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# Non-invasive Retrieval of the Transmission Matrix for Optical Imaging Deep Inside a Multiple Scattering Medium

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1    **Non-invasive Retrieval of the Transmission Matrix for Optical**  
2            **Imaging Deep Inside a Multiple Scattering Medium**

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Abstract

As light travels through a disordered medium such as biological tissues, it undergoes multiple scattering events. This phenomenon is detrimental to in-depth optical microscopy, as it causes a drastic degradation of contrast, resolution and brightness of the resulting image beyond a few scattering mean free paths. However, the information about the inner reflectivity of the sample is not lost; only scrambled. To recover this information, a matrix approach of optical imaging can be fruitful. Here we report on the compressed measurement of a high-dimension reflection matrix  $R$  via low coherence interferometry. Then, we show how the inner transmission matrix linking each camera sensor and each medium voxel can be extracted through an iterative multi-scale analysis of wave distortions contained in  $R$ . This transmission matrix is the Holy Grail for volumetric imaging since it enables an optimal compensation of forward multiple scattering paths and provides a three-dimensional confocal image of the sample as the latter one had become digitally transparent. The proof-of-concept experiment is performed on a human opaque cornea and an extension of the penetration depth by a factor five is demonstrated compared to the state-of-the-art.

7 Multiple scattering of waves concerns many domains of physics, ranging from optics or  
8 acoustics to solid-state physics, seismology, medical imaging, or telecommunications. In an  
9 inhomogeneous medium where the refractive index  $n$  depends on the spatial coordinates  $\mathbf{r}$ ,  
10 several physical parameters are relevant to characterize wave propagation: (i) the scattering  
11 mean free path  $\ell_s$ , which is the average distance between two successive scattering events;  
12 (ii) the transport mean free path  $\ell_t$ , which is the distance after which the wave has lost the  
13 memory of its initial direction. For a penetration depth  $z$  smaller than  $\ell_s$ , ballistic light is  
14 predominant and standard focusing methods can be employed; for  $z > \ell_s$ , multiple scattering  
15 events result in a gradual randomization of the propagation direction before reaching the  
16 diffusive regime for  $z > \ell_t$ . Although it gives rise to fascinating interference phenomena such  
17 as perfect transmission<sup>1,2</sup> or Anderson localization<sup>3,4</sup>, multiple scattering still represents a  
18 major obstacle to deep imaging and focusing of light inside complex media<sup>5,6</sup>.

19 In the past decades, there have been many proposals for the harnessing of waves through  
20 complex media. With the emergence of multi-element technology, wave-fields can be tamed  
21 in order to take advantage of the complexity of propagation media to focus waves through  
22 them or image objects hidden behind them. This has been realized in acoustics using  
23 the concept of the time reversal mirror or in optics using wave-front shaping techniques<sup>7</sup>.  
24 More fundamentally, a matrix formalism is particularly appropriate in wave physics<sup>8,9</sup> when  
25 the wave field can be controlled by transmission<sup>10</sup> and/or reception<sup>11</sup> arrays of independent  
26 elements.

27 While a subsequent amount of work has considered the transmission matrix  $\mathbf{T}$  for op-  
28 timizing wave control and focusing through complex media<sup>12-17</sup>, this configuration is not  
29 the most relevant for imaging purposes since only one side of the medium is accessible  
30 for most *in-vivo* applications. Moreover, in all the aforementioned works, the scattering  
31 medium is usually considered as a black box, while imaging requires to open it. To that  
32 aim, a *reflection matrix approach of wave imaging* (RMI) has been developed for the last  
33 few years<sup>18-21</sup>. The objective is to determine, from the reflection matrix  $\mathbf{R}$ , the  $\mathbf{T}$ -matrix  
34 between sensors outside the medium and voxels mapping the sample<sup>22</sup> (Fig. 1). Previous  
35 works have mainly considered the imaging of highly reflecting structures (*e.g* myelin fibers)  
36 through an aberrating layer (*e.g* mouse skull)<sup>20</sup>, in a wavelength range that limits scattering  
37 and aberration from tissues<sup>23</sup>. On the contrary, here, we want to address the extremely  
38 challenging case<sup>24</sup> of three-dimensional imaging of biological tissues themselves (cells, collagen,

39 extracellular matrix etc.) at large penetration depth ( $z \sim 5\ell_s$ ), regime in which aberration  
40 and scattering effects are spatially-distributed over multiple length-scales.

41 In previous studies, coherent measurements of  $\mathbf{R}$  have been performed either in a plane  
42 wave basis<sup>25</sup>, point-to-point basis<sup>20</sup>, or between those dual bases<sup>26,27</sup>. In this paper, a  
43 low coherence interferometry set up, derived from full-field optical coherence tomography  
44 (FFOCT)<sup>28,29</sup>, is used to record the  $\mathbf{R}$ -matrix in a de-scanned basis. Interestingly, this frame  
45 drastically reduces the number of input wave-fronts required for a full measurement of  $\mathbf{R}$ .

46 Another advantage of this basis is the direct access to the distortion matrix  $\mathbf{D}$  through  
47 a Fourier transform. This matrix basically connects any focusing point with the distorted  
48 part of the associated reflected wavefront<sup>19,21</sup>. A multi-scale analysis of  $\mathbf{D}$  is here proposed  
49 to estimate the forward scattering component of the  $\mathbf{T}$ -matrix at an unprecedented spatial  
50 resolution ( $\sim 6 \mu\text{m}$ ). Once the latter matrix is known, one can actually unscramble, in  
51 post-processing, all wave distortions and multiple scattering events undergone by the incident  
52 and reflected waves for each voxel. A three-dimensional confocal image of the medium can  
53 then be retrieved as if the medium had been made digitally transparent.

54 The experimental proof-of-concept presented in this paper is performed on a human  
55 *ex-vivo* cornea that we chose deliberately to be extremely opaque. Its overall thickness is of 5  
56  $\ell_s$ . FFOCT shows an imaging depth limit of  $1\ell_s$  due to aberration and scattering. Strikingly,  
57 RMI enables to recover a full 3D image of the cornea at a resolution close to  $\lambda/4$  ( $\sim 230\text{nm}$ )  
58 and a penetration depth enhanced by, at least, a factor five.

59

## 60 **Focused Reflection Matrix in De-Scanned Basis**

61

62 The  $\mathbf{R}$ -matrix is generally defined as containing responses between a recording basis  
63 (camera pixels) and an illumination basis, that corresponds to the set of incident waves  
64 used to probe the sample (Fig. 1a). Once recorded,  $\mathbf{R}$  can be easily projected by simple  
65 matrix products in: (i) the pupil plane ( $\mathbf{u}_{\text{in/out}}$ ) where input-output correlations can be  
66 exploited for full-field aberration compensation<sup>20,30</sup>; (ii) the focused basis ( $\boldsymbol{\rho}_{\text{in/out}}$ ) where  
67 an image of the sample can be formed. Compared to confocal imaging where focusing is  
68 performed at input and output on the same point ( $\boldsymbol{\rho}_{\text{in}} = \boldsymbol{\rho}_{\text{out}}$ ), RMI enables the decoupling  
69 of input and output focal spots. Interestingly, the off-diagonal elements of the focused  
70  $\mathbf{R}$ -matrix,  $\mathbf{R}_{\boldsymbol{\rho}\boldsymbol{\rho}} = [R(\boldsymbol{\rho}_{\text{in}}, \boldsymbol{\rho}_{\text{out}})]$ , enable to probe locally the impact of aberrations and



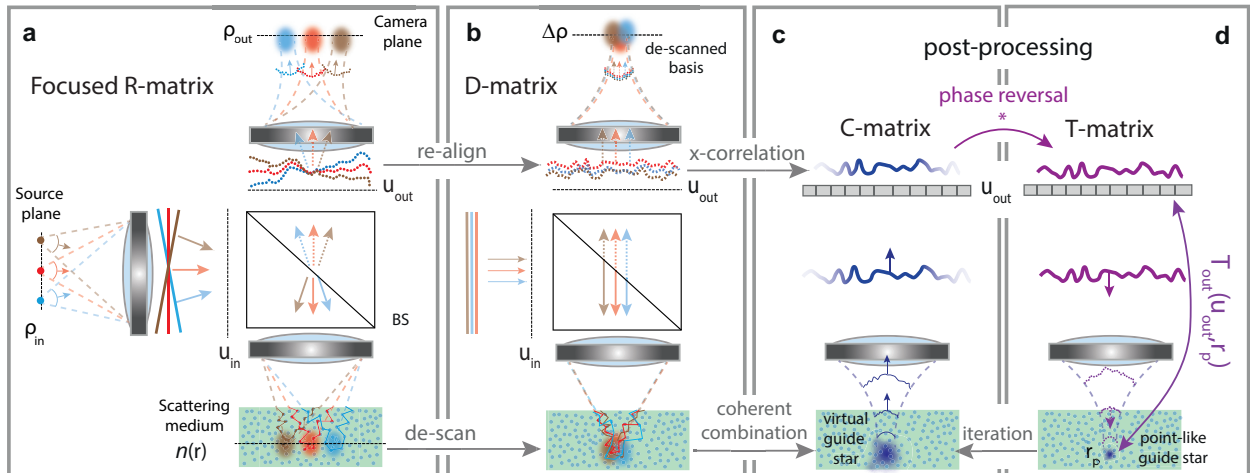


FIG. 1. **Principle of Matrix Imaging.** **a.** A set of focused wave-fronts is used to illuminate the sample. They can be generated by an array of point-like sources  $\rho_{\text{in}}$  conjugated with the focal plane of the microscope objective or by a set of plane waves in the pupil plane ( $\mathbf{u}_{\text{in}}$ ). The reflected wave-fronts are recorded by the pixels  $\rho_{\text{out}}$  of a camera also conjugated with the focal plane. The set of impulse responses  $R(\rho_{\text{in}}, \rho_{\text{out}})$  between each source  $\rho_{\text{in}}$  and detector  $\rho_{\text{out}}$  form the focused reflection matrix  $\mathbf{R}_{\rho\rho} = [R(\rho_{\text{in}}, \rho_{\text{out}})]$ . **b.** The reflected wave-fields can be de-scanned as if they were generated by virtual sources all shifted at the origin thereby forming the de-scanned matrix  $\mathbf{D}_{\Delta\rho}$  (Eq. 2). In the output pupil plane ( $\mathbf{u}_{\text{out}}$ ), this operation is equivalent to a realignment of wave-fronts, thereby forming the so-called distortion matrix  $\mathbf{D}_{\mathbf{u}\mathbf{r}}$ . **c.** The correlation matrix  $\mathbf{C}_{\text{out}}$  between those wave-fronts mimics the time-reversal operator associated with a virtual guide star that results from a coherent average of all the de-scanned focal spots (see Supplementary Section S4). **d.** An iterative phase reversal algorithm is then applied (see Methods). It first consists in a phase conjugation of the wave-front induced by the virtual guide star. The impinging wave-front compensates for aberrations and scattering inside the medium to produce a sharper guide star. The process is iterated and ultimately provides an estimation of  $\mathbf{T}_{\text{out}}(\mathbf{r}_p)$ , the column of  $\mathbf{T}_{\text{out}}$  corresponding to the common mid-point  $\mathbf{r}_p$  of the input focal spots considered in panel **a**.

71 multiple scattering.

72 In that perspective, a de-scan operation is of particular interest (Fig. 1b). Mathematically,  
 73 it only consists in the following change of coordinates:  $(\rho_{\text{in}}, \rho_{\text{out}}) \rightarrow (\rho_{\text{in}} - \rho_{\text{out}}, \rho_{\text{out}})$ . The

74 interest of a de-scanned reflection matrix  $\mathbf{D}$  is twofold. First, it constitutes a flexible starting  
75 block for a local compensation of wave distortions through a simple change of basis. Second,  
76 the dimension of  $\mathbf{D}$  is much smaller than the size of  $\mathbf{R}$ . In the following, we show how low  
77 coherence interferometry enables a compressed measurement of  $\mathbf{R}$ <sup>31,32</sup>.

79

## 80 Compressed Sensing of the Reflection Matrix

81

82 Inspired by FFOCT<sup>28</sup>, the experimental set up consists in a Linnik interferometer (Fig. 2a).  
83 In the first arm, a reference mirror is placed in the focal plane of a microscope objective  
84 (MO). The second arm contains the scattering sample to be imaged. In FFOCT, the same  
85 broadband incoherent light source is used to illuminate the entire field of the microscope  
86 objectives. Because of the broad spectrum of the incident light, interferences occur between  
87 the two arms provided that the optical path difference through the interferometer is close to  
88 zero. The length of the reference arm determines the slice of the sample (coherence volume)  
89 to be imaged and is adjusted in order to match with the focal plane of the MO in the sample  
90 arm. The backscattered light from each voxel of the coherence volume can only interfere with  
91 the light coming from the conjugated point of a reference mirror. The spatial incoherence of  
92 the light source actually acts as a physical confocal pinhole (Fig. 2c). All these interference  
93 signals are recorded in parallel by the pixels of the camera in the imaging plane. The FFOCT  
94 signal is thus equivalent to a time-gated confocal image of the sample but without any  
95 lateral raster scanning<sup>33</sup>. It yields the diagonal coefficients,  $R(\boldsymbol{\rho}_{\text{out}}, \boldsymbol{\rho}_{\text{out}}, z)$ , of  $\mathbf{R}_{\boldsymbol{\rho}\boldsymbol{\rho}}(z)$ , where  
96  $z$  denotes the depth of the coherence volume. Figures 3b and c show en-face and axial  
97 FFOCT images of the opaque cornea at different depths. A dramatic loss in contrast is  
98 found beyond the epithelium ( $z > 70 \mu\text{m}$ , see Fig. 3g). It highlights the detrimental effect of  
100 multiple scattering for deep optical imaging.

