Compensation of sample-induced optical aberrations is crucial for visualizing microscopic structures deep within biological tissues. However, strong multiple scattering poses a fundamental limitation for identifying and correcting the tissue-induced aberrations. Here, we introduce a label-free deep-tissue imaging technique termed dimensionality reduction adaptive-optical microscopy (DReAM) to selectively attenuate multiple scattering. We established a theoretical framework in which dimensionality reduction of a time-gated reflection matrix can attenuate uncorrelated multiple scattering while retaining a single-scattering signal with a strong wave correlation, irrespective of sample-induced aberrations. We performed mouse brain imaging in vivo through the intact skull with the probe beam at visible wavelengths. Despite the strong scattering and aberrations, DReAM offered a 17-fold enhancement of single-scattering-to–multiple scattering ratio and provided high-contrast images of neural fibers in the brain cortex with the diffraction-limited spatial resolution of 412 nanometers and a 33-fold enhanced Strehl ratio.

INTRODUCTION

In deep-tissue optical imaging, spatial heterogeneity in biological tissues leads to complex wavefront distortion. This blurs the point-spread function (PSF), thereby obscuring the image of fine structures. Therefore, resolving the wavefront distortion has been a notable issue for enhancing the depth of high-resolution optical imaging (1–6). For the past couple of decades, various adaptive optic (AO) microscopes have been developed to compensate for sample-induced aberrations. They can largely be classified into hardware- and software-based approaches. In the former, a wavefront sensor is installed in the pupil plane to measure the aberration, and a wavefront shaping device is used to compensate for the measured aberration (7–9). Alternatively, the wavefront shaping device is controlled to optimize the image quality metrics, such as the image brightness and sharpness, without wavefront sensing (10–14). They have been used to visualize synaptic structures in deep cortical and subcortical areas of the mouse brain and somatosensory-evoked calcium responses in the mouse spinal cord (14).

Software-based AO approaches measure the wavefront of backscattered waves, and computational postprocessing is applied to optimize image quality metrics (15–18). They have been applied to visualize photoreceptor cells in the retina of a living human by the correction of ocular aberrations. Methods measuring a time-gated reflection matