larger than 1.5, both methods are giving similarly good results, as the average
322 displacement error is null in both cases (Fig.5a). However, as the SBR decreases, the centroid
323 method is more impacted. A large dispersion of the shift measurement was observed in the case
324 of the centroid method, as shown by the large error bars (Fig.5a) and Standard Error of the Mean
325 (Fig.4b). Thus, the number of images over which the displacement calculation is done has to
326 be increased by a factor 25 at a SBR of 1.2 to achieve the same precision on the displacement
327 measurement with the centroid approach as with the correlation method. This variability in
328 the displacement measurement affects directly the quality of the aberration correction that can
329 be achieved with an AO loop. At SBR smaller than 1.2, the noise also impacts the estimate of
330 the average displacement obtained through the centroid process (at SBR = 1.09, Student t-test
331 $\alpha = 0.05$, $p = 0.009$). On the contrary, the mean value of the shift evaluated by the correlation-
332 based measurement is not significantly different from the one imposed numerically at all SBR
333 values assessed (at SBR = 1.09, Student t-test $\alpha = 0.05$, $p = 0.355$). As the results provided
334 in Fig.4a,b were obtained for a specific threshold of 0.1 for the centroid-based measurement
335 (see Methods), we checked if they depend on the threshold value. For this purpose, the same
336 simulation is computed for thresholds varying between 0.07 and 0.2 and the standard deviation
337 and the mean values of the retrieved displacement error are represented respectively on Fig.5c and
338 Fig.5d, as a function of the SBR. It shows that the threshold value of 0.1 was roughly optimal and
339 that independently of the threshold on the centroid measurement, the shift retrieval performances
340 of the correlation remain better, in low SBR conditions, whatever the threshold used in the
341 centroid measurement. Our simulation proves thus that a much more accurate measurement of