101 To overcome the multiple scattering phenomenon, the illumination scheme is slightly  
102 modified compared with FFOCT (Fig. 2a). The incident wave-fields are still identical in  
103 each arm but are laterally shifted with respect to each other by a transverse position  $\Delta\boldsymbol{\rho}$ .  
104 Their spatial incoherence now acts as a de-scanned pinhole that gives access to the cross-  
105 talk between distinct focusing points (Fig. 2b). The interferogram recorded by the camera

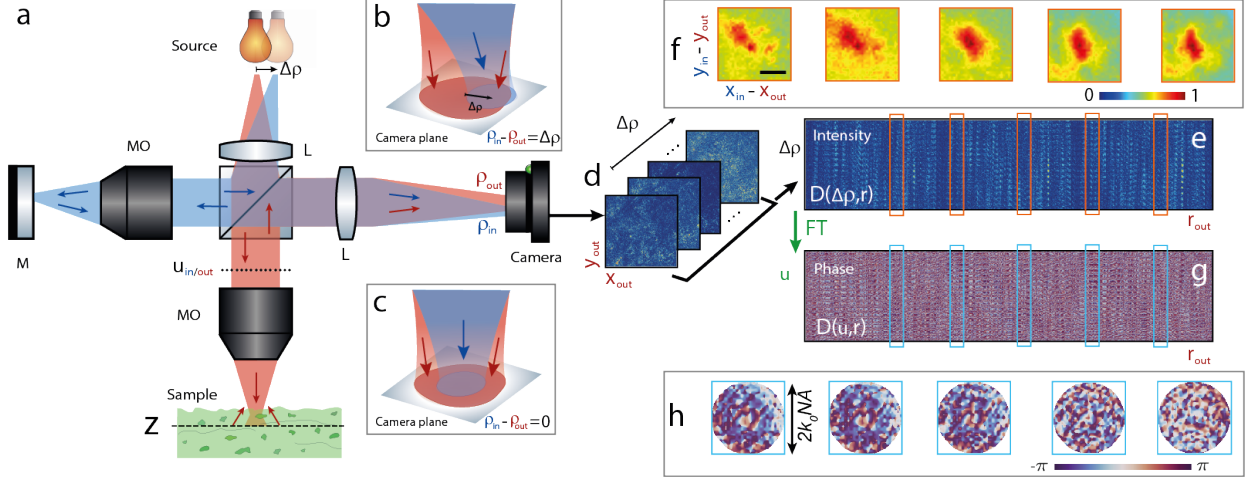


FIG. 2. **Compressed Sensing of the Reflection Matrix.** **a** Experimental setup (L: lenses, MO: microscope objectives, and M: reference mirror.). Light from an incoherent source is split into two replica laterally shifted with respect to each other by a relative position  $\Delta\boldsymbol{\rho}$  (see Supplementary Section S1). By a game of polarization, each replica illuminates one arm of a Linnik interferometer. The sample beam (in red) illuminates the scattering sample through a microscope objective ( $NA = 1.0$ ). The reference beam (in blue) is focused on a dielectric mirror through an identical microscope objective. Both reflected beams interfere on a CMOS camera whose surface is conjugated with focal planes of the MO. The amplitude and phase of the interference term are retrieved by phase-shifting interferometry. **b** Each pixel of the camera, depicted by its position  $\boldsymbol{\rho}_{\text{out}}$ , measures the reflection coefficient  $R(\boldsymbol{\rho}_{\text{in}}, \boldsymbol{\rho}_{\text{out}}, z)$  between de-scanned focusing points,  $\mathbf{r}_{\text{out}} = (\boldsymbol{\rho}_{\text{out}}, z)$  and  $\mathbf{r}_{\text{in}} = (\boldsymbol{\rho}_{\text{out}} + \Delta\boldsymbol{\rho}, z)$ , at depth  $z$  of the sample. **c** For  $\Delta\boldsymbol{\rho} = \mathbf{0}$ , the experimental set up is equivalent to a FFOCT apparatus and the interferogram directly provides a time-gated confocal image of the sample. **d** The set of interferograms are stored in the de-scanned reflection matrix  $\mathbf{D}_{\Delta\boldsymbol{\rho}}(z) = [D(\Delta\boldsymbol{\rho}, \boldsymbol{\rho}_{\text{out}}, z)]$  displayed in panel **e**. **f** Each column of this matrix yields a reflection point-spread function (RPSF) associated with the focusing quality at point  $\boldsymbol{\rho}_{\text{out}}$  (scale bar:  $2 \mu\text{m}$ ). **g** The Fourier transform of each de-scanned wave-field provides the distortion matrix  $\mathbf{D}_{\mathbf{u}\mathbf{r}}(z) = [D(\mathbf{u}_{\text{in}}, \boldsymbol{\rho}_{\text{out}}, z)]$ . **h** Each column of this matrix displays the distorted wave-front associated with each point  $\boldsymbol{\rho}_{\text{out}}$  in the field-of-view. The optical data shown in panels d-h correspond to the acquisition performed at depth  $z = 150 \mu\text{m}$ .

106 (Fig. 2d) directly provides one line of the de-scanned matrix  $\mathbf{D}$ , such that

$$D(\Delta\boldsymbol{\rho}, \boldsymbol{\rho}_{\text{out}}, z) = R(\boldsymbol{\rho}_{\text{in}} + \Delta\boldsymbol{\rho}, \boldsymbol{\rho}_{\text{out}}, z). \quad (1)$$

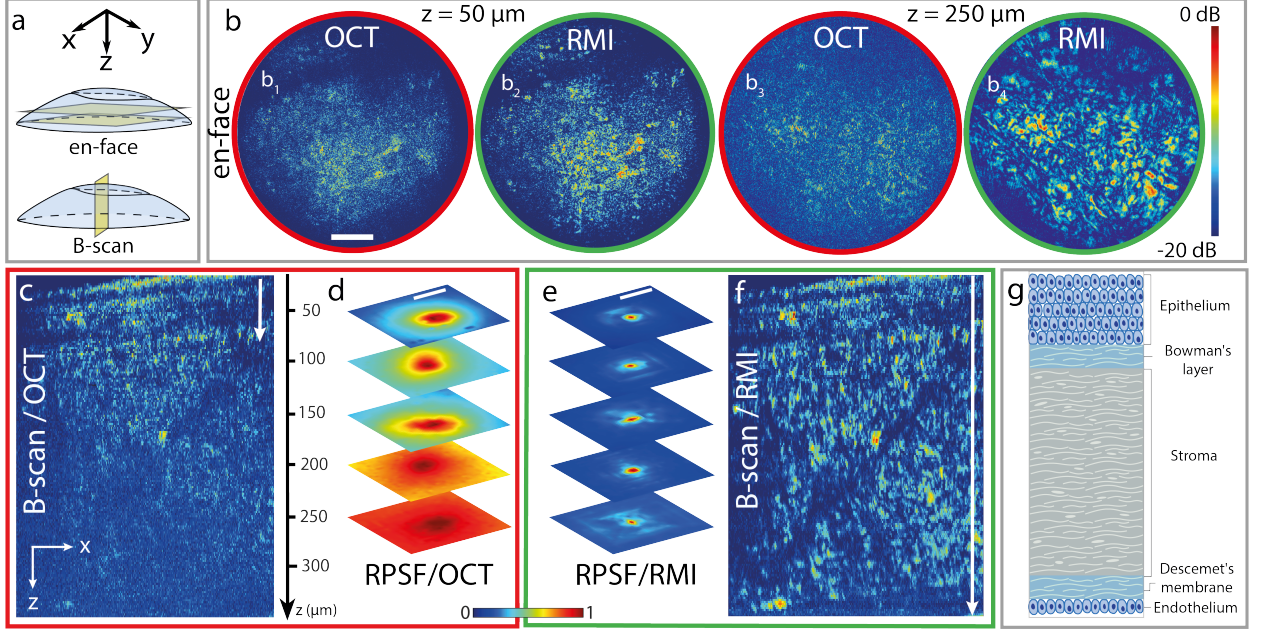


FIG. 3. **Volumetric matrix imaging of an opaque cornea.** **a.** Schematic of the imaging planes in the cornea. **b.** *En-face* confocal images before ( $\mathbf{b}_1, \mathbf{b}_3$ ) and after ( $\mathbf{b}_2, \mathbf{b}_4$ ) the matrix imaging process for  $z = 50 \mu\text{m}$  and  $250 \mu\text{m}$ , respectively (scale bar:  $50 \mu\text{m}$ ). **c.** Longitudinal ( $x, z$ ) section of the initial confocal image. **d.** Original RPSFs from  $z = 50$  to  $250 \mu\text{m}$  (scale bar:  $1 \mu\text{m}$ ). **e.** Corresponding RPSFs after the matrix imaging process. **f.** Longitudinal ( $x, z$ ) section of the volumetric image at the end of the matrix imaging process. **g.** Schematic of a healthy human cornea.

107 Scanning the relative position  $\Delta\boldsymbol{\rho}$  is equivalent to recording the  $\mathbf{R}$ -matrix diagonal-by-diagonal  
 108 when expressed in a canonical basis (Fig. 1a). However, while a canonical (column-by-column)  
 109 acquisition of  $\mathbf{R}$  requires to illuminate the sample over a field-of-view  $\Omega$  with  $N = (\Omega/\delta_0)^2$   
 110 input wave-fronts, the de-scanned basis implies a much smaller number of illuminations.

111 This sparsity can be understood by expressing theoretically the  $\mathbf{D}$ -matrix (Supplementary  
 112 Section S2):

$$D(\Delta\boldsymbol{\rho}, \boldsymbol{\rho}_{\text{out}}, z) = \int_{\Omega} d\boldsymbol{\rho} H_{\text{in}}(\boldsymbol{\rho} + \Delta\boldsymbol{\rho}, \mathbf{r}_{\text{in}}) \gamma(\boldsymbol{\rho} + \boldsymbol{\rho}_{\text{out}}, z) H_{\text{out}}(\boldsymbol{\rho}, \mathbf{r}_{\text{out}}) \quad (2)$$

113 where  $\gamma$  is the sample reflectivity.  $H_{\text{in}}(\boldsymbol{\rho}, \mathbf{r}_{\text{in}})$  and  $H_{\text{out}}(\boldsymbol{\rho}, \mathbf{r}_{\text{out}})$  are the local input and output  
 114 point spread functions (PSFs) at points  $\mathbf{r}_{\text{in}} = (\boldsymbol{\rho}_{\text{in}}, z)$  and  $\mathbf{r}_{\text{out}} = (\boldsymbol{\rho}_{\text{out}}, z)$ , respectively. This  
 115 last equation confirms that the central line of  $\mathbf{D}_{\Delta\boldsymbol{\rho}}$  ( $\Delta\boldsymbol{\rho} = \mathbf{0}$ ), i.e. the FFOCT image, results  
 116 from a convolution between the sample reflectivity  $\gamma$  and the local confocal PSF,  $H_{\text{in}} \times H_{\text{out}}$ .

117 The de-scanned elements allow us to go far beyond standard imaging. In particular, they

118 will be exploited to unscramble the local input and output PSFs in the vicinity of each focal  
 119 point. As a preliminary step, they can also be used to quantify the level of aberrations and  
 120 multiple scattering. In average, the de-scanned intensity,  $I(\Delta\boldsymbol{\rho}, \mathbf{r}_{\text{out}}) = |D(\Delta\boldsymbol{\rho}, \boldsymbol{\rho}_{\text{out}}, z)|^2$ , can  
 121 actually be expressed as the convolution between the incoherent input and output PSFs<sup>34</sup>:

$$\langle I(\Delta\boldsymbol{\rho}, \mathbf{r}_{\text{out}}) \rangle \propto |H_{\text{in}}|^2 \overset{\Delta\boldsymbol{\rho}}{\otimes} |H_{\text{out}}|^2(\Delta\boldsymbol{\rho}, \mathbf{r}_{\text{out}}) \quad (3)$$

122 where the symbol  $\otimes$  stands for correlation product and  $\langle \dots \rangle$  for ensemble average. This  
 123 quantity will be referred to as RPSF in the following (acronym for reflection PSF). Figure 2e  
 124 displays examples of RSPF extracted in depth of the opaque cornea. Their spatial extension  
 125  $\delta_R$  of the RPSF indicates the focusing quality and dictates the number  $M$  of central lines of  
 126  $\mathbf{D}_{\Delta\boldsymbol{\rho}}(z)$  that contain the relevant information for imaging:

$$M \sim (\delta_R/\delta_0)^2 \quad (4)$$

127 with  $\delta_0 \sim \lambda/(4NA)$ , the confocal maximal resolution of the imaging system. For a field-of-  
 128 view much larger than the spatial extension of the RPSF ( $\Omega \gg \delta_R$ ), the de-scanned basis is  
 129 thus particularly relevant for the acquisition of  $\mathbf{R}$  ( $M \ll N$ ).

130

## 131 Quantifying the Focusing Quality

132

133 Figure 3d shows the depth evolution of the RPSF. It exhibits the following characteristic  
 134 shape: a distorted and enlarged confocal spot on top of an incoherent background<sup>34</sup>. The  
 135 former component is a manifestation of aberrations; the latter contribution is due to multiple  
 136 scattering. Figure 3d clearly highlights two regimes. In the epithelium ( $z < 70 \mu\text{m}$ ), the  
 137 confocal component is predominant and the image of the cornea is reliable although its  
 138 resolution is affected by aberrations (Fig. 3b<sub>1</sub>). Beyond this depth, the multiple scattering  
 139 background is predominant and drastically blurs the image (Fig. 3b<sub>3</sub>). The axial evolution  
 140 of the confocal-to-multiple scattering ratio enables the measurement of the scattering mean  
 141 free path  $\ell_s$ <sup>35</sup> (Supplementary Section S3). We find  $\ell_s \sim 70 \mu\text{m}$  in the stroma (Fig. 3g),  
 142 which confirms the strong opacity of the cornea. The penetration depth limit thus scales as  
 143  $\ell_S$ . This value is modest compared with theoretical predictions<sup>24</sup> ( $\sim 3\ell_S$ ) but is explained by  
 144 the occurrence of strong aberrations at shallow depths, partially due to the index mismatch  
 145 at the cornea surface (Fig. 3d).

146

147 The RSPF also fluctuates in the transverse direction. To that aim, a map of local RPSFs  
 148 (Fig. 4c) can be built by considering the back-scattered intensity over limited spatial windows  
 149 (Methods). This map shows important fluctuations of aberrations and multiple scattering  
 150 across the field-of-view due to the lateral variations of the optical index upstream of the  
 151 focal plane. Such complexity implies that any point in the medium will be associated with  
 152 its own distinct focusing law. Nevertheless, spatial correlations subsist between RSPFs in  
 153 adjacent windows (Fig. 4c). Such correlations can be explained by a physical phenomenon  
 154 often referred to as isoplanatism in adaptive optics<sup>36</sup> and that results in a locally-invariant  
 155 PSF<sup>37</sup>. We will now see how this local isoplanicity can be exploited for the estimation of the  
 156 **T**-matrices.

158

## 159 Iterative Phase Reversal of Wave Distortions

160

161 Beyond a direct quantification of aberration and scattering problems, the **D**-matrix can  
 162 be leveraged for their compensation. Indeed, a Fourier transform over the coordinate  $\Delta\rho$  of  
 163 each de-scanned wave-field,  $D(\Delta\rho, \mathbf{r}_{\text{out}})$ , directly yields the wave distortions seen from the  
 164 input pupil plane :

$$\mathbf{D}_{\text{ur}} = \mathbf{F} \times \mathbf{D}_{\Delta\mathbf{r}} \quad (5)$$

165 where **F** denotes the Fourier transform operator,  $F(\mathbf{u}, \Delta\rho) = \exp(-i2\pi\mathbf{u}\cdot\Delta\rho/\lambda f)$ ,  $\lambda$  the  
 166 central wavelength and  $f$  the MO focal length.  $\mathbf{D}_{\text{ur}} = \mathbf{D}_{\mathbf{u}\rho}(z) = [D(\mathbf{u}_{\text{in}}, \rho_{\text{out}}, z)]$  is the  
 167 distortion matrix that connects any voxel ( $\mathbf{r}_{\text{out}}$ ) in the field-of-view to wave-distortions in  
 168 the input pupil plane ( $\mathbf{u}_{\text{in}}$ ).

169 As the RPSF displayed in Fig. 4c, this matrix exhibits local correlations that can be  
 170 also understood in light of the optical memory effect<sup>38,39</sup>. Waves produced by nearby points  
 171 inside a complex medium generate highly correlated random speckle patterns in the far  
 172 field<sup>37,40,41</sup>. Figure 2 illustrates this fact by displaying an example of distortion matrix  
 173 (Fig. 2g) and reshaped distorted wave-fields for different points  $\mathbf{r}_{\text{out}}$  (Fig. 2h). A strong  
 174 similarity can be observed between distorted wave-fronts associated with neighboring points  
 175 but this correlation tends to vanish when the two points are too far away.

176 The next step is to extract and exploit the local memory effect in **D** for imaging. To that  
 177 aim, a set of correlation matrices  $\mathbf{C}_{\text{in}}(\mathbf{r}_{\text{p}})$  shall be considered between distorted wave-fronts  
 178 in the vicinity of each point  $\mathbf{r}_{\text{p}}$  in the field-of-view (Methods). Under the hypothesis of



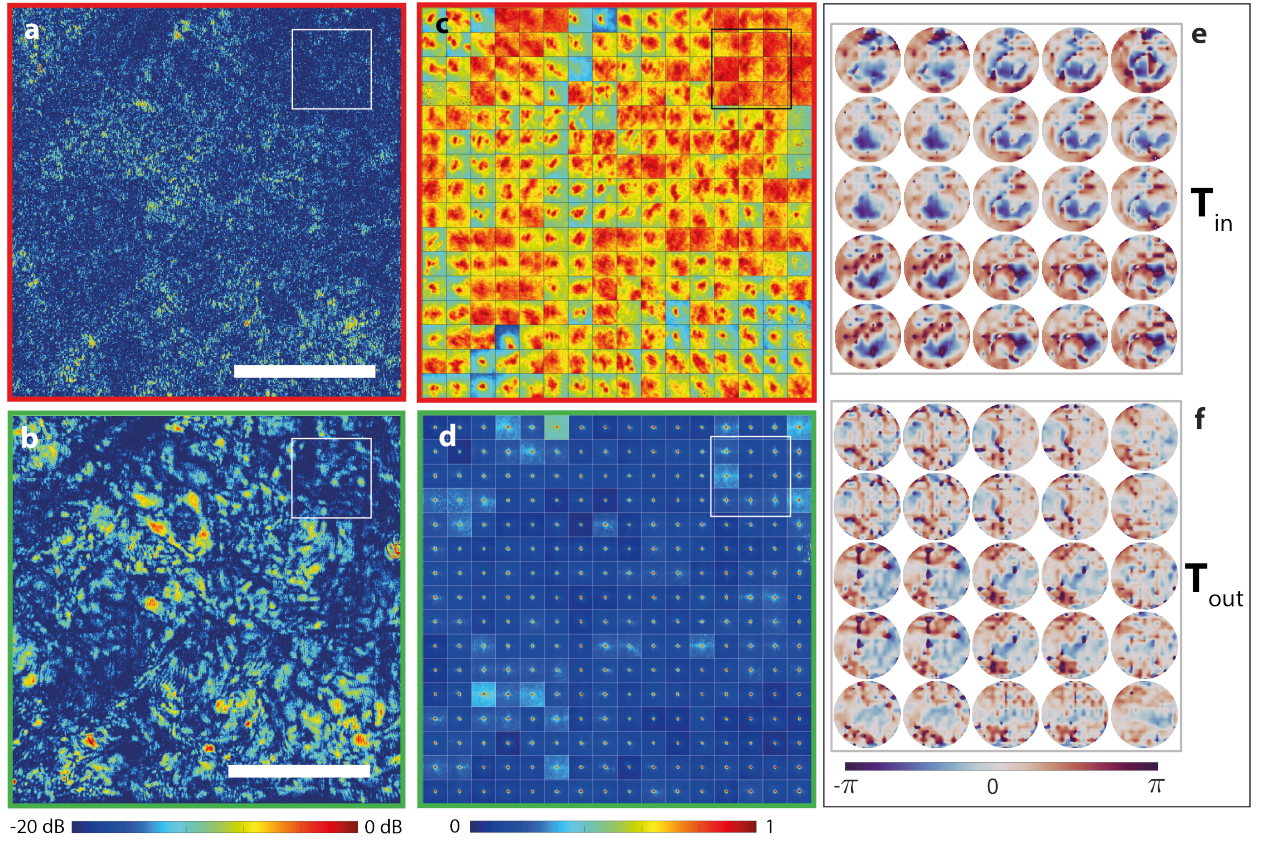


FIG. 4. **Inner Transmission matrix for Local Compensation of Forward Multiple Scattering.** **a,b.** Confocal field of view before and after the correction process at 200  $\mu\text{m}$ -depth, respectively (scale bar: 50  $\mu\text{m}$ ). **c,d.** Maps of the local reflection point-spread functions (RPSFs) (de-scan field-of-view:  $7 \times 7 \mu\text{m}^2$ ) over the field of view, before and after the correction process, respectively. **e,f.** Sub-part of matrices,  $\hat{\mathbf{T}}_{\text{in}}$  and  $\hat{\mathbf{T}}_{\text{out}}$ , respectively, for the area delimited by the square box in panels **a-d**.

179 local isoplanicity, each matrix  $\mathbf{C}_{\text{in}}(\mathbf{r}_p)$  is analogous to a  $\mathbf{R}$ -matrix associated with a virtual  
 180 reflector synthesized from the set of output focal spots<sup>21</sup> (see Fig. 1c and Supplementary  
 181 Section S4). In this fictitious experimental configuration, an iterative phase-reversal process  
 182 can be performed to converge towards the incident wave front that focuses perfectly through  
 183 the heterogeneities of the medium onto this virtual scatterer (see Fig. 1d and Methods).

184 This iterative phase reversal algorithm, repeated for each point  $\mathbf{r}_p$ , yields an estimator  
 185  $\hat{\mathbf{T}}_{\text{in}}$  of the transmission matrix,  $\mathbf{T}_{\text{in}} = \mathbf{F} \times \mathbf{H}_{\text{in}}$ . Its digital phase conjugation enables a local  
 186 compensation of aberration and multiple scattering. An updated de-scanned matrix can then

187 be built:

$$\mathbf{D}_{\Delta r} = \mathbf{F}^\dagger \times \left[ \hat{\mathbf{T}}_{\text{in}}^* \circ \mathbf{D}_{ur} \right] \quad (6)$$

188 where the symbol  $\dagger$  stands for transpose conjugate and  $\circ$  for the Hadamard product. The  
189 same process can be repeated by exchanging input and output to estimate the output  
190 transmission matrix  $\mathbf{T}_{\text{out}}$  (Methods).

191

## 192 **Multi-Scale analysis of the Distortion Matrix**

193

194 A critical aspect of RMI is the choice of the spatial window over which wave distortions shall  
195 be analyzed. On the one hand, the isoplanatic assumption is valid for low-order aberrations  
196 that are associated with extended isoplanatic patches. Forward multiple scattering, on  
197 the other hand, gives rise to high-order aberration that exhibits a coherence length that  
198 decreases with depth until reaching the size of a speckle grain beyond  $\ell_t^{37}$ . However, the  
199 spatial windows should be large enough to encompass a sufficient number of independent  
200 realizations of disorder<sup>42</sup>. Indeed, this number should be one order of magnitude larger than  
201 the size  $M$  of the aberrated focal spot in terms of resolution cells. If the latter condition is  
202 not fulfilled, the iterative phase reversal process indeed cannot converge towards a correct  
203 estimator (Supplementary Section S5).

204 To satisfy these two contradictory conditions, an iterative multi-scale strategy is proposed  
205 for the analysis of the  $\mathbf{D}$ -matrix. It consists in iterating the RMI process while dividing by  
206 two the size of overlapping spatial windows at each iterative step (Fig. a). At each iteration,  
207 the RPSF extension decreases (Fig. b) and the spatial window can be reduced accordingly at  
208 the next step. It enables the capture of finer angular and spatial details of the  $\mathbf{T}$ -matrix at  
209 each step ( Fig. c) while ensuring the convergence of the iterative phase reversal algorithm.  
210 At the end of the process (Supplementary Section S5), each individual patch covers an area  
211 of  $6 \times 6 \mu\text{m}^2$  which provides the spatial resolution of the  $\mathbf{T}$ -matrix estimator.

212

## 213 **Transmission Matrix and Memory Effect**

214

215 Figures 4e and f show a sub-part of the  $\mathbf{T}$ -matrices measured at depth  $z = 200 \mu\text{m}$ . Spatial  
216 reciprocity should imply equivalent input and output aberration phase laws. This property is  
217 not checked by our estimators. Indeed, the input aberration phase law accumulates not only



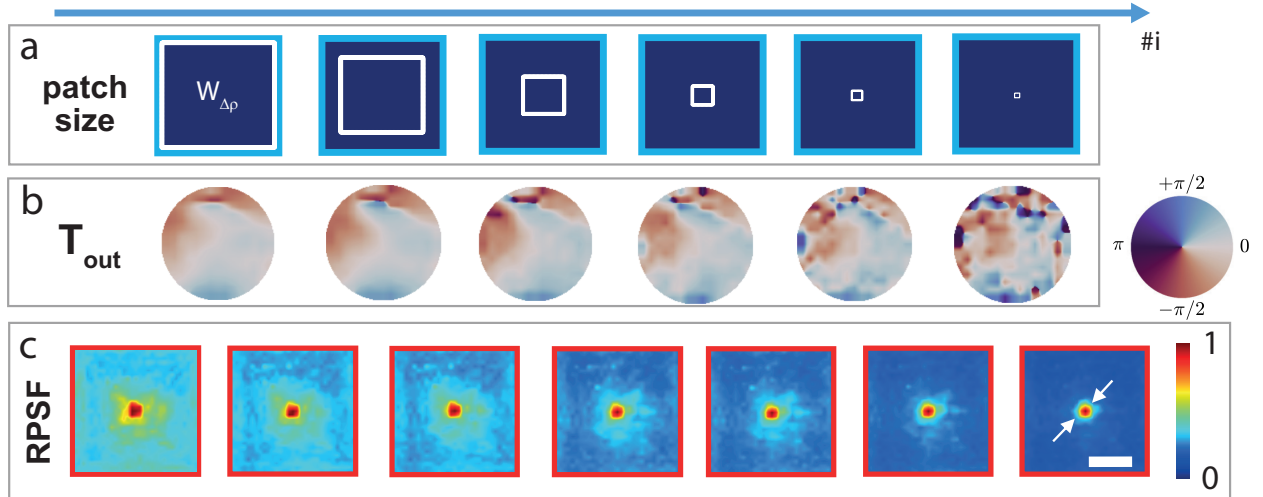


FIG. 5. **Multi-scale analysis of wave distortions.** **a.** The entire field-of-view is  $138 \times 138 \mu\text{m}^2$ . At each step, it is divided into a set of spatial windows whose dimension gradually decreases: from 138, 100, 50, 25, 13 to  $6 \mu\text{m}$  **b.** Evolution of the transmitted wave-front  $\hat{T}(\mathbf{u}_{\text{out}}, \mathbf{r}_p)$  for one point  $\mathbf{r}_p$  of the field-of-view at each iteration step. **c.** Corresponding local RPSF at  $\mathbf{r}_p$  before and after compensation of aberration and scattering using digital phase-conjugation of the optical transfer function displayed in panel **b** (scale bar:  $2 \mu\text{m}$ ). Data are from the cross-section at  $200 \mu\text{m}$  depth within the sample.

