Controlling light in scattering media non-invasively using the photoacoustic transmission matrix

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Optical wavefront shaping has emerged as a powerful tool for manipulating light in strongly scattering media. It enables diffraction-limited focusing and imaging at depths where conventional microscopy techniques fail. However, to date, most examples of wavefront shaping have relied on direct access to the targets or implanted probes, and the challenge is to apply it non-invasively inside complex samples. Recently, ultrasonic-tagging techniques have been utilized successfully, but these allow only small acoustically tagged volumes to be addressed at each measurement. Here, we introduce an approach that allows the non-invasive measurement of an optical transmission matrix over a large volume, inside complex samples, using a standard photoacoustic imaging set-up. We demonstrate the use of this matrix for detecting, localizing and selectively focusing light on absorbing targets through diffusive samples, as well as for extracting the scattering medium properties. Combining the transmission-matrix approach with the advantages of photoacoustic imaging opens a path towards deep-tissue imaging and light delivery utilizing endogenous optical contrast.

The microscopic-scale inhomogeneity of complex samples such as biological tissues leads to light scattering, thereby limiting the penetration depth of current optical imaging and light delivery techniques. Light scattering results in a decrease in intensity and a loss of spatial resolution, thus limiting optical penetration. On a micrometre scale, interference between scattered-light components is manifested as random speckle patterns. Although usually disregarded in deep-tissue, diffused-light imaging techniques, these complex random interferences are deterministic. They can therefore be manipulated coherently and exploited for focusing and imaging by controlling the input wavefront.

Focusing scattered light with wavefront shaping requires a feedback signal from the targeted focal point. Such feedback allows one to maximize the intensity on the target through iterative optimization, phase conjugation or by calculating the complex relations between input incident modes and output target modes. The last of these, the ‘optical transmission matrix’ approach, essentially parallelizes the acquisition of information from multiple target points in a large field of view, allowing the generation of a desired focus on one or several targets at will, as well as the study of important properties of the scattering medium such as the optical memory effect and transmission eigenchannels.

In most wavefront-shaping demonstrations to date, the feedback signal has been provided by a camera or a detector placed at the target plane, behind the scattering sample. To focus inside a scattering sample, one has to find an alternative method to monitor the optical intensity at the target point(s). Implanting fluorescent or second-harmonic ‘guide stars’ is one successfully studied option, but, as well as being invasive, this only allows focusing in the vicinity of a single static target. Ultrasound tagging offers dynamic and flexible control over the probe position. However, each such acousto-optical measurement is limited to a single ultrasonically tagged volume. Thus, utilizing ultrasonic tagging for imaging large volumes requires scanning of the ultrasonic focus and therefore requires long acquisition times.

Here, we present an approach to non-invasively measure a transmission matrix inside a scattering medium by exploiting the photoacoustic effect. Our approach allows us to optically investigate a large volume (containing many ultrasonic resolution cells) using a standard photoacoustic imaging set-up in conjunction with a spatial light modulator (SLM) (Fig. 1a). Our approach is non-invasive thanks to the fact that the feedback signals are acoustic waves, generated by light absorption at the targets and measured by an ultrasonic transducer placed outside the sample (Fig. 1a). We demonstrate that the information contained in this matrix can be used directly to detect, localize and selectively focus light on any absorbing target within the field of view of the photoacoustic imaging system, using a measurement sequence that is independent of the number of investigated targets, resolution cells or the size of the probed volume. Kong and colleagues first investigated photoacoustic feedback for focusing on a single target and using an optimization-based approach, but here we are able to investigate a large field of view and multiple targets using a considerably shorter acquisition sequence. In addition to focusing through various scattering samples including 0.5-mm-thick chicken-breast tissue, we retrieve the optical ‘memory effect’ of the scattering medium, and discriminate and localize targets using singular value decomposition (SVD) analysis.

Principle

To selectively focus light on any single target from a large number of target points inside or through a scattering medium, one has to know the input–output phase relations between each optical input mode (that is, the optical field on an SLM pixel \( E_{n}^{\text{in}} \); \( n=1, \ldots, N_{\text{SLM}} \)) and the field at each target point output mode (\( E_{m}^{\text{out}} \); \( m=1, \ldots, M \)), where \( N_{\text{SLM}} \) is the number of controlled SLM pixels. For a linear propagation medium, this relationship is given by the complex-valued \( M \times N \) optical transmission matrix \( T \) with elements \( t_{mn} = \langle E_{m}^{\text{out}} | E_{n}^{\text{in}} \rangle \). The complex optical field at the \( m \)th output point is then given by \( E_{m}^{\text{out}} = \sum_{n=1}^{N_{\text{SLM}}} t_{mn} E_{n}^{\text{in}} \), and the SLM phase pattern required to focus light on this point is given by \( \varphi_{\text{SLM}} = \arg \{ E_{m}^{\text{out}} \} = -\varphi_{m} \).

In all works performed to date, measuring the transmission matrix \( T \) has been realized by imaging the target plane on a

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camera\textsuperscript{12–15}. The use of a camera enables the influence of each SLM pixel on a large number of output modes (limited by the number of camera pixels) to be mapped. This is an advantage over iterative optimization\textsuperscript{5,22} or measurements with a single-pixel detector\textsuperscript{31}, where only a single output point is investigated (that is, a single row of $T$). To date, the main limitation of the transmission-matrix approach has been the requirement to directly (and thus invasively) optically image the output plane. Here, we demonstrate that this limitation can be removed by replacing the camera with a photoacoustic imaging set-up\textsuperscript{26,28}.

In photoacoustic imaging\textsuperscript{26,28}, when a laser pulse hits an absorbing portion of the sample (for example, a small blood vessel\textsuperscript{27}), the local increase in temperature generates an ultrasound pulse that propagates away from the absorber. The amplitude of the ultrasound wave is proportional to the light intensity on the absorber.

Figure 1 | Measuring a photoacoustic transmission matrix. a, Experimental set-up. A nanosecond pulsed laser beam is shaped by an SLM and illuminates a phantom placed behind a scattering sample. A 30 MHz spherically focused ultrasonic transducer with an elongated pencil-shaped field of view measures the photoacoustic signals from absorbing targets embedded in the phantom. b, Measured photoacoustic (PA) trace following a single laser shot through a scattering diffuser; signals at different time delays correspond to targets at different axial positions. c, The measured complex-valued transmission matrix gives the influence of each SLM pixel (vertical axis) on each acoustic voxel (horizontal axis). Hue represents phase, and brightness represents amplitude. d, Measurements. The phase of each SLM input mode (in the Hadamard basis) is scanned from 0 to $2\pi$ in $K$ steps, and the corresponding photoacoustic trace is measured for each phase step. The unshaped part of the circular input beam serves as a fixed reference field\textsuperscript{12}. The signal peak-to-peak value is then analysed for a 90 ns time window scanned across the signal to obtain the influence of every input mode on each of the time windows (all acoustic resolution cells). e, Measurement process. The peak-to-peak signal of each time window closely follows a cosine modulation as a function of the phase of an input mode (Supplementary equation (2)); the photoacoustic transmission-matrix elements are retrieved directly from these cosine modulation phases $\theta$ and amplitudes $\beta$ for all input-output mode pairs $j$. The matrix is converted to the SLM-pixel input basis by a basis transformation.
negligible scattering, it can be measured non-invasively by an ultrasonic transducer (or a set of transducers\textsuperscript{28,29}) placed outside the sample\textsuperscript{28} (Fig. 1a). After a single laser shot, the photoacoustic signals from all absorbing targets in the transducer’s field of view are recorded with times of arrival corresponding to their distance from the transducer (Fig. 1b). An image of the distribution of the absorbers is then formed from the time-resolved signal(s). Using a standard one-dimensional array of transducers\textsuperscript{29}, a single laser pulse yields a two-dimensional image\textsuperscript{29}. Alternatively, a single spherically focused transducer with an elongated pencil-shaped beam provides a one-dimensional image within its depth of field directly from the signals’ times of arrival using a single laser pulse\textsuperscript{28} (Fig. 1b). Thus, a photoacoustic imaging system serves, in essence, as a virtual camera sensing the light absorption distribution inside the medium, with a resolution limited by the size of the acoustic focus.