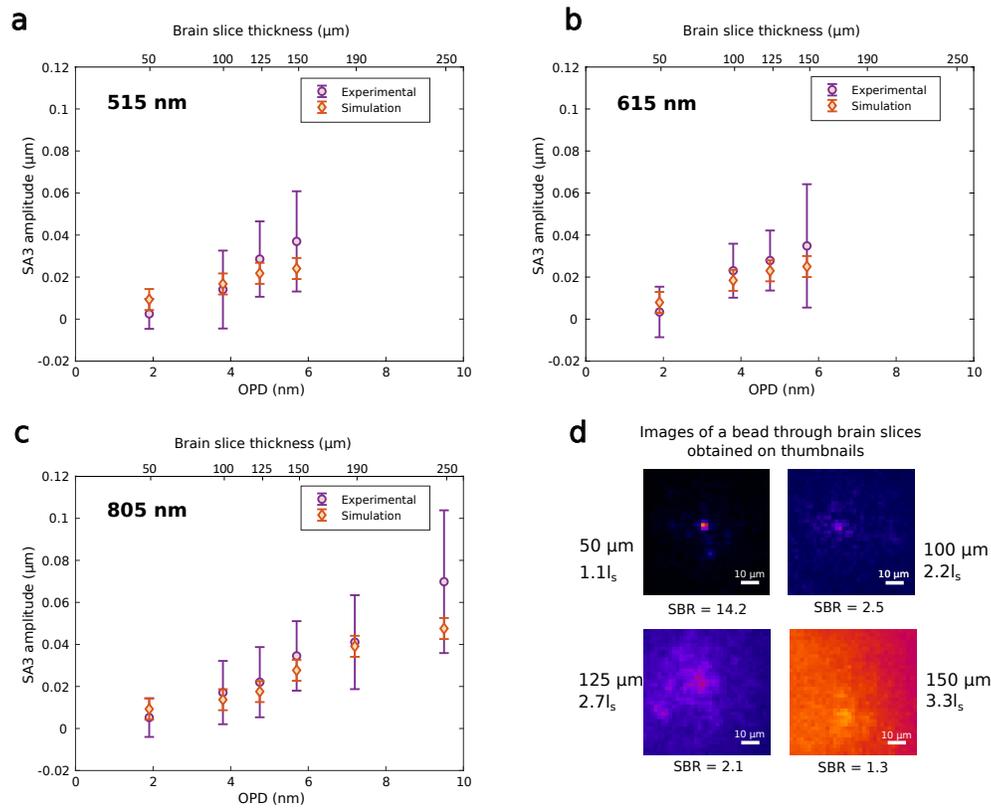


Fig. 4. Spherical Aberration measurement on scattering sample with ESSH. (a)-(c) 3rd order Spherical Aberration amplitude increases with the Optical Path Difference (i.e. the brain slice thickness) induced by mouse brain slice at 3 wavelengths, mean and standard deviation plotted. The experimental data are compared to simulation data (described in Methods). (d) Examples of signal on external thumbnails for 4 different thicknesses of brain slice, at 615 nm. The corresponding values of slice thicknesses are given in terms of number of scattering lengths.

342 the shifts between thumbnails induced by aberration could be obtained at lower SBR with the
 343 correlation method rather than with the centroid-based method. We also tested the accuracy
 344 of subpixelic shift retrieval on experimentally acquired thumbnails to take into account real
 345 scattering conditions from biological samples (see Methods). Results are reported on Fig.4a.
 346 Identically good shift retrieval is obtained with the two methods for SBR larger than 2; however,
 347 when the SBR drops, the error on the measured displacement remains within ± 0.1 pixel with
 348 the correlation method, whereas the centroid-based measurements deviates from zero, and this
 349 error increases significantly at SBRs smaller than 1.3. These results based on experimental data
 350 confirm the robustness of the shift retrieval for low SBR using the correlation method.

351 3.5. ESSH allows closed-loop aberration correction for enhanced imaging through 352 scattering media

353 We finally tested the possibility to correct aberration and improve image quality in a closed-loop
 354 AO set up using the ESSH sensor (see Methods), with a model sample that resembles *in vivo*
 355 biological samples in term of structures and photon flux. The sample consists in fluorescently-
 356 labelled neurons placed under a fixed unlabelled mouse brain slice (Fig.6a and Fig.6h, see

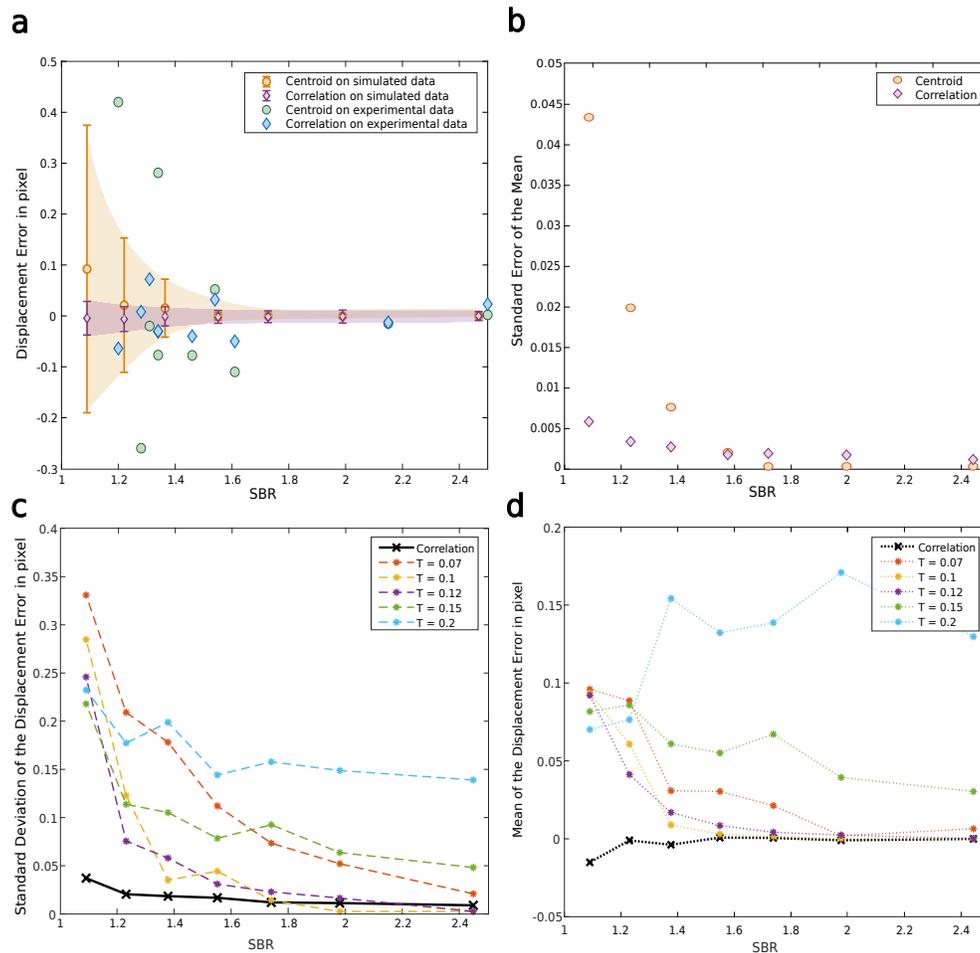


Fig. 5. Comparison of shift retrieval accuracy using centroid and correlation method. (a) Numerical simulation of cross-correlation and centroid method to retrieve a subpixelic displacement induced numerically as a function of the SBR. Coloured dots with error bars correspond respectively to the mean and standard deviation for 50 simulations at each SBR. Single dots and diamonds represent shift retrieval for experimental images with both methods (b) Standard Error of the Mean (SEM) as a function of the SBR. The SEM values correspond to the simulated data in (a). (c-d) Standard deviation (c) and mean values (d) of the displacement error for 50 simulations at each SBR, for several thresholds (T) applied for the centroid method, T varying between 0.07 and 0.2

357 Methods). Aberrations are induced by the brain slice and by the coverslip placed on top of
 358 it. The latter is generating a 210 nm SA3, prevailing to the 10 to 20 nm SA3 generated by
 359 the brain slice, the brain tissue mainly enabling the introduction of significant scattering. The
 360 geometry of the sample was chosen to mimic in depth optical sectioning in a scattering biological
 361 medium. The AO-loop, running at 2Hz, improves significantly the image quality. The initial
 362 images (Fig.6b and Fig.6i) are corrected for the microscope aberrations, so that only correction
 363 of the sample-induced aberrations is performed to retrieve the images in Fig.5c and Fig.6j. For
 364 the thinnest sample, the signal from the soma is increased by 28%, between Fig.5b and Fig.5c
 365 and the signal from dendrites is increased by 34% (Fig.6f and Fig.6g). This contrast and signal
 366 enhancement reveals some dendrites that were not visible before the correction (Fig.6c). In the

367 case of the thicker sample (thickness of $2.5l_s$), the correction, which is achieved with a very
 368 low amount of ballistic photons (8% of the incident light), increases the signal and contrast of
 369 the image even in these conditions (Fig.6j). In this proof of principle experiment, the gain in
 370 image quality is even significantly lower than the one expected using two-photon microscopy,
 371 as in the latter scattering on the emission path doesn't affect image quality. This experiment
 372 illustrates the fact that a correction based on the ESSH wavefront measurement obtained through
 373 a scattering tissue and using fluorescently-labelled biological samples allows a significant image
 374 improvement, that is crucial especially in functional imaging where sensitivity is a key parameter.

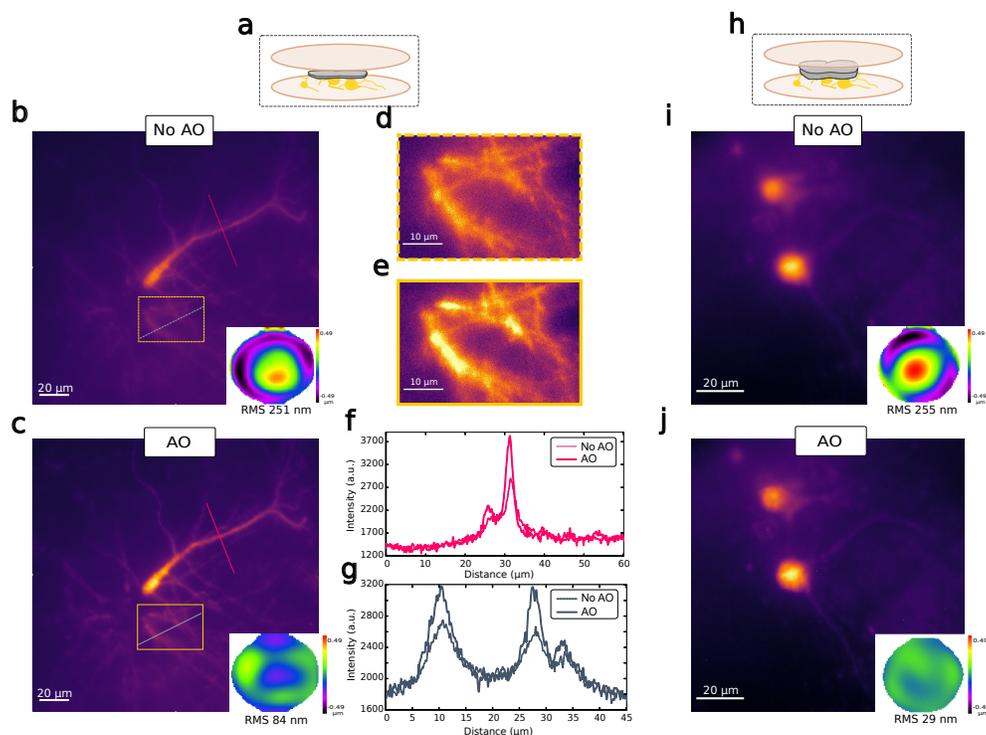


Fig. 6. ESSH based AO correction loop enables to retrieve microscope performances through scattering samples. (a) Fixed mVenus neuron culture placed under a 50- μm fixed mouse brain slice, corresponding to $1.2l_s$. Wide-field images before (b) and after the correction (c) with the corresponding wavefronts. Signal profiles along the black and pink lines in (b) and (c) are plotted in (f) and (g). Zoom on dendrites before (d) and after (e) correction, with adjusted contrast. (h) Same as (a) but using a 100- μm fixed mouse brain slice, corresponding to $2.5l_s$. Images before (i) and after (j) correction, with corresponding wavefronts.

375 4. Discussion

376 We showed that our innovative Shack-Hartmann sensor, based on an extended-source wavefront
 377 measurement, is a reliable tool to measure quantitatively aberrations in scattering samples up
 378 to $3.9l_s$ -thick. We have shown that wavefront measurements using Shack-Hartmann sensors
 379 is ultimately limited by the SBR on the thumbnails at the edge of the sensor, due to the
 380 increased scattering of the rays reaching them. However, by computing simulations at low
 381 SBR, corresponding to real scattering conditions, we obtained more accurate displacement
 382 measurements with the correlation-based method than with the centroid-based approach. This

383 study demonstrates the robustness of the ESSH wavefront measurement approach in depth in
 384 biological samples with significant levels of scattering. This can be attributed to the joint use of
 385 correlation and Laplacian of Gaussian filter, instead of centroid to estimate shifts. Concerning
 386 correlation, we currently make use of conventional correlation algorithms (see Methods), whereas
 387 more advanced correlation algorithms such as phase correlation are known to provide better
 388 sensitivity and/or accuracy, depending on the characteristics of the signal, but at the cost of
 389 more computational complexity and time. There is as a consequence still some room for further
 390 improvement of the approach.

391 By digging into the characteristics of the ESSH signal, we have also shown that the use of the
 392 ESSH allows the measurement of the scattering lengths of these samples with a single image
 393 acquisition. Indeed, it allows to measure in a single shot using a single sample the attenuation
 394 through 7 effective thicknesses, corresponding to the 7 microlenses conjugated along the objective
 395 back focal plane radius. It facilitates therefore the measurement of l_s as it can be obtained directly
 396 from a single measurement in one sample. Indeed, it doesn't require multiple measurements at
 397 multiple depths [35, 36] or the use of multiple samples of different thicknesses, which would
 398 introduce additional uncertainties associated with the estimation of each sample thickness. This
 399 single shot measurement of l_s is therefore both accurate and easy to implement on a wide range of
 400 samples. A specific design of the ESSH with dedicated number of microlenses and characteristics
 401 of the camera could be realized to optimize the performance of the l_s measurement. The accuracy
 402 of the measurement will depend on the sample thickness, as the relative attenuation between
 403 the central and border thumbnails in our case decreases as $\exp(-0.5z_c/l_s) - 1$. Therefore,
 404 increasing the sample thickness is beneficial, until attenuation of the ballistic light is too strong
 405 to be separated from the background on each thumbnail. In our hand thicknesses of the order of
 406 l_s to $3l_s$ in fixed tissues seemed optimal. The values obtained can be compared with the ones
 407 reported in the literature (see Table 1). Since scattering lengths measured *in vivo* are 2 to 3 times
 408 higher than the one realized on fixed tissues [35, 36], we used for comparison values reported
 409 only in fixed slices [35] and extrapolated linearly their measurement to the wavelengths used in
 410 our study. Our measurements match well the values reported in literature and confirm the ability
 411 of the ESSH sensor to provide a consistent measurement of l_s . The slight discrepancy can be
 412 related to differences in slice preparation or animal age.