218 the input aberrations of the sample-arm but also those of the reference arm (Supplementary  
 219 Section S2). Therefore, the sample-induced aberrations can be investigated independently  
 220 from the imperfections of the experimental set up by considering the output matrix  $\hat{\mathbf{T}}_{\text{out}}$ .

221 An analysis of its spatial correlations clearly shows that wave distortions induced by the  
 222 cornea are made of two contributions (Methods): *(i)* a spatially-invariant aberrated component  
 223 (Fig. 6a) associated with long-scale fluctuations of the refractive index (Fig. 6c) ; *(ii)* a  
 224 forward multiple scattering component (Fig. 6d) associated with isoplanatic patches whose  
 225 size drastically decreases in depth (Fig. 6a,e).

226

## 228 Deep Volumetric Imaging

229

230 Eventually, the estimated  $\mathbf{T}$ -matrices can be used to compensate for local aberrations over  
 231 the whole field-of-view, by digital phase conjugation performed at input and output (Eq. 6).  
 232 The comparison between the initial and resulting images (Figs. 4a,b) demonstrates the benefit

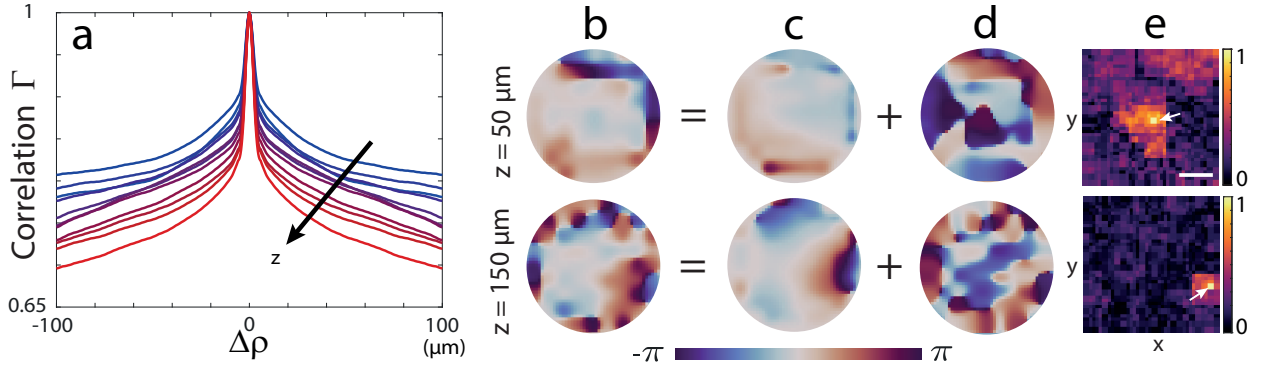


FIG. 6. Revealing the memory effect exhibited by the  $\mathbf{T}$ -matrix. **a.** Transverse evolution of the mean correlation function of the transmitted wave-field from shallow (blue) to large (red) depths. **b.** The phase of each transmitted wave-field is the sum of: **c.** a spatially-invariant aberration phase function; **d.** a complex scattering law exhibiting high spatial frequencies. **e.** The spatial correlation of the latter component with the  $\mathbf{T}$ -matrix provides a map of the corresponding isoplanatic patch (scale bar:  $50\mu\text{m}$ ).

233 of a local compensation of aberration and scattering. The drastic gain in resolution and  
 234 contrast provided by RMI enables to reveal a rich arrangement of biological structures (cells,  
 235 striae, *etc.*) that were completely blurred by scattering in the initial image. For instance,  
 236 a stromal stria, indicator of keratoconus<sup>43</sup>, is clearly revealed on the RMI B-scan (Fig. 3f)  
 237 while it was hidden by the multiple scattering fog on the initial image (Fig. 3c). The B-scan  
 238 shows that RMI provides a full image of the cornea with the recovery of its different layers  
 239 throughout its thickness ( $350\ \mu\text{m} \sim 5\ell_s$ , see also Supplementary Movies).

240 The gain in contrast and resolution can be quantified by investigating the RSPF after  
 241 RMI. A close-to-ideal confocal resolution ( $230\ \text{nm}$  vs.  $\delta_0 \sim 215\ \text{nm}$ ) is reached throughout  
 242 the cornea thickness (Fig. 3e). The confocal-to-diffuse ratio is increased by a factor up to 15  
 243 dB in depth (Supplementary Section S6). Furthermore, the map of local RPSFs displayed in  
 244 Fig. 4d shows the efficiency of RMI for addressing extremely small isoplanatic patches.

245

## 246 Discussion

247

248 Although this experimental proof-of-concept is spectacular and promising for deep optical  
 249 imaging of biological tissues, it suffers from several limitations that need to be addressed in

250 future works. First, FFOCT is not very convenient for 3D in-vivo imaging since it requires  
251 an axial scan of the sample. Another possibility would be to move the reference arm and  
252 measure  $\mathbf{R}$  as a function of the time-of-flight.

253 An access to the time (or spectral) dependence of the  $\mathbf{R}$ -matrix is actually critical to  
254 reach a penetration depth larger than  $\ell_t$ . Indeed, the aberration phase law extracted from  
255 a time-gated  $\mathbf{R}$ -matrix is equivalent in the time domain to a simple application of time  
256 delays between each angular component of the wave-field. Yet, the diffusive regime requires  
257 to address independently each frequency component of the wave-field to make multiple  
258 scattering paths of different lengths constructively interfere on any focusing point in depth.

259 Beyond the diffusive regime, another blind spot of this study is the medium movement  
260 during the experiment<sup>44,45</sup>. In that respect, the matrix formalism shall be developed to  
261 include the medium dynamics. Moving speckle can actually be an opportunity since it  
262 can give access to a large number of speckle realizations for each voxel. A high resolution  
263  $\mathbf{T}$ -matrix could be, in principle, extracted without relying on any isoplanatic assumption<sup>46</sup>.

264 To conclude, this study is a striking illustration of a pluri-disciplinary approach in wave  
265 physics. A passive measurement of the  $\mathbf{R}$ -matrix is indeed an original idea coming from  
266 seismology<sup>47</sup>. The  $\mathbf{D}$ -matrix is inspired by stellar speckle interferometry in astronomy<sup>48</sup>. The  
267  $\mathbf{T}$ -matrix is a concept that has emerged both from fundamental studies in condensed matter  
268 physics<sup>8</sup> and more applied fields such as MIMO communications<sup>10</sup> and ultrasound therapy<sup>12</sup>.  
269 The emergence of high-speed cameras and the rapid growth of computational capabilities  
270 now makes matrix imaging mature for deep in-vivo optical microscopy.

## 271 **Methods**

272

### 273 **Experimental set up**

274 The full experimental setup is displayed in Supplementary Figure S1. It is made of two  
275 parts: (i) a polarized Michelson interferometer illuminated by a broadband LED source  
276 (Thorlabs M850LP1,  $\lambda_o = 850$  nm,  $\Delta\lambda = 35$  nm) in a pseudo-Kohler configuration, thereby  
277 providing at its output two identical spatially-incoherent and broadband wave-fields of  
278 orthogonal polarization, the reference one being shifted by a lateral position  $\Delta\boldsymbol{\rho}$  by tilting  
279 the mirror in the corresponding arm; (ii) a polarized Linnik interferometer with microscope  
280 objectives (Nikon N60X-NIR,  $M = 60\times$ ,  $NA = 1.0$ ) in the two arms and a CMOS camera  
281 (Adimec Quartz 2A-750, 2Mpx) at its output. The de-scanned beam at the output the first  
282 interferometer illuminates the reference arm of the second interferometer and is reflected by  
283 the reference mirror placed in the focal plane of the MO. The other beam at the output of  
284 the first interferometer illuminates the sample placed in the focal plane of the other MO.  
285 The CMOS camera, conjugated with the focal planes of the MO, records the interferogram  
286 between the beams reflected by each arm of the Linnik interferometer. The spatial sampling  
287 of each recorded image is  $\delta_0 = 230$  nm and the field-of-view is  $275\times 275 \mu\text{m}^2$

### 288 **Experimental procedure**

289 The experiment consists in the acquisition of the de-scanned reflection matrix  $\mathbf{D}_{\Delta\mathbf{r}}$ . To  
290 that aim, an axial scan of the sample is performed over the cornea thickness ( $350 \mu\text{m}$ ) with a  
291 sampling of  $2 \mu\text{m}$  (i.e 185 axial positions). For each depth, a transverse scan of the de-scanned  
292 position  $\Delta\boldsymbol{\rho}$  is performed over a  $2.9 \times 2.9 \mu\text{m}^2$  area with a spatial sampling  $\delta_0 = 230$  nm  
293 (that is to say 169 input wave-fronts instead of  $10^6$  input wave-fronts in a canonical basis).  
294 For each scan position  $(\Delta\boldsymbol{\rho}, z)$ , a complex-reflected wave field is extracted by phase shifting  
295 interferometry from four intensity measurements. This measured field is averaged over 5  
296 successive realisations (for denoising). The integration time of the camera is set to 5 ms.  
297 Each wave-field is stored in the de-scanned reflection matrix  $\mathbf{D}_{\Delta\mathbf{r}} = [D(\Delta\boldsymbol{\rho}, \boldsymbol{\rho}_{\text{out}})]$  (Fig. 2).  
298 The duration time for the recording of  $\mathbf{D}_{\Delta\boldsymbol{\rho}}$  is of  $\sim 30$  s at each depth. The post-processing  
299 of the reflection matrix (iterative phase reversal and multi-scale analysis) to get the final  
300 image took only a few minutes on Matlab. The experimental results displayed in Fig. 4 and  
301 at a single depth  $z = 200 \mu\text{m}$  have been obtained by performing a de-scan over a  $7 \times 7 \mu\text{m}^2$   
302 area with a spatial sampling  $\delta_0 = 230$  nm (961 input wave-fronts).

303

### 304 **Local RPSF**

305 To probe the local RPSF, the field-of-view is divided into regions that are defined by  
 306 their central midpoint  $\mathbf{r}_p$  and their spatial extension  $L$ . A local average of the back-scattered  
 307 intensity can then be performed in each region:

$$I(\Delta\boldsymbol{\rho}, \mathbf{r}_p) = \langle |D(\Delta\boldsymbol{\rho}, \mathbf{r}_{\text{out}})|^2 W_L(\mathbf{r}_{\text{out}} - \mathbf{r}_p) \rangle_{\mathbf{r}_{\text{out}}} \quad (7)$$

308 where  $W_L(\mathbf{r}_{\text{out}} - \mathbf{r}_p) = 1$  for  $|\boldsymbol{\rho}_{\text{out}} - \boldsymbol{\rho}_p| < L$ , and zero otherwise.

309

### 310 **Local correlation matrix**

311 A set of correlation matrices shall be considered between distorted wave-fronts associated  
 312 with different regions of the field-of-view:

$$C_{\text{in}}(\mathbf{u}_{\text{in}}, \mathbf{u}'_{\text{in}}, \mathbf{r}_p) = \langle D(\mathbf{u}_{\text{in}}, \mathbf{r}_{\text{out}}) D^*(\mathbf{u}'_{\text{in}}, \mathbf{r}_{\text{out}}) W_L(\mathbf{r}_p - \mathbf{r}_{\text{out}}) \rangle_{\mathbf{r}_{\text{out}}} \quad (8)$$

### 313 **Iterative phase reversal algorithm.**

314 The iterative phase reversal algorithm is a computational process that provides an  
 315 estimator of the transmit wave-field  $T(\mathbf{u}, \mathbf{r}_p)$  that links each point  $\mathbf{u}$  of the pupil plane with  
 316 each voxel  $\mathbf{r}_p$  of the cornea volume. To that aim, the correlation matrix  $\mathbf{C}$  computed over  
 317 the spatial window  $W_L$  centered around each point  $\mathbf{r}_p$  is considered (Eq. 8). Mathematically,  
 318 the algorithm is based on the following recursive relation:

$$\hat{\mathbf{T}}^{(n)}(\mathbf{r}_p) = \exp \left[ i \arg \left\{ \mathbf{C}(\mathbf{r}_p) \times \hat{\mathbf{T}}^{(n-1)}(\mathbf{r}_p) \right\} \right] \quad (9)$$

319 where  $\hat{\mathbf{T}}^{(n)}$  is the estimator of  $\mathbf{T}$  at the  $n^{\text{th}}$  iteration of the phase reversal process.  $\hat{\mathbf{T}}^{(0)}$  is an  
 320 arbitrary wave-front that initiates the iterative phase reversal process (typically a flat phase  
 321 law) and  $\hat{\mathbf{T}} = \lim_{n \rightarrow \infty} \hat{\mathbf{T}}^{(n)}$  is the result of this iterative phase reversal process.

322

### 323 **Aberration and Scattering compensation at output.**

324 The output de-scanned matrix  $\mathbf{D}_{\Delta\boldsymbol{\rho}}(z)$  is deduced from the input de-scanned matrix  
 325  $\mathbf{D}_{\Delta\boldsymbol{\rho}}(z)$  using the following change of variable:

$$D(\boldsymbol{\rho}_{\text{in}}, \Delta\boldsymbol{\rho}', z) = D(-\Delta\boldsymbol{\rho}', \boldsymbol{\rho}_{\text{in}} + \Delta\boldsymbol{\rho}', z) \quad (10)$$

326 with  $\Delta\boldsymbol{\rho}' = \boldsymbol{\rho}_{\text{out}} - \boldsymbol{\rho}_{\text{in}} = -\Delta\boldsymbol{\rho}$ .

327 An output distortion matrix is then built by applying a Fourier transform over the  
 328 de-scanned coordinate:

$$\mathbf{D}_{\mathbf{ru}} = \mathbf{D}_{\Delta\mathbf{r}} \times \mathbf{F}^T \quad (11)$$

329 where the superscript  $T$  stands for matrix transpose. From  $\mathbf{D}_{\mathbf{ru}}$ , one can build a correlation  
 330 matrix  $\mathbf{C}_{\text{out}}$  for each point  $\mathbf{r}_p$ :

$$C_{\text{out}}(\mathbf{u}_{\text{out}}, \mathbf{u}'_{\text{out}}, \mathbf{r}_p) = \langle D(\mathbf{r}_{\text{in}}, \mathbf{u}_{\text{out}}) D^*(\mathbf{r}_{\text{in}}, \mathbf{u}'_{\text{out}}) W_L(\mathbf{r}_p - \mathbf{r}_{\text{out}}) \rangle_{\mathbf{r}_{\text{out}}} \quad (12)$$

331 The iterative phase reversal algorithm described above is then applied to each matrix  $\mathbf{C}_{\text{out}}(\mathbf{r}_p)$   
 332 to derive an estimator  $\hat{\mathbf{T}}_{\text{out}}$  of the output  $\mathbf{T}$ -matrix.

333

### 334 Aberration and Scattering Components of the $\mathbf{T}$ -matrix.

335 The spatial correlation of transmitted wave-fields are investigated at each depth  $z$  by  
 336 computing the correlation matrix of  $\mathbf{T}_{\text{out}}$ :  $\mathbf{C}_{\mathbf{T}} = \mathbf{T}_{\text{out}} \times \mathbf{T}_{\text{out}}^\dagger$ . A mean correlation function  $\Gamma$   
 337 can be computed by performing the following average:

$$\Gamma(\Delta\boldsymbol{\rho}, z) = \langle C_T(\boldsymbol{\rho}_{\text{in}}, \boldsymbol{\rho}_{\text{in}} + \Delta\boldsymbol{\rho}, z) \rangle_{\boldsymbol{\rho}_{\text{in}}} \quad (13)$$

338 The correlation function  $\Gamma$  displayed in Fig. 6a shows that the matrix  $\mathbf{T}_{\text{out}}$  can be decomposed  
 339 as a spatially-invariant component  $\mathbf{T}_{\text{out}}^{(a)}$  and a short-range correlated component  $\mathbf{T}_{\text{out}}^{(m)}$ . Each  
 340 component can be separated by performing a singular value decomposition of  $\mathbf{T}_{\text{out}}$ , such that

341

$$\mathbf{T}_{\text{out}} = \sum_{p=1}^N s_p \mathbf{U}_p \mathbf{V}_p^\dagger \quad (14)$$

342 where  $s_p$  are the positive and real singular values of  $\mathbf{T}_{\text{out}}$  ranged in decreasing order,  $\mathbf{U}_p$   
 343 and  $\mathbf{V}_p$  are unitary matrices whose columns correspond to the input and output singular  
 344 vectors of  $\mathbf{T}_{\text{out}}$ . The first eigenspace of  $\mathbf{T}_{\text{out}}$  provides its spatially-invariant component:  
 345  $\mathbf{T}_{\text{out}}^{(a)} = s_1 \mathbf{U}_1 \mathbf{V}_1^\dagger$ . The higher rank eigenstates provide the multiple scattering component  $\mathbf{T}_{\text{out}}^{(m)}$ .  
 346 Lines or columns of the associated correlation matrix  $\mathbf{C}_T^{(m)} = \mathbf{T}_{\text{out}}^{(m)} \times \mathbf{T}_{\text{out}}^{(m)\dagger}$  provides the map  
 347 of isoplanatic patches displayed in Fig. 6e.

348

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352

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359

360 **Author Contributions.** A.A. initiated and supervised the project. A.C.B., V.B. and  
361 A.A. designed the experimental setup. U.N., V.B. and P.B. built the experimental set up.  
362 U.N. and V.B. developed the post-processing tools. U.N. performed the corneal imaging  
363 experiment. U.N. and A.A. analyzed the experimental results. V.B. and A.A. performed the  
364 theoretical study. A.A. and U.N. prepared the manuscript. U.N., V.B., P.B., M.F., A.C.B.,  
365 and A.A. discussed the results and contributed to finalizing the manuscript.

366

367 **Competing interests.** A.A., M.F., A.C.B. and V.B. are inventors on a patent related to  
368 this work held by CNRS (no. US11408723B2, published August 2022). All authors declare  
369 that they have no other competing interests.

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**Supplementary Information for**  
**Non-invasive Retrieval of the Transmission Matrix for Optical**  
**Imaging Deep Inside a Multiple Scattering Medium**

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**Abstract**

This document provides further information on: (i) the experimental set up; (ii) the theoretical expression of the de-scanned matrix; (iii) the measurement of the scattering mean free path; (iv) the theoretical expression of the correlation matrix; (v) the estimation of the transmission matrix; (vi) the contrast enhancement provided by reflection matrix imaging.

7 S1. DETAILED EXPERIMENTAL SET-UP

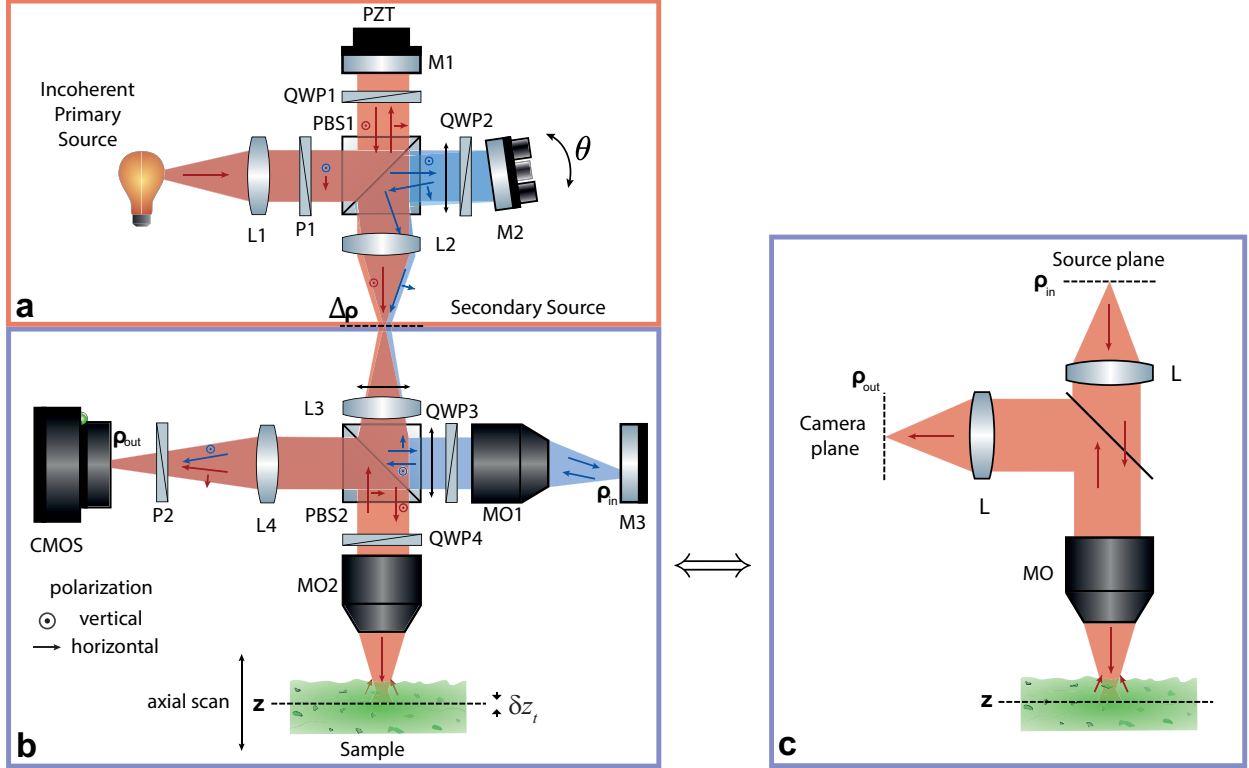


FIG. S1. **Passive measurement of the de-scanned reflection matrix.** P: polarizer, L: lens, QWP: quarter-wave plate, M: mirror, PZT: piezo-electric actuator, PBS: polarisation beam splitter, MO: microscope objective. The apparatus is made up of two parts. **a.** Michelson interferometer illuminated by incoherent light source at its input and generating two twin incoherent beams of orthogonal polarization and laterally shifted from each other at its output. The polarised beam splitters (PBS1) separates the impinging light into a reference path (in blue) and a sample path (in red). The tilt  $\delta\theta$  of mirror M2 controls the shift  $\Delta\rho$  between the twin beams in the secondary source plane. **b.** Michelson interferometer with microscope objectives (MO) in both arms (Linnik configuration). Both beams have orthogonal polarizations and each interferometer arm includes a quarter-wave plate (QWP). The output beams are collected by the L4 lens and interfere on the camera after having been projected on a  $45^\circ$ -rotated polarizer (P2). **c.** Equivalent layout in the case of a coherent measurement. The source plane, the focal plane, and the camera planes are conjugated. Displacing a point source  $\rho_{in}$  in the source plane discretely scans the focal plane inside the sample. The illuminated area is imaged in the camera plane; in an epi-detection configuration.

8 The full experimental set up is displayed in Fig. S1. The setup is divided into two building  
9 blocks, labelled (a) and (b). The first component is a Michelson interferometer [Fig. S1a].  
10 The light source is a broadband LED (Thorlabs M850LP1,  $\lambda_o = 850$  nm,  $\Delta\lambda = 35$  nm),  
11 which, under a pseudo-Kohler configuration, ensures a spatially-incoherent, yet uniform,  
12 illumination of the field-of-view. The incident light is collimated using a convergent lens (L1)  
13 with a focal length  $f_1 = 150$  mm. The beam transmitted through this lens (L1) is linearly  
14 polarized at  $45^\circ$  by a polarizer (P1) so that it is then equally reflected (sample arm) and  
15 transmitted (reference arm) by the polarized beam splitter (PBS1).

16 The sample beam reflected by PBS1 is horizontally polarized. It propagates through a  
17 quarter-wave plate (QWP1), is reflected by a plane mirror (M1), whose normal lies along  
18 the optical axis and that is mounted on a piezoelectric actuator (PZT). The reflected beam  
19 passes again through the quarter-wave plate (QWP1). This sequence induces a polarization  
20 rotation by  $90^\circ$  of the reflected beam with respect to the incident beam in the sample arm.  
21 The reflected wave can be then transmitted through the beam splitter (PBS1) with a vertical  
22 polarization and finally focused in a secondary source plane conjugated with the source plane  
23 by means of the lens (L2) of focal length  $f_2 = 125$  mm.

24 The reference beam, vertically polarized at the exit of the polarizer (P1), is transmitted by  
25 the beam splitter (PBS1), propagates through a quarter-wave plate (QWP2), is reflected by  
26 a set of galvanometric scan mirrors (M2) that enables a 2D rotation of the incident wave-field  
27 by angles  $\boldsymbol{\theta} = (\theta_x, \theta_y)$  with respect to the optical axis. The reflected beam then propagates  
28 again through the quarter wave plate (QWP2). This round trip through (QWP2) enables a  
29  $90^\circ$  rotation of the light polarization. The reflected beam is therefore reflected by the beam  
30 splitter (PBS1) before being focused by the lens (L2) in the secondary source plane.

31 Finally, in the secondary source plane, the wave-field is made of two images of the incident  
32 light orthogonally polarized and translated with respect each other by a relative position  $\Delta\boldsymbol{\rho}$ .  
33 This lateral shift is dictated by the tilt  $\boldsymbol{\theta} = (\theta_x, \theta_y)$  of the reference beam:  $\Delta x = f_2 \tan \theta_x$   
34 and  $\Delta y = f_2 \tan \theta_y$ . Note also that the optical path difference between the two arms is set to  
35 zero by equalizing the length of sample and reference arms for  $\Delta\boldsymbol{\theta} = 0$ .

36 After the Michelson interferometer, these two orthogonally polarized beams enter a Michel-  
37 son interferometer with two identical microscope objectives in both arms (a configuration  
38 known as a Linnik interferometer) [Fig. S1b]. They are again collimated by a lens (L3)  
39 of focal length  $f_3=200$  mm. The two lenses (L2) and (L3) thus constitute a  $4f$  system

40 which compensates the effects of diffraction between the two interferometers. The vertically  
 41 polarized light (sample beam) is transmitted by a polarized beam splitter cube (PBS2),  
 42 propagates through a quarter-wave plate (QWP4) before being focused in the focal plane  
 43 of an immersion microscope objective (MO2, Nikon, 60×, NA=1.0). The light reflected  
 44 by the sample is then collected by (MO2) and propagates again through the quarter-wave  
 45 plate (QWP4). Because single scattering tends to preserve polarization, the corresponding  
 46 wave-field undergoes a 90° polarization rotation and gets reflected by the beam splitter  
 47 (PBS2) before being focused in the plane of the camera using the convergent lens (L4) of  
 48 focal length  $f_4 = 200$  mm. The combination of this lens (L4) with the microscope objective  
 49 (MO1) entails a magnification  $M_4$  of 60.

50 Regarding the horizontally-polarized beam at the exit of the lens (L3), it is reflected  
 51 by the beam splitter (PBS2), passes through the quarter-wave plate (QWP3) before being  
 52 focused by the microscope objective (MO1) identical to (MO2). The light is then reflected by  
 53 the reference mirror (M3) placed in the focal plane of MO2 before being collected again by  
 54 the same microscope objective (MO2). The reflected light comes through the quarter-wave  
 55 plate (QWP3). As in the other arm, the polarization of the reflected beam exhibits a 90°  
 56 rotation of its polarization. The beam is now vertically polarized and transmitted by the  
 57 beam splitter (PBS2), before being focused on the camera with the lens (L4).

58 The CMOS camera (Adimec Quartz 2A-750, 2Mpx) records the interferogram with a  
 59 spatial sampling equal to  $\delta_0 = 230$  nm given the magnification  $M_4$ . The volume of the sample  
 60 from which photons can interfere with the reference beam is called the "coherence volume".  
 61 Its position is dictated by the optical path difference between the reference and sample arms.  
 62 Its thickness is inversely proportional to the light spectrum bandwidth [1]:

$$\delta z_t = \frac{2 \ln 2}{n\pi} \left( \frac{\lambda^2}{\Delta\lambda} \right) \quad (\text{S1})$$

63 with  $\lambda$  the central wavelength of the light source and  $\Delta\lambda$  its spectral bandwidth. In the  
 64 present case,  $\delta z_t \sim 10$   $\mu\text{m}$ . A critical tuning of the experimental set up consists in adjusting  
 65 the coherence volume with the focal plane of the microscope objective. In a volumetric  
 66 sample, whose refractive index differs from that of water, the coherence volume no longer  
 67 coincides with the focusing plane. This focusing defect accumulates with the transverse  
 68 aberrations generated by the heterogeneities of the medium. However, it is possible to  
 69 compensate for it by a fine tuning of the length of the reference arm.

70 The experimental procedure then consists in recording the de-scanned reflection matrix  
71  $\mathbf{D}_{\Delta\rho}(z)$  at each depth  $z$  of the sample. This latter parameter is swept by means of a motorized  
72 axial displacement of the sample carrier. The scan of the relative position  $\Delta\rho$  between the  
73 incident wave-fields in the sample and reference arms is controlled by the tilt imposed by  
74 the galvanometer (M2). For each couple  $(\Delta\rho, z)$ , the CCD camera conjugated with the MO  
75 focal plane records the output intensity:

$$I_\alpha(\Delta\rho, \rho_{\text{out}}, z) = \int_0^T |e^{i\alpha} E_{\text{out}}(\rho_{\text{out}}, t) + E_{\text{out}}^{(\text{ref})*}(\Delta\rho, \rho_{\text{out}}, t)|^2 dt \quad (\text{S2})$$

76 with  $t$  the absolute time,  $\mathbf{r}$  the position vector on the CCD screen,  $E_{\text{out}}(\mathbf{r}, \tau)$  the scattered wave  
77 field associated with the sample arm,  $E_{\text{out}}^{(\text{ref})}(\mathbf{r}, \tau)$  the reference wave field;  $T$  the integration  
78 time of the CCD camera, and  $\alpha$  an additional phase term controlled with a piezoelectric  
79 actuator placed on mirror (M1) of the first interferometer [Fig. S1a]. The interference term  
80 is extracted from the four intensity patterns (Eq. S2) recorded at  $\alpha = 0, \pi/2, 3\pi/2$  and  $\pi$   
81 (“four phase method” [2]):

$$D(\Delta\rho, \rho_{\text{out}}, z) = \frac{1}{T} \int_0^T E_{\text{out}}(\rho_{\text{out}}, t) E_{\text{out}}^{(\text{ref})*}(\Delta\rho, \rho_{\text{out}}, t) dt \quad (\text{S3})$$

82 As we will see in the next section, under certain conditions, this interference term tends  
83 towards the coefficients of the focused reflection matrix  $\mathbf{R}_{\mathbf{r}\mathbf{r}}$  that would be recorded by the  
84 coherent set up displayed in Fig. S1c [Eq.1 of the accompanying article].

85 In particular, for  $\Delta\rho = \mathbf{0}$  (FFOCT set up), the de-scanned wave-field is equivalent to a  
86 time-gated confocal image [3]. On the one hand, the confocal nature of the recorded wave-field  
87 implies a transverse resolution  $\delta\rho_0 \sim \lambda/4NA$ . On the other hand, the axial resolution is  
88 either controlled by the thickness  $\delta z_t$  of the coherence volume or the depth-of-field  $\delta z_0$  of the  
89 microscope objective:  $\delta z_0 = n\lambda/NA^2$ . In the present case,  $\delta z_0 \sim 1 \mu\text{m} < \delta z_t \sim 10 \mu\text{m}$ . The  
90 axial resolution is thus given by the depth-of-field.  $\delta\rho_0$  and  $\delta z_0$  thus dictate the values of the  
91 transverse and axial sampling of matrix  $\mathbf{D}$  in our experiment.

92 **S2. THEORETICAL EXPRESSION OF THE DE-SCANNED MATRIX**

93 In this section, we investigate to which extent the de-scanned matrix recorded by the  
 94 experimental set up in Figs. S1a,b can be considered equivalent to the focused reflection  
 95 matrix that would be recorded by the fictitious coherent set up displayed in Fig. S1c.

96 To that aim, we will rely on the simple Fourier optics model proposed in a recent paper [3]  
 97 to describe the manifestation of aberrations in FFOCT. For the sake of simplicity, this model  
 98 is scalar. The large numerical aperture imposes that the recorded wave-field is associated  
 99 with single scattering events taking place in the focal plane of the MO.

100 The wave field  $E_{\text{out}}(\boldsymbol{\rho}_{\text{out}}, z)$  reflected by the sample arm in the camera plane can then be  
 101 expressed as follows [3]:

$$E_{\text{out}}(\boldsymbol{\rho}_{\text{out}}, z, \omega) = \int_{\Sigma_0} \int_{\Sigma_\rho} H_{\text{out}}(\boldsymbol{\rho}_{\text{out}}, \boldsymbol{\rho}, z) \gamma(\boldsymbol{\rho}, z) H_{\text{in}}(\boldsymbol{\rho}, \boldsymbol{\rho}_0, z) E_s(\boldsymbol{\rho}_0, \omega) d\mathbf{r} d\boldsymbol{\rho}_0. \quad (\text{S4})$$

102  $E_{\text{in}}(\boldsymbol{\rho}_0, \omega)$  is the incident wave-field in the secondary source plane  $\Sigma_0$  at frequency  $\omega$ . Light  
 103 propagation between  $\Sigma_0$  and the focal plane  $\Sigma_\rho$  is described by the impulse response  $H(\boldsymbol{\rho}_0, \boldsymbol{\rho})$   
 104 between a point in the secondary source plane at transverse coordinate  $\boldsymbol{\rho}_0$  and a point in the  
 105 focal plane at coordinate  $\boldsymbol{\rho}$ . It accounts for sample-induced aberrations.  $\gamma(\boldsymbol{\rho}, z)$  represents  
 106 the sample reflectivity at depth  $z$ . By spatial reciprocity, the propagation of the reflected  
 107 wave-field from the sample to the detector plane is also modelled by the impulse response  
 108  $H(\boldsymbol{\rho}, \boldsymbol{\rho}_{\text{out}})$ . The relatively narrow bandwidth ( $\Delta\lambda \ll \lambda$ ) of the light source and the use of  
 109 achromatic optical elements (lens, beam splitter, quarter wave plate) allows us to neglect the  
 110 dependence of  $H$  on frequency  $\omega$ .

111 Replacing  $\gamma(\boldsymbol{\rho}, z)$  by a uniform reflectivity in Eq. S4 and taking into account the lateral  
 112 shift of the reference wave-field induced by the galvanometer M2 [Fig. S1] leads to the  
 113 following previous expression for  $E_{\text{out}}^{(\text{ref})}(\boldsymbol{\rho}_{\text{out}}, z)$  [3]:

$$E_{\text{out}}^{(\text{ref})}(\boldsymbol{\rho}_{\text{out}}, \Delta\boldsymbol{\rho}, z) = \int_{\Sigma_0} H_{\text{ref}}(\boldsymbol{\rho}_{\text{out}} - \boldsymbol{\rho}_0) E_0(\boldsymbol{\rho}_0 + \Delta\boldsymbol{\rho}) d\mathbf{r} d\boldsymbol{\rho}_0. \quad (\text{S5})$$

114 where  $H_{\text{ref}}$  is the impulse response associated with the reference arm (way and return path)  
 115 that we assume as spatially-invariant [ $H_{\text{ref}}(\boldsymbol{\rho}_{\text{out}}, \boldsymbol{\rho}_0) = H_{\text{ref}}(\boldsymbol{\rho}_{\text{out}} - \boldsymbol{\rho}_0)$ ].

116 The de-scanned wave-field is obtained by extracting the interference term between the  
 117 reflected wave-fields coming from the sample and reference arms:

$$D(\boldsymbol{\rho}_{\text{out}}, \Delta\boldsymbol{\rho}, z) = \langle E_{\text{out}}(\boldsymbol{\rho}_{\text{out}}, \omega) E_{\text{out}}^{(\text{ref})*}(\boldsymbol{\rho}_{\text{out}}, \omega) \rangle \quad (\text{S6})$$



118 Assuming a spatially-incoherent incident wave-field [ $\langle E_0(\mathbf{r}_0)E_0^*(\mathbf{r}'_0) \rangle = I_0\delta(\mathbf{r}_0 - \mathbf{r}'_0)$ ] and  
 119 injecting Eqs. S4 and S5 into the last equation leads to the following expression for  $\mathbf{D}$ -matrix  
 120 coefficients:

$$D(\boldsymbol{\rho}_{\text{out}}, \Delta\boldsymbol{\rho}, z) = I_0 \iiint H(\boldsymbol{\rho}_{\text{out}}, \boldsymbol{\rho}, z)\gamma(\boldsymbol{\rho}, z) [H \odot H_{\text{ref}}^*](\boldsymbol{\rho}, \boldsymbol{\rho}_{\text{out}} + \Delta\boldsymbol{\rho}, z)d\boldsymbol{\rho} \quad (\text{S7})$$

121 where the symbol  $\odot$  stands for the convolution product over the variable  $\boldsymbol{\rho}_{\text{out}} + \Delta\boldsymbol{\rho}$ .

122 The coefficients of a focused reflection matrix recorded by the fictitious coherent set up  
 123 displayed in Fig. S1 can be expressed as:

$$R(\boldsymbol{\rho}_{\text{out}}, \boldsymbol{\rho}_{\text{in}}, z) = I_0 \iiint H(\boldsymbol{\rho}_{\text{out}}, \boldsymbol{\rho}, z)\gamma(\boldsymbol{\rho}, z)H(\boldsymbol{\rho}, \boldsymbol{\rho}_{\text{in}}, z)d\boldsymbol{\rho} \quad (\text{S8})$$

124 Only, a perfect reference arm would imply  $\mathbf{T}_{\text{ref}} \equiv \mathbf{1}_{k < NA}$  and  $H \odot H_{\text{ref}}^* \equiv H$ . Equations S7  
 125 and S8 are then strictly identical in this ideal case: The incoherent set up of Fig. S1a is  
 126 equivalent to the fictitious coherent set up of Fig. S1b. In reality, the reference arm can  
 127 exhibit aberrations such as a slight defocus of the reference mirror M3 in Fig. S1b or a  
 128 slight defocus of the reference beam in the secondary source plane at the output of first  
 129 interferometer.

130 The comparison between Eq. S7 and Eq.2 of the accompanying paper leads to the  
 131 following identification:  $H_{\text{out}} \equiv H$  and  $H_{\text{in}} \equiv H \odot H_{\text{ref}}^*$ . In other words, while the output  
 132 transmission matrices ( $\mathbf{H}_{\text{out}}$ , or equivalently,  $\mathbf{T}_{\text{out}}$ ) only grasp the sample-induced aberrations,  
 133 the input transmission matrices ( $\mathbf{H}_{\text{in}}$  and  $\mathbf{T}_{\text{in}}$ ) also contain the aberrations undergone by  
 134 the incident and reflected reference beams. This feature explains the difference between the  
 135 transmission matrices  $\hat{\mathbf{T}}_{\text{in}}$  and  $\hat{\mathbf{T}}_{\text{in}}$  shown in Fig.4 of the accompanying paper in which the  
 136 input transmission matrix  $\hat{\mathbf{T}}_{\text{in}}$  (Fig. 4e) shows an additional spherical aberration on top of  
 137 the sample-induced aberrations exhibited by  $\hat{\mathbf{T}}_{\text{out}}$  (Fig. 4f).