A photoacoustic imaging set-up can thus replace the camera for the non-invasive measurement of the transmission matrix inside a scattering medium. The main difference is then that, unlike an optical camera, the resolution of this ‘acoustic camera’ may not be able to resolve a single optical mode (that is, speckle grain). In such a case, the photoacoustic signal amplitude from a given position results from several optical modes within the ultrasound resolution cell (determined by the numerical aperture (NA) and the frequency response of the acoustic transducer). However, the same phase-shifting transmission-matrix measurement protocol of Popoff et al.\textsuperscript{12} can be used to retrieve and modulate the local absorbed energy, which corresponds to the sum of acoustic emissions of all absorbers contained in the probed resolution cell (for a detailed analysis see Supplementary equations (1) to (6)). As in the all-optical transmission-matrix measurement\textsuperscript{13}, thanks to the linearity of the photoacoustic generation process, when phase-shifting the input modes, the photoacoustic signal from each ultrasound resolution cell is cosine-modulated (Supplementary Fig. 2b). From these modulations, the photoacoustic transmission matrix is retrieved (Fig. 1e).

For a proof-of-principle experimental demonstration of the approach, an agarose gel phantom containing 30-μm-diameter absorbing nylon wires was illuminated through a scattering sample by a 5 ns pulsed laser beam, shaped by a phase-only SLM (Fig. 1a; see Methods for details). These absorbing wires could, for instance, be considered to mimic capillary vessels, one of the targets studied using photoacoustics\textsuperscript{27,28}. A 30 MHz spherically focused ultrasonic transducer detected the photoacoustic signals generated by the absorbing targets. We chose to use a high-frequency single focused transducer with an elongated pencil-shaped beam to directly obtain one-dimensional photoacoustic cross-sections of the phantom with high resolution\textsuperscript{28}. The raw measured signals in a time-resolved photoacoustic trace from such a focused transducer have time delays that directly correspond to the targets’ axial positions (that is, depths) along the axis of the transducer over its depth of field (Fig. 1b).

To acquire the photoacoustic transmission matrix we used a procedure based on the phase-shifting technique presented by Popoff and colleagues\textsuperscript{12}. The measurement process was composed of three steps (for details see Methods): (1) sequential phase-shifting of each input mode from 0 to 2\(\pi\) and recording the corresponding photoacoustic images (Fig. 1d); (2) recording, within each resolution cell (a time window in this one-dimensional experiment), the peak-to-peak voltage (Fig. 1d); and (3) by analysing the peak-to-peak cosine modulation, extracting the amplitude and phase of each coefficient of the transmission matrix (Fig. 1e). The photoacoustic transmission matrix \(TPA\) was directly obtained for all time windows (thus all absorbing targets), simultaneously from a number of measurements independent of the number of targets or the acoustic field of view.

An example of a photoacoustic transmission matrix obtained experimentally through an optical diffuser is presented in Fig. 1c. This matrix describes the influence of each input mode (SLM pixel, vertical axis) on each ultrasound resolution cell (time window in photoacoustic trace, horizontal axis).

### Controlled focusing using the transmission matrix

As a first demonstration for utilizing the measured photoacoustic transmission matrix we present deterministic selective light focusing on several absorbers along the focus of the ultrasonic transducer (Fig. 2). The results for focusing through an optical diffuser are presented in Fig. 2a–c, and the results obtained through a sample of \(\sim 500\)-μm-thick chicken-breast tissue are presented in Fig. 2d–f. Figure 2a,d shows the experimentally measured transmission matrices in the two experiments. The SLM phase pattern required to selectively focus on a target located at a specific axial position \(z_m = c_t \tau_m\) is simply the phase conjugate of the transmission-matrix column corresponding to time delay \(\tau_m\) (Fig. 2b,e, Supplementary equation (6))\textsuperscript{12}. The photoacoustic signals obtained when displaying two of these focusing phase patterns on the SLM are presented in Fig. 2c,f. These confirm a selective enhancement of the photoacoustic signals of the selected targets.