Sample	λ [nm]	l_s [μm]	Reference
Fixed brain	775	55.2	Kobat et al. [35]
	1280	106.4	
Fixed slice	515	38 ± 2	Results
	615	46 ± 2	
	805	77 ± 5	

Table 1. Measured scattering lengths l_s of fixed brain slices at different wavelengths with the ESSH analyzer compared to literature reported values.

413 One possible limitation of the scattering measurement method is the possible impact of the
 414 geometry of the sample on the measurement, in particular its surface. A non-flat surface will
 415 lead to some measurement artifacts, different local heights leading to differences in measured
 416 local scattering values. A proper use of the technique is then conditioned to the use of samples
 417 with flattened surfaces, for examples using glass slides or coverslips, which corresponds to a
 418 significant part of experimental conditions, such as e.g. transcranial imaging in the rodent using
 419 optical windows, which minimizes movement artifacts.

420 In this study we realized quantitative aberration measurement and correction in scattering
421 conditions with an extended-source wavefront sensor, paving the way to the combination of
422 adaptive optics based on this method to optical sectioning microscopy, such as two-photon
423 microscopy or light-sheet microscopy, for deep imaging. We have shown here that the ESSH
424 allows to perform quantitative aberration measurement deep inside biological tissues up to
425 $3.9l_s$ -thick. Using standard GFP labelling ($\lambda_{em} = 514$ nm), and since l_s is of the order of $60 \mu\text{m}$
426 *in vivo* [36], the ESSH sensor would give access to quantitative aberration measurements, and
427 then correction, up to $3.9 \times 60 \mu\text{m} \approx 250 \mu\text{m}$ deep inside a live mouse brain at this wavelength.
428 To make ESSH-based wavefront measurement compatible with the maximum imaging depths in
429 2-photon microscopy (up to $800 \mu\text{m}$ in the mouse brain [13, 14]) or in light-sheet microscopy, a
430 dual-labelling approach is relevant. It allows to keep the commonly used GFP-based functional
431 labelling in the visible range, and to shift the wavelength used for the wavefront measurement
432 towards the near-IR. This strategy presents two advantages: first, the impact of scattering is
433 minimized on the ESSH thumbnails; secondly, the photon budget is optimized as all photons
434 from functional dyes are kept for the neuronal activity recording. Using dye emitting around
435 900 nm, where $l_s \approx 170 \mu\text{m}$ (studies at 920nm are reporting l_s between $170 \mu\text{m}$ [37] and 200
436 μm [38]), AO corrections at depths of $3.9 \times 170 \mu\text{m} \approx 700 \mu\text{m}$ are reachable.

437 There are however several limitations to the present study that will require further experimental
438 confirmation. First of all, the experimental demonstration realized in this study only partially
439 confirms the prediction based on simulation. Indeed, the approach used to prepare the sample
440 did not allow a precise enough control of the SBR of the wavefront measurement to correspond
441 to extreme values (about 1.7 in our experiment), enabling only partial confirmation of the
442 capabilities of the ESSH measurement to work with very low SBR. Also, the sample used does
443 not reflect the behavior of signal and background for both light-sheet and 2-photon microscopy,
444 since for example the main effect of aberrations in non-linear microscopy is a decrease of signal.
445 Considering this, the proof of principle experiment is more representative of the behavior of the
446 AO loop in a light-sheet experiment. This particular aspect will have to be investigated using a
447 dedicated 2-photon setup. The present experiment is nevertheless a clear demonstration of the
448 capability of an ESSH-driven AO loop to provide a correction in low SBR situations, whatever
449 the origin of such SBR is, and on real biological objects of interest such as neurons. Also, there
450 was no consideration here of the impact of the isoplanetic patch with the use of SA3, since this
451 topic was already discussed in detail in our previous work [23]. Finally, our ESSH sensor doesn't
452 require descanning of the fluorescence, as in centroid measurement [14], nor requires the use of a
453 specific scanning arrangement and of an ultrafast laser for the wavefront measurement when the
454 technique is applied outside non-linear microscopy [22]. This is advantageous in term of photon
455 budget, as the ESSH can be placed closer to the microscope objective. Furthermore, it makes it
456 perfectly suited for advanced scanning methods as acousto-optic deflectors, where descanning is
457 difficult to implement [39].

458 **Acknowledgments.** We thank Marine Depp, Ombeline Hoa, Alexandra Bogicevic, Rémi Carminati and
459 Thomas Pons for technical assistance and fruitful discussion. This work was supported by grants from
460 France's Agence Nationale de la Recherche (INOVAO, ANR-18-CE19-0002). This work has received
461 also support under the program « Investissements d'Avenir » launched by the French Government and
462 implemented by ANR with the references ANR-10-LABX-54 (MEMOLIFE), ANR-10-IDEX-0001-02
463 (Université PSL) and ANR-10-INSB-04- 01 (France-BioImaging Infrastructure).

464 **Disclosures.** F.H. is employed by the company Imagine Optic and A.H.'s doctoral research is funded by
465 Imagine Optic. The other authors declare no competing interests.

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

468 **References**

- 469 1. A. Gilad, Y. Gallero-Salas, D. Groos, and F. Helmchen, "Behavioral strategy determines frontal or posterior location
470 of short-term memory in neocortex," *Neuron* **99**, 814–828.e7 (2018).
- 471 2. C. Dussaux, V. Szabo, Y. Chastagnier, J. Fodor, J.-F. Léger, L. Bourdieu, J. Perroy, and C. Ventalon, "Fast confocal
472 fluorescence imaging in freely behaving mice," *Sci. Reports* **8**, 16262 (2018).
- 473 3. M. B. Ahrens, M. B. Orger, D. N. Robson, J. M. Li, and P. J. Keller, "Whole-brain functional imaging at cellular
474 resolution using light-sheet microscopy," *Nat. Methods* **10**, 413–420 (2013).
- 475 4. E. M. Hillman, V. Voleti, W. Li, and H. Yu, "Light-sheet microscopy in neuroscience," *Annu. Rev. Neurosci.* **42**,
476 295–313 (2019).
- 477 5. S. Wolf, W. Supatto, G. Debrégeas, P. Mahou, S. G. Kruglik, J.-M. Sintes, E. Beaurepaire, and R. Candelier,
478 "Whole-brain functional imaging with two-photon light-sheet microscopy," *Nat. Methods* **12**, 379–380 (2015).
- 479 6. F. Helmchen and W. Denk, "Deep tissue two-photon microscopy," *Nat. Methods* **2**, 932–940 (2005).
- 480 7. J. Lecoq, N. Orlova, and B. F. Grewe, "Wide. fast. deep: Recent advances in multiphoton microscopy of in vivo
481 neuronal activity," *The J. Neurosci.* **39**, 9042–9052 (2019).
- 482 8. W. Yang and R. Yuste, "In vivo imaging of neural activity," *Nat. Methods* **14**, 349–359 (2017).
- 483 9. M. J. Booth, "Adaptive optical microscopy: the ongoing quest for a perfect image," *Light. Sci. & Appl.* **3**, e165–e165
484 (2014).
- 485 10. N. Ji, "The practical and fundamental limits of optical imaging in mammalian brains," *Neuron* **83**, 1242–1245 (2014).
- 486 11. C. Rodríguez and N. Ji, "Adaptive optical microscopy for neurobiology," *Curr. Opin. Neurobiol.* **50**, 83–91 (2018).
- 487 12. W. Sun, Z. Tan, B. D. Mensh, and N. Ji, "Thalamus provides layer 4 of primary visual cortex with orientation- and
488 direction-tuned inputs," *Nat. Neurosci.* **19**, 308–315 (2016).
- 489 13. R. Liu, Z. Li, J. S. Marvin, and D. Kleinfeld, "Direct wavefront sensing enables functional imaging of infragranular
490 axons and spines," *Nat. Methods* **16**, 615–618 (2019).
- 491 14. K. Wang, W. Sun, C. T. Richie, B. K. Harvey, E. Betzig, and N. Ji, "Direct wavefront sensing for high-resolution in
492 vivo imaging in scattering tissue," *Nat. Commun.* **6**, 7276 (2015).
- 493 15. T.-L. Liu, S. Upadhyayula, D. E. Milkie, V. Singh, K. Wang, I. A. Swinburne, K. R. Mosaliganti, Z. M. Collins,
494 T. W. Hiscock, J. Shea, A. Q. Kohrman, T. N. Medwig, D. Dambournet, R. Forster, B. Cunniff, Y. Ruan, H. Yashiro,
495 S. Scholpp, E. M. Meyerowitz, D. Hockemeyer, D. G. Drubin, B. L. Martin, D. Q. Matus, M. Koyama, S. G. Megason,