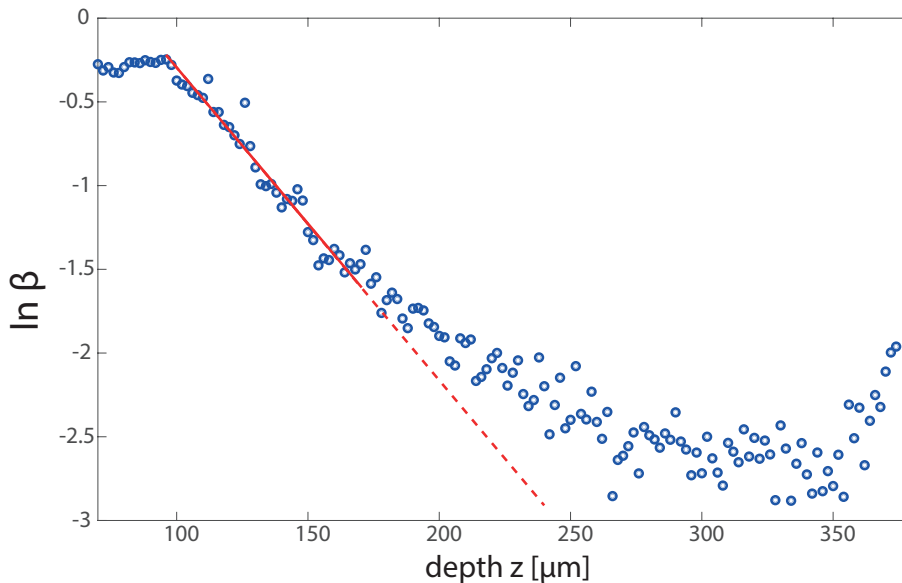


FIG. S2. Confocal scattering ratio  $\ln \beta$  versus depth (blue dots) fitted with Eq. S9 (red line).

139 In a previous work [4], the scattering mean free path  $\ell_s$  in the cornea has been measured  
 140 by investigating the depth evolution of the confocal intensity. Indeed, in the single scattering  
 141 regime, under the paraxial approximation and for an homogeneous reflectivity, the time-gated  
 142 confocal intensity is supposed to decrease as  $\exp(-2z/\ell_s)$  if we neglect absorption losses [5, 6].

143 Unfortunately, here, the cornea is not healthy but oedematous. The depth evolution of  
 144 the confocal intensity in the stroma is thus strongly impacted by multiple scattering and  
 145 cannot be used for a measurement of  $\ell_s$ . Moreover, in the epithelium, the different layers of  
 146 cell make the cornea reflectivity too heterogeneous to provide an exponential decrease of the  
 147 confocal intensity.

148 Recently, an alternative strategy has been proposed in presence of multiple scattering. It  
 149 consists in investigating the depth evolution of the ratio  $\beta$  between the confocal surintensity  
 150 and the total intensity [7]. For a medium statistically homogeneous in terms of disorder,  
 151 numerical simulations have shown empirically that this quantity scales as [8]:

$$\beta(z) \sim \exp(-4z/(3\ell_s)) \quad (\text{S9})$$

152 In the present case, this confocal ratio  $\beta$  has been measured as follows:

$$\hat{\beta}(z) = 1 - \frac{\min_{\Delta\rho} \{I(\Delta\rho, z)\}}{\max_{\Delta\rho} \{I(\Delta\rho, z)\}} \quad (\text{S10})$$

153 This estimator  $\hat{\beta}$  relies on the fact that the multiple scattering component of the RPSF  
154 exhibits a flat background such that it can be estimated with the minimum of  $I(\Delta\rho, z)$ . This  
155 hypothesis is wrong at shallow depth since the diffuse halo grows as  $\sqrt{Dt}$ . Nevertheless,  
156 beyond  $\ell_s$  or so (here  $100 \mu\text{m}$ ), the multiple scattering background can be considered as flat  
157 as illustrated by Fig. 3d of the accompanying paper.

158 Figure S2 displays the depth evolution of the estimator  $\hat{\beta}(z)$ . It exhibits an exponential  
159 decay in the stroma beyond  $z = 100 \mu\text{m}$ . The decay rate decreases beyond  $z = 170 \mu\text{m}$   
160 because our estimator of  $\beta(z)$  starts to be impacted by the experimental noise [see Fig. 3d of  
161 the accompanying paper]. Therefore, the fit of  $\hat{\beta}(z)$  with Eq. S9 is performed from  $z = 100$   
162 to  $z = 170 \mu\text{m}$ . We find  $\ell_s \sim 70 \mu\text{m}$ .

163 **S4. THEORETICAL EXPRESSION OF THE CORRELATION MATRIX**

164 The multi-scale analysis of  $\mathbf{D}$  allows an estimation of the  $\mathbf{T}$ -matrix at an increasingly  
 165 finer resolution, by iteratively reducing the area over which each aberration phase law is  
 166 estimated. At each step, the iterative phase reversal (IPR) algorithm assumes the convergence  
 167 of the correlation matrix  $\mathbf{C}(\mathbf{r}_p)$  (Eq.8) towards its ensemble average  $\langle \mathbf{C} \rangle (\mathbf{r}_p)$ , the so-called  
 168 covariance matrix [9, 10]. In fact, this convergence is never fully realized and  $\mathbf{C}$  should be  
 169 decomposed as the sum of this covariance matrix  $\langle \mathbf{C} \rangle (\mathbf{r}_p)$  and a perturbation term  $\delta\mathbf{C}(\mathbf{r}_p)$ :

$$\mathbf{C}(\mathbf{r}_p) = \langle \mathbf{C} \rangle (\mathbf{r}_p) + \delta\mathbf{C}(\mathbf{r}_p). \quad (\text{S11})$$

170 The intensity of the perturbation term scales as the inverse of the number  $N_L = (L/\delta_0)^2$  of  
 171 resolution cells in each sub-region [10, 11]:

$$\langle |\delta C(\mathbf{u}, \mathbf{u}', \mathbf{r}_p)|^2 \rangle = \frac{\langle |C(\mathbf{u}, \mathbf{u}', \mathbf{r}_p)|^2 \rangle}{N_L} \quad (\text{S12})$$

172 This perturbation term can thus be reduced by increasing the size  $L$  of the spatial window  
 173  $W_L$ , but at the cost of a resolution loss. In the following, we express theoretically the bias  
 174 induced by this perturbation term on the estimation of  $\mathbf{T}$ . In particular, we will show how  
 175 it scales with the parameter  $L$  and the focusing quality. To that aim, we will consider the  
 176 input correlation matrix  $\mathbf{C}_{\text{in}}$  but a similar demonstration can be performed at output. For  
 177 sake of lighter notation, the dependence over  $\mathbf{r}_p$  will be omitted in the following.

178 Under assumptions of local isoplanicity and random reflectivity, the covariance matrix  
 179 can be expressed as follows [9]:

$$\langle \mathbf{C}_{\text{in}} \rangle = \mathbf{T}_{\text{in}} \times \mathbf{C}_H \times \mathbf{T}_{\text{in}}^\dagger, \quad (\text{S13})$$

180 or in terms of matrix coefficients,

$$\langle \mathbf{C}_{\text{in}} \rangle (\mathbf{u}, \mathbf{u}') = T_{\text{in}}(\mathbf{u}) T_{\text{in}}^*(\mathbf{u}') \underbrace{\int d\rho |H_{\text{out}}(\rho)|^2 e^{-i2\pi \frac{(\mathbf{u}-\mathbf{u}') \cdot \rho}{\lambda_f}}}_{=C_H(\mathbf{u}, \mathbf{u}')}. \quad (\text{S14})$$

181  $\mathbf{C}_H$  is a reference correlation matrix associated with a virtual reflector whose scattering  
 182 distribution corresponds to the output focal spot intensity  $|H_{\text{out}}(\rho)|^2$ . This scatterer plays  
 183 the role of virtual guide star in the RMI process.

184 **S5. ESTIMATION OF THE T–MATRIX**

185 For such an experimental configuration, an iterative time reversal process converges  
 186 towards a wavefront that maximizes the energy back-scattered by the reflector [12, 13].  
 187 Mathematically, this iterative time reversal process writes as follows

$$\mathbf{U}_{\text{in}}^{(n+1)} = \sigma \mathbf{C}_{\text{in}} \times \mathbf{U}_{\text{in}}^{(n)} \quad (\text{S15})$$

188 with  $\mathbf{U}_{\text{in}}^{(n)}$ , the wave-front at iteration  $n$  of the iterative time reversal process and  $\sigma$ , the  
 189 scatterer reflectivity. Iterative time reversal converges towards a wave front,  $\mathbf{U}_{\text{in}} = \lim_{n \rightarrow +\infty} \mathbf{U}_{\text{in}}^{(n)}$ ,  
 190 that is none other than the first eigenvector of  $\mathbf{C}_{\text{in}}$ .

191 If the virtual reflector was point-like, this wave-front would be a perfect estimator of  
 192  $\mathbf{T}_{\text{in}}$ . Its phase conjugate perfectly compensates for aberrations and focuses through the  
 193 heterogeneous medium onto the point-like target [12, 13]. However, here the virtual guide  
 194 star is enlarged compared to the diffraction limit. Iterative time reversal thus converges  
 195 towards a wave-front  $\mathbf{U}_{\text{in}}$  of finite angular support  $\delta u_c$  that tends to focus on the center of  
 196 the virtual reflector but with a resolution width  $\delta \rho_c \sim \lambda f / \delta u_c$  larger than the diffraction  
 197 limit [10].

198 To circumvent that issue, the iterative phase reversal algorithm has been developed. It  
 199 consists in modifying the iterative time reversal process as follows:

$$\hat{\mathbf{T}}_{\text{in}}^{(n+1)} = \exp \left[ i \arg \left\{ \mathbf{C}_{\text{in}} \times \hat{\mathbf{T}}_{\text{in}}^{(n)} \right\} \right] \quad (\text{S16})$$

200 where  $\hat{\mathbf{T}}_{\text{in}}^{(n)}$  is the estimator of  $\mathbf{T}_{\text{in}}$  at the  $n^{\text{th}}$  iteration of the phase reversal process.  $\hat{\mathbf{T}}_{\text{in}}^{(0)}$  is  
 201 an arbitrary wave-front that initiates the iterative phase reversal process (typically a flat  
 202 phase law).  $\hat{\mathbf{T}}_{\text{in}} = \lim_{n \rightarrow \infty} \hat{\mathbf{T}}_{\text{in}}^{(n)}$  is the result of this iterative phase reversal process. The  
 203 iterative phase reversal algorithm differs from an iterative time reversal process by imposing  
 204 the  $\mathbf{T}$ -matrix coefficients to be of constant modulus in the pupil plane. In other words,  
 205 unlike the iterative time reversal process, the resulting wave-front here equally addresses each  
 206 angular component of the imaging process to reach a diffraction-limited resolution. While  
 207 the iterative time reversal process is guided by a maximization of the back-scattered energy,  
 208 the iterative phase reversal process is dictated by a minimization of the resolution length.

209 In practice, however, the  $\mathbf{T}$ –matrix estimator is still impacted by the blurring of the  
 210 synthesized guide star and the presence of diffusive background and/or noise. Therefore the

211 whole process shall be iterated at input and output in order to gradually refine the guide  
 212 star and reduce the bias on our  $\mathbf{T}$ -matrix estimator. Moreover, the spatial window  $W_L$   
 213 over which the  $\mathbf{C}$ -matrix is computed [Eq. 8 in the accompanying paper] shall be gradually  
 214 decreased in order to address the forward multiple scattering component, the latter one being  
 215 associated with smaller isoplanatic patches.