The expected intensity enhancement when focusing with the all-optical transmission matrix is given by\textsuperscript{23} \(\eta = 0.5N_{\text{SLM}}/N_{\text{modes}}\). This is proportional to the number of controlled degrees of freedom, \(N_{\text{SLM}}\), and inversely proportional to the number of optical modes (speckle grains) whose intensity is enhanced, \(N_{\text{modes}}\). In our experiments, \(N_{\text{modes}}\) corresponds to the number of modes enclosed within the focusing resolution, \(\Delta x \approx 100\ \mu m\), that is, the number of speckles contained in the target absorbing area intersecting the acoustic focus. In the results presented in Fig. 2, \(N_{\text{modes}}\approx 6\), and the expected intensity enhancement is \(\eta \approx 0.5 \times 140/6 \approx 11.5\) (Supplementary Section 3), which is close to the experimental enhancement factors of \(\eta \approx 6\). We attribute the lower experimental enhancement to factors such as optical speckle decorrelation during the experiments, the presence of noise in the measurements, and the uneven contribution of the SLM pixels to the photoacoustic signal at a given position, leading to a lower effective number of degrees of freedom. We note that, with knowledge of the transmission matrix, one is not limited to focusing on a single target, and any intensity distribution on the targets can be generated\textsuperscript{12,17}.

### Probing the optical ‘memory effect’

The information encoded in the optical transmission matrix is much richer than that simply required to focus on a single or multiple targets. Here, we experimentally demonstrate that it allows the scattering sample’s optical memory effect to be probed\textsuperscript{25}.

The memory effect for speckle correlations is, in a nutshell, the effect whereby a multiply scattering sample of thickness \(L\) retains a range of isoplanatism; that is, the speckle patterns generated by plane waves at different incident angles \(\theta_1, \theta_2\) are correlated as long as \(\Delta \theta = \theta_1 - \theta_2\) is smaller than \(\sim \lambda/2\pi L\), where \(\lambda\) is the wavelength of the incident light\textsuperscript{25}. Similarly, in weakly aberrating samples this is known as isoplanatism, both in optics\textsuperscript{12} and acoustics\textsuperscript{29}. The memory effect is important not just because it is a mesoscopic property of the medium\textsuperscript{13}, but also because it can serve to scan the focus obtained by wavefront shaping\textsuperscript{7,11} and allow real-time imaging within its range\textsuperscript{6}.

In the measured photoacoustic transmission matrix, as presented in Fig. 1c, the phases of each column \(m\) represent the SLM phase pattern \(\varphi_{\text{SLM}}(x, z)\) required to focus on a target absorber at an axial position \(z_m = c_t \tau_m\) from the ultrasonic transducer (Figs 2b,3a). If these targets are within the sample’s memory-effect range, the difference between each such two SLM phase patterns would typically be just a tilt (Fig. 3b). The \(x\)-space representation of this tilt would then be a shifted delta function. Thus, to
examine the memory-effect range one can plot the $k$-space representation of the phase differences between the transmission-matrix columns (Fig. 3, Supplementary Section 4). In the case of perfect memory effect, such analysis would give a diagonal matrix, representing the angular (that is, $k$-space) correlations. We performed this analysis on the experimental transmission matrix measured through a thin diffuser (Fig. 1c) for a tilt along the $z$-direction, and obtained the result presented in Fig. 3c. The result reveals the $k$-space correlations of the transmission-matrix columns, highlighting the memory effect expected from such a thin scatterer. The apparent wrapping of the large $k$-space components ($k$-space aliasing, or equivalently grating lobes) is a result of the low SLM resolution used in this experiment. Only two absorbing wires are in the focus of the transducer in this experiment.

Target localization by SVD

A powerful tool in the analysis of the scattering matrix is SVD. Recently, SVD of the optical transmission matrix was used to identify transmission eigenchannels\textsuperscript{12} and maximize energy transport in multiply scattering samples\textsuperscript{24}, and the SVD of the backscattering matrix was utilized to discriminate and selectively focus on individual nanoparticles through aberrating media\textsuperscript{30}, a result analogous to works carried out for ultrasound\textsuperscript{34,35}. Similarly, here we show that the SVD of the photoacoustic transmission matrix enables the identification and discrimination of individual absorbing targets and gives the wavefronts required for selective focusing without any \textit{a priori} information on the targets’ number or positions.

To this end, we computed the SVD of the photoacoustic transmission matrix measured through a diffuser. We present the results of this analysis in Fig. 4. Performing an SVD of a matrix $T$ consists of writing $T = U \Sigma V^*$, where $\Sigma$ is a rectangular diagonal matrix containing the real positive singular values $\lambda_i$ in descending order, and $U$ and $V$ are unitary matrices whose columns correspond to the output and input singular vectors $U_i$ and $V_j$, respectively. In the case of the photoacoustic transmission matrix, each input singular vector $V_j$ corresponds to the SLM phase pattern for focusing on a
target with photoacoustic modulation intensity $I$. The corresponding output singular vector $U_i$ is the expected photoacoustic modulated temporal trace for this singular value, that is, the corresponding absorber’s position.

Plotting the obtained singular values in descending order (Fig. 4b), one can observe that the distribution has two parts, forming a continuum of low singular values (associated with background noise) and a few higher singular values (associated with strong absorbing targets) (highlighted in Fig. 4b). Plotting the output singular vectors, $U_i$, that correspond to these singular values reveals temporally localized photoacoustic responses for the large singular values (Fig. 4a). This shows that SVD can be used to give the positions of the absorbing targets within the limitation of the ultrasound resolution, that is, one absorbing target per ultrasound resolution cell. Such an approach advantageously replaces the subjective and imprecise visual inspection of the transmission matrix used to select the time delays for focusing in Fig. 2. The corresponding input singular vectors $V_i$ can be displayed on the SLM to ‘automatically’ guide light to these targets.

**Discussion**

We have demonstrated a non-invasive approach to optically interrogate a large number of absorbing targets and selectively focus light inside scattering media, using a standard photoacoustic imaging setup. Although the focusing dimensions reported here were limited by the photoacoustic imaging resolution (see Methods), interestingly, in the case of an isolated small absorber, light can be concentrated onto the absorber in principle down to the optical speckle-grain dimensions. Related to this issue of resolution, it has been shown very recently that the acoustic resolution limit can be surpassed by analysing the variance of different ultrasonically tagged optical fields. It would be appealing to find ways to introduce similar notions of this novel approach (acronym TROVE) to photoacoustics, to complement the advantage of its drastically improved resolution with the large field of view and parallel acquisition of the presented technique. An additional advantage of the presented technique is its simple experimental implementation. It makes use of a standard photoacoustic imaging setup and does not require a reference arm, careful timing of acoustic and optical pulses, or precise positioning of the SLM.

Interestingly, one may point out that all wavefront-shaping techniques are fundamentally based on characterizing a transmission matrix. In both optimization and acousto-optic tagging (‘TRUE’) approaches, a single output mode is targeted and a single row (‘vector’) of the transmission matrix is acquired per acquisition sequence. TROVE has the additional advantages that it essentially measures a whole matrix, with optical resolution. In all these techniques, the acquisition time scales with the number of targets or resolution cells. In the photoacoustic transmission matrix technique presented here, the measurement time depends only on the number of input modes.

It is important to note that, unlike acousto-optic techniques, photoacoustics requires the presence of an absorber. However, absorption is perhaps the most common optical contrast mechanism; it is present in all fluorescent markers and often also in unstained tissues. As such, it forms the basis for the very large field of photoacoustic biomedical imaging. Although the photoacoustic transmission matrix enables light to be delivered only to locations where absorption is present, these are exactly the positions that are targeted by laser therapy and fluorescence imaging techniques. Our proof of principle demonstrated focusing through scattering samples, but the proposed approach could similarly be applied to focusing inside scattering media. To focus with high efficiency inside scattering samples one has to maintain a high ratio between the number of controlled degrees of freedom and the number of optical modes on each target within the acoustic focus. Given the submicrometre diffraction-limited dimensions of optical modes inside tissues, this goal can be met by the combined use of a high-resolution SLM, absorbing targets of small dimensions and a high-frequency and large-NA acoustic detection system.

Another practical aspect is the reduction of the acquisition time. In this work, the matrix acquisition time of a few tens of minutes was limited by the repetition rate of the laser used (10 Hz) and the signal-to-noise ratio (SNR). Using higher-repetition-rate lasers and fast SLMs with a higher optical energy damage threshold is expected to significantly reduce the acquisition time, potentially reaching the subsecond acquisition times of the optical transmission matrix. Because the total acquisition time is dictated by the number of measurements per input mode and the number of controlled input modes, a trade-off exists between the acquisition...
time and focusing efficiency, which can be advantageous in certain applications.

We used photoacoustic feedback to perform light focusing at depth, but the method could also benefit the field of photoacoustic imaging. Photoacoustic imaging is now a mature technique for tissue imaging at large optical depths\(^28\), but it currently relies on diffuse homogeneous illumination. Our method enables us to spatially tailor the illumination to the regions of interest, providing the possibility to reveal features at even larger depths and with reduced light exposure levels. The advantage of the presented technique lies in its generality. In particular, by replacing the reduced light exposure levels. The advantage of the presented method enables us to spatially tailor the illumination to the regions of interest, providing the possibility to reveal features at even larger depths and with reduced light exposure levels. The advantage of the presented approach lies in its generality. In particular, by replacing the single ultrasonic transducer with a standard array of transducers\(^28,29\), a direct two-dimensional mapping would be obtained with the same acquisition time and number of measurements reported here\(^29\). One may also imagine extending the matrix method to other contrast mechanisms that can be probed locally and non-invasively, such as the real-time probing of local heat deposition via magnetic resonance imaging\(^36\).

Methods

Experimental set-up. The complete experimental set-up is presented in Supplementary Fig. 1. An agarose gel phantom containing 30-μm-diameter absorbing nylon wires (NYL02DS, VetSuture) was placed behind the scattering sample. The scattering samples were provided by a 0.5-μm-diameter Newport light-shaping diffuser, which spread the light evenly with no notable ballistic component, and a 0.5-mm-thick chicken breast sample, partially dried and sandwiched between two glass slides. The phantom was illuminated through the scattering sample by an attenuated beam from a 5 ns pulsed laser source (Continuum Surelight, 10 Hz repetition rate, <10 mJ pulse energy at a wavelength of 532 nm), shaped by a phase-only SLM (Boston Micromachines Multi-DM). The SLM was a 12 × 12 controllable segmented micromirror array, where the corner mirrors of the array were fixed and we controlled the 140 remaining mirrors. The photoacoustic signals generated by the absorbing wires were detected by a focused ultrasonic transducer (SNXI10509, HF113, Sonaxis) with a central frequency of \(f \approx 28\) MHz, pulse-echo bandwidth \(B = 27\) MHz (\(\approx 6\) dB), diameter \(D = 3.25\) mm and focal length \(F = 7.5\) mm. The corresponding imaging spatial resolution is given transversely by \(\Delta x = (c_f / f) \times (F / D) \approx 100\) μm, and axially by the transducer’s impulse response, \(\Delta z \approx c_s \Delta \tau \approx (c_s / B) \approx 60\) μm.

The signal from the transducer was amplified by a preamplifier (Model 5900PR, Sofranel) and recorded on an oscilloscope (Lecroy WaveSurfer 104 MXs-B). The oscilloscope signal was sampled at 1 GHz, and digitally filtered by a bandpass filter between 2 and 60 MHz. To minimize the effects of laser pulse-to-pulse intensity fluctuations, the signal from each pulse was normalized by the laser pulse intensity as measured by a photodiode (Supplementary Fig. 1).

Transmission-matrix measurement. To acquire the photoacoustic transmission matrix we used a procedure based on the phase-shifting technique presented by Popoff and colleagues, where the unshaped part of the circular input beam outside the active area of the SLM serves as a fixed reference field\(^12\). Our procedure was composed of three steps. (1) As depicted in Fig. 1d, the phase of each input mode was sequentially shifted from 0 to \(2\pi\) in \(K = 16\) steps. To achieve maximal modulation, the Hadamard input basis was used rather than the SLM pixel basis\(^12\).

Figure 4 | Localizing absorbing targets by SVD of the transmission matrix. Results for SVD of the photoacoustic matrix measured through a diffuser (Fig. 1c). a, The first 25 output singular vectors \(U_i\), weighted by their corresponding singular values \(l_i\). b, The first 25 singular values \(l_i\). A few singular values rise above the continuum of background-noise singular values. c, Raw average photoacoustic time trace, showing an excellent correspondence between the output singular vectors of a and the time delays where absorbing targets can be subjectively identified.
To span the 140 controlled degrees of freedom in the Hadamard basis we used the smallest Hadamard basis of size $\geq 140$ generated by MathWorks' MATLAB's 'Hadamard' function, which is $160$. (2) The recorded signals were then analysed to retrieve the influence of each SLM input mode on the intensity value of each 'voxel' in the photoacoustic image. Here, we defined the voxels' values as the signal's peak-to-peak voltage at each 90 ns time window (Fig. 1d, 1st time windows, peak-to-peak, are depicted by red, green and blue arrows). The time window width was carefully chosen to match the typical impulse response of the transducer for a single isolated absorber, maximizing the signal-to-noise while maintaining spatial resolution (Supplementary Fig. 2). For each phase step, the photoacoustic signals corresponding to five successive laser pulses were recorded and averaged. (3) The modulation of each voxel value as a function of the $K$ phase shifts of each SLM input mode was analysed by computing a discrete Fourier transform (Supplementary equation 4)). For each voxel $m$ and input mode $n$, the amplitude and phase of this modulation, $B_m^n$ and $\theta_m^n$, respectively, give the photoacoustic transmission matrix element value $u_{mn} = B_m^n e^{i\theta_m^n}$ (Fig. 1e, Supplementary Section 2). In principle, this analysis requires $K \geq 3$ steps to satisfy Nyquist rate sampling. We used $K = 16$ to validate the assumption of signal linearity and to improve the SNR (Supplementary Fig. 2b). Experimentally, the peak-to-peak signals closely followed the expected cosine modulation as a function of each input-mode phase (Supplementary Fig. 2b), confirming the linearity assumption (Supplementary equations 1 to (3)). Equivalently, $K = 3$ could be used and the SNR could be improved by averaging more pulses per phase step. In total, the photoacoustic transmission matrix is obtained from $N_{\text{SLM}}$ measurements, resulting in a total acquisition time of $\sim 40$ min in our experiments ($K = 16$, $N_{\text{SLM}} = 160$ acquired at a 10 Hz laser repetition rate) including all latencies, triggering and communication delays. Once the transmission matrix was measured in the Hadamard input basis, it was converted to the canonical (input SLM pixels) basis by a unitary transformation (Hadamard–canonical basis transformation$^5$).

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Author contributions

S.G. and E.B. designed the initial experimental set-up. O.K. proposed the photoacoustic transmission matrix approach and analysis. S.G., E.B., T.C. and O.K. discussed the experimental implementation. T.C. and O.K. performed the experiments and analysed the results. S.G., E.B., T.C., O.K., A.C.B. and M.F. contributed to discussing the results and writing the manuscript.

Additional information

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Competing financial interests

The authors declare no competing financial interests.