496 T. Kirchhausen, and E. Betzig, "Observing the cell in its native state: Imaging subcellular dynamics in multicellular
497 organisms," *Science* **360**, eaaq1392 (2018).
- 498 16. R. Jorand, G. Le Corre, J. Andilla, A. Maandhui, C. Frongia, V. Lobjois, B. Ducommun, and C. Lorenzo, "Deep and
499 clear optical imaging of thick inhomogeneous samples," *PLoS ONE* **7**, e35795 (2012).
- 500 17. A. Masson, P. Escande, C. Frongia, G. Clouvel, B. Ducommun, and C. Lorenzo, "High-resolution in-depth imaging
501 of optically cleared thick samples using an adaptive spim," *Sci. Reports* **5**, 16898 (2015).
- 502 18. D. Débarre, E. J. Botcherby, T. Watanabe, S. Srinivas, M. J. Booth, and T. Wilson, "Image-based adaptive optics for
503 two-photon microscopy," *Opt. Lett.* **34**, 2495 (2009).
- 504 19. A. Facomprez, E. Beaurepaire, and D. Débarre, "Accuracy of correction in modal sensorless adaptive optics," *Opt.*
505 *Express* **20**, 2598 (2012).
- 506 20. C. Wang, R. Liu, D. E. Milkie, W. Sun, Z. Tan, A. Kerlin, T.-W. Chen, D. S. Kim, and N. Ji, "Multiplexed aberration
507 measurement for deep tissue imaging in vivo," *Nat. Methods* **11**, 1037–1040 (2014).
- 508 21. C. Rodríguez, A. Chen, J. A. Rivera, M. A. Mohr, Y. Liang, R. G. Natan, W. Sun, D. E. Milkie, T. G. Bifano, X. Chen,
509 and N. Ji, "An adaptive optics module for deep tissue multiphoton imaging in vivo," *Nat. Methods* **18**, 1259–1264
510 (2021).
- 511 22. X. Tao, O. Azucena, M. Fu, Y. Zuo, D. C. Chen, and J. Kubby, "Adaptive optics microscopy with direct wavefront
512 sensing using fluorescent protein guide stars," *Opt. Lett.* **36**, 3389 (2011).
- 513 23. A. Hubert, F. Harms, R. Juvénal, P. Treimany, X. Leveq, V. Loriette, G. Farkouh, F. Rouyer, and A. Fragola,
514 "Adaptive optics light-sheet microscopy based on direct wavefront sensing without any guide star," *Opt. Lett.* **44**,
515 2514 (2019).
- 516 24. J. Li, D. R. Beaulieu, H. Paudel, R. Barankov, T. G. Bifano, and J. Mertz, "Conjugate adaptive optics in widefield
517 microscopy with an extended-source wavefront sensor," *Optica* **2**, 682 (2015).
- 518 25. J. Li, T. G. Bifano, and J. Mertz, "Widefield fluorescence microscopy with sensor-based conjugate adaptive optics
519 using oblique back illumination," *J. Biomed. Opt.* **21**, 121504 (2016).
- 520 26. W. Zheng, Y. Wu, P. Winter, R. Fischer, D. D. Nogare, A. Hong, C. McCormick, R. Christensen, W. P. Dempsey, D. B.
521 Arnold, J. Zimmerberg, A. Chitnis, J. Sellers, C. Waterman, and H. Shroff, "Adaptive optics improves multiphoton
522 super-resolution imaging," *Nat. Methods* **14**, 869–872 (2017).
- 523 27. Z. Li, Q. Zhang, S.-W. Chou, Z. Newman, R. Turcotte, R. Natan, Q. Dai, E. Y. Isacoff, and N. Ji, "Fast widefield
524 imaging of neuronal structure and function with optical sectioning in vivo," *Sci. Adv.* **6**, eaz3870 (2020).
- 525 28. E. J. Fernández, L. Vabre, B. Hermann, A. Unterhuber, B. Považay, and W. Drexler, "Adaptive optics with a magnetic
526 deformable mirror: applications in the human eye," *Opt. Express* **14**, 8900–8917 (2006).
- 527 29. N. Anugu, P. J. V. Garcia, and C. M. Correia, "Peak-locking centroid bias in shack–hartmann wavefront sensing,"
528 *Mon. Notices Royal Astron. Soc.* **476**, 300–306 (2018).
- 529 30. M. E. Siemons, N. A. K. Hanemaaijer, M. H. P. Kole, and L. C. Kapitein, "Robust adaptive optics for localization
530 microscopy deep in complex tissue," *Nat. Commun.* **12**, 3407 (2021).