216 To understand the parameters controlling the bias  $\delta\hat{\mathbf{T}}_{\text{in}}$  between  $\hat{\mathbf{T}}_{\text{in}}$  and  $\mathbf{T}_{\text{in}}$ , one can  
 217 express  $\hat{\mathbf{T}}_{\text{in}}$  as follows:

$$\hat{\mathbf{T}}_{\text{in}} = \exp\left(j\arg\left\{\mathbf{C}_{\text{in}} \times \hat{\mathbf{T}}_{\text{in}}\right\}\right) = \frac{\mathbf{C}_{\text{in}} \times \hat{\mathbf{T}}_{\text{in}}}{\|\mathbf{C}_{\text{in}} \times \hat{\mathbf{T}}_{\text{in}}\|} \quad (\text{S17})$$

218 By injecting Eq. S11 into the last expression,  $\hat{\mathbf{T}}_{\text{in}}$  can be expressed, at first order, as the sum  
 219 of its expected value  $\mathbf{T}_{\text{in}}$  and a perturbation term  $\delta\hat{\mathbf{T}}_{\text{in}}$ :

$$\hat{\mathbf{T}}_{\text{in}} = \underbrace{\frac{\langle \mathbf{C}_{\text{in}} \rangle \times \mathbf{T}_{\text{in}}}{\|\langle \mathbf{C}_{\text{in}} \rangle \times \mathbf{T}_{\text{in}}\|}}_{=\mathbf{T}_{\text{in}}} + \underbrace{\frac{\delta \mathbf{C}_{\text{in}} \times \mathbf{T}_{\text{in}}}{\|\langle \mathbf{C}_{\text{in}} \rangle \times \mathbf{T}_{\text{in}}\|}}_{\simeq \delta\hat{\mathbf{T}}_{\text{in}}}. \quad (\text{S18})$$

220 The bias intensity can be expressed as follows:

$$\|\delta\hat{\mathbf{T}}_{\text{in}}\|^2 = \frac{\mathbf{T}_{\text{in}}^\dagger \times \delta \mathbf{C}_{\text{in}}^\dagger \times \delta \mathbf{C}_{\text{in}} \times \mathbf{T}_{\text{in}}}{\mathbf{T}_{\text{in}}^\dagger \times \langle \mathbf{C}_{\text{in}} \rangle^\dagger \times \langle \mathbf{C}_{\text{in}} \rangle \times \mathbf{T}_{\text{in}}} \quad (\text{S19})$$

221 Using Eq. S12, the numerator of the previous equation can be expressed as follows:

$$\mathbf{T}_{\text{in}}^\dagger \times \delta \mathbf{C}_{\text{in}}^\dagger \times \delta \mathbf{C}_{\text{in}} \times \mathbf{T}_{\text{in}} = M^2 \delta u_0^2 \langle |\delta C_{\text{in}}(\mathbf{u}, \mathbf{u}')|^2 \rangle = M^2 \delta u_0^2 \langle |C_{\text{in}}(\mathbf{u}, \mathbf{u})|^2 \rangle / N_L. \quad (\text{S20})$$

222 with  $\delta u_0 \sim \lambda f / \delta_R$ , the resolution of the  $\mathbf{T}$ -matrix in the pupil plane and  $\delta_R$ , the de-scan  
 223 field-of-view. Injecting Eq. S14 into the last equation leads to the following expression for  
 224 the numerator of Eq. S19:

$$\mathbf{T}_{\text{in}}^\dagger \times \delta \mathbf{C}_{\text{in}}^\dagger \times \delta \mathbf{C}_{\text{in}} \times \mathbf{T}_{\text{in}} = M^2 \delta u_0^2 \left| T_{\text{out}} \overset{\mathbf{u}_{\text{out}}}{\otimes} T_{\text{out}}(\mathbf{0}) \right|^2 / N_L. \quad (\text{S21})$$

225 The denominator of Eq. S19 can be expressed as follows:

$$\mathbf{T}_{\text{in}}^\dagger \times \langle \mathbf{C}_{\text{in}} \rangle^\dagger \times \langle \mathbf{C}_{\text{in}} \rangle \times \mathbf{T}_{\text{in}} = M^2 \left| \sum_{\mathbf{u}} T_{\text{out}} \overset{\mathbf{u}_{\text{out}}}{\otimes} T_{\text{out}}(\mathbf{u}) \right|^2 \quad (\text{S22})$$

226 The bias intensity is thus given by:

$$\|\delta\hat{\mathbf{T}}_{\text{in}}\|^2 = \frac{\delta u_0^2 \left| T_{\text{out}} \overset{\mathbf{u}_{\text{out}}}{\otimes} T_{\text{out}}(\mathbf{0}) \right|^2}{\left| \sum_{\mathbf{u}} T_{\text{out}} \overset{\mathbf{u}_{\text{out}}}{\otimes} T_{\text{out}}(\mathbf{u}) \right|^2} = \frac{\delta_R^{-2} \int d\mathbf{r} |H_{\text{out}}(\mathbf{r})|^2}{|H_{\text{out}}(\mathbf{r} = \mathbf{0})|^2} \quad (\text{S23})$$

227 The bias thus scales as the ratio between the mean incoherent input intensity and the  
 228 coherent intensity (energy deposited exactly at focus). It is thus inversely proportional to the  
 229 focusing quality at output. In terms of order of magnitude, we have  $\left| \sum_{\mathbf{u}} T_{\text{out}} \overset{\mathbf{u}_{\text{out}}}{\otimes} T_{\text{out}}(\mathbf{u}) \right|^2 \sim$   
 230  $\delta u_{\text{out}}^2 \left| T_{\text{out}} \overset{\mathbf{u}_{\text{out}}}{\otimes} T_{\text{out}}(\mathbf{0}) \right|^2$ , with  $\delta u_{\text{out}} \sim \lambda f / \delta r_{\text{out}}$  the coherence length of the  $\mathbf{T}$ -matrix in the  
 231 output pupil plane and  $\delta r_{\text{out}}$ , the spatial extension of the output PSF. The bias intensity  
 232 thus exhibits the following scaling law,

$$\|\delta \hat{\mathbf{T}}_{\text{in}}\|^2 \sim \frac{D_{\text{out}}}{MN_L}. \quad (\text{S24})$$

233 where  $D_{\text{out}} = (\delta r_{\text{out}} / \delta_0)^2$  is the characteristic size of the output focal spot in terms of  
 234 resolution cells.

235 This last expression justifies the multi-scale analysis proposed in the accompanying paper.  
 236 A gradual decrease of the aberration level, quantified by  $D_{\text{out}}$ , is required to address smaller  
 237 spatial windows that scale as  $N_L$ . Following this scheme, the bias of the  $\mathbf{T}$ -matrix can be  
 238 minimized and the iterative phase reversal algorithm converges towards a satisfying estimator.

239 However, the spatial window cannot be reduced to a speckle grain otherwise the method  
 240 would lead to a bucket image that consists in an incoherent summation of each de-scanned  
 241 wave-field. Figure S3c illustrates this fact by displaying the en-face image obtained when the  
 242 spatial window reaches a size  $L = 3 \mu\text{m}$ . Compared with  $L = 6 \mu\text{m}$  [Figure S3b], the image  
 243 has clearly lost some contrast, which is a manifestation of an incoherent compensation of  
 244 aberrations and scattering (bucket image).

245 The iteration should therefore be stopped at some point. In practice, the end of the  
 246 process can be determined by a careful look at the image. An incoherent compensation  
 247 of aberrations induces a loss of contrast on the final image. Figure S3 illustrates those  
 248 detrimental effects by comparing the original image (Fig. S3a), the RMI image obtained  
 249 with a  $\mathbf{T}$ -matrix of optimal resolution ( $6 \times 6 \mu\text{m}^2$ , see Fig. S3b) and a RMI image relying  
 250 on too small spatial windows  $W_L$  ( $3 \times 3 \mu\text{m}^2$ , see Fig. S3c). The contrast of each image  
 251  $I(\boldsymbol{\rho}, z)$ ,  $\mathcal{C}(z) = \text{std}[I(\boldsymbol{\rho}, z)] / \langle I(\boldsymbol{\rho}, z) \rangle$ , tends to gradually increase when the estimator  $\hat{\mathbf{T}}$   
 252 approaches  $\mathbf{T}$  (see comparison between Figs. S3a and b) and decrease when the compensation  
 253 of aberrations and scattering becomes bucket-like (see comparison between Figs. S3b and c).  
 254 For the images displayed in Figs. S3a, b and c, we find  $\mathcal{C} \sim 1.48$ ,  $\mathcal{C} \sim 1.61$  and  $\mathcal{C} \sim 1.37$ ,  
 255 respectively. Nevertheless, an optimization criterion only based on the image contrast can be  
 256 misleading since the contrast also depends on the sample reflectivity distribution.

257 A more reliable observable is the spatial correlation of the  $\mathbf{T}$ -matrix between neighbor  
 258 patches as displayed in Fig.6e of the accompanying paper. While a spatial window of  $6 \times 6$   
 259  $\mu\text{m}^2$  preserves a short-range correlation between neighbor windows (see inset of Fig. S3b), a  
 260 spatial window of  $3 \times 3 \mu\text{m}^2$  leads to a fully spatially incoherent estimator  $\hat{\mathbf{T}}$  (see inset of  
 261 Fig. S3c). This observable clearly shows whether the estimator  $\hat{\mathbf{T}}$  leads to a coherent (i.e  
 262 physical) or incoherent (i.e bucket-like) compensation of multiple scattering. The number of  
 263 iterations in the phase reversal algorithm has thus been based on this  $\mathbf{T}$ -matrix correlation  
 264 criterion.

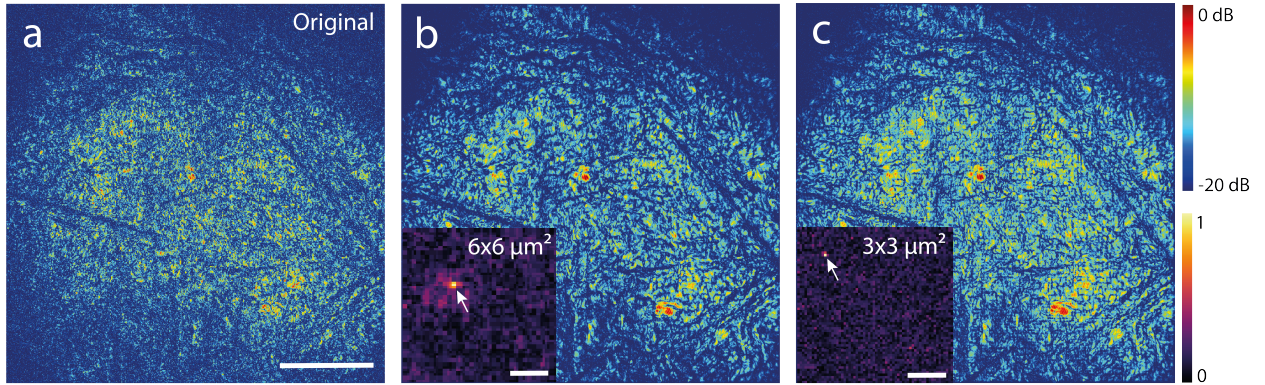


FIG. S3. **Confocal images at several steps of the multi-scale analysis.** **a** Initial en-face image of the cornea at depth  $z \sim 100 \mu\text{m}$ . **b** RMI image based on a  $\mathbf{T}$ -matrix estimator of spatial resolution  $L = 6 \mu\text{m}$ . **c** RMI image based on a  $\mathbf{T}$ -matrix estimator of spatial resolution  $L = 3 \mu\text{m}$ . The spatial correlation of  $\hat{\mathbf{T}}$  with respect to one reference location (white arrow) is displayed in insets of panels b and c. Scale bars :  $50 \mu\text{m}$ .



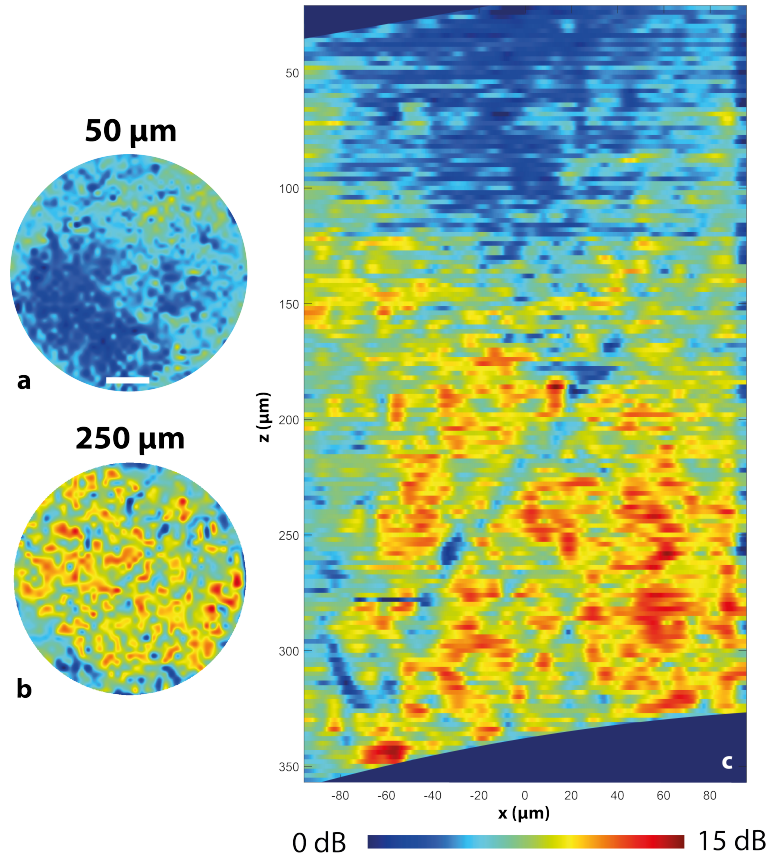


FIG. S4. **Confocal gain provided by the matrix imaging process.** **ab.** Transverse cross-section of the confocal gain observed for the *en-face* images displayed in Fig. 3b at depths 50 μm and 250 μm within the cornea [scale bar: 50 μm]. **c.** Longitudinal cross-section of the confocal gain observed by comparing the B-scan displayed in Fig.3f with its original version shown in Fig. 3c. In each panel, the color scale is in dB.

266 Figure S4 shows the enhancement of the confocal peak before and after RMI. It reaches a  
 267 maximal value of 30. This gain should scale, in amplitude, as the number  $P_c$  of independent  
 268 coherence grains exhibited by the  $\mathbf{T}$ -matrix in the pupil plane (see, for instance, Figs. 4e and  
 269 f) and that RMI tends to realign in phase by means of a digital optical phase conjugation.  
 270 Figure S4b clearly shows that the confocal gain increases with depth  $z$ . Indeed, multiple  
 271 scattering becomes predominant in depth and the transmission phase laws become more and  
 272 more complex. Note, however, that given the complexity of phase laws displayed in Figs. 4e  
 273 and f, we could have expected a larger confocal intensity enhancement. This moderate gain

274 in contrast is explained by the fact that a part of the multiple scattering background is not  
275 addressed by RMI.

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