- 531 31. N. P. d. Sert, V. Hurst, A. Ahluwalia, S. Alam, M. T. Avey, M. Baker, W. J. Browne, A. Clark, I. C. Cuthill, U. Dirnagl,
532 M. Emerson, P. Garner, S. T. Holgate, D. W. Howells, N. A. Karp, S. E. Lazic, K. Lidster, C. J. MacCallum,
533 M. Macleod, E. J. Pearl, O. H. Petersen, F. Rawle, P. Reynolds, K. Rooney, E. S. Sena, S. D. Silberberg, T. Steckler,
534 and H. Würbel, “The arrive guidelines 2.0: Updated guidelines for reporting animal research,” *PLOS Biol.* **18**,
535 e3000410 (2020).
- 536 32. T. Pons, S. Bouccara, V. Lorient, N. Lequeux, S. Pezet, and A. Fragola, “In vivo imaging of single tumor cells in
537 fast-flowing bloodstream using near-infrared quantum dots and time-gated imaging,” *ACS Nano* **13**, 3125–3131
538 (2019).
- 539 33. Y. A. Yang, H. Wu, K. R. Williams, and Y. C. Cao, “Synthesis of cdse and cdte nanocrystals without precursor
540 injection,” *Angewandte Chemie Int. Ed.* **44**, 6712–6715 (2005).
- 541 34. N. Lue, J. Bewersdorf, M. D. Lessard, K. Badizadegan, R. R. Dasari, M. S. Feld, and G. Popescu, “Tissue refractometry
542 using hilbert phase microscopy,” *Opt. letters* **32**, 3522–3524 (2007).
- 543 35. D. Kobat, M. E. Durst, N. Nishimura, A. W. Wong, C. B. Schaffer, and C. Xu, “Deep tissue multiphoton microscopy
544 using longer wavelength excitation,” *Opt. Express* **17**, 13354 (2009).
- 545 36. M. Wang, C. Wu, D. Sinefeld, B. Li, F. Xia, and C. Xu, “Comparing the effective attenuation lengths for long
546 wavelength in vivo imaging of the mouse brain,” *Biomed. Opt. Express* **9**, 3534 (2018).
- 547 37. K. Charan, B. Li, M. Wang, C. P. Lin, and C. Xu, “Fiber-based tunable repetition rate source for deep tissue
548 two-photon fluorescence microscopy,” *Biomed. Opt. Express* **9**, 2304 (2018).
- 549 38. P. Theer, M. T. Hasan, and W. Denk, “Two-photon imaging to a depth of 1000 μm in living brains by use of a ti:al2o3
550 regenerative amplifier,” *Opt. Lett.* **28**, 1022–1024 (2003).
- 551 39. W. Akemann, S. Wolf, V. Villette, B. Mathieu, A. Tangara, J. Fodor, C. Ventalon, J.-F. Léger, S. Dieudonné, and
552 L. Bourdieu, “Fast optical recording of neuronal activity by three-dimensional custom-access serial holography,” *Nat.*
553 *Methods* **19**, 100–110 (2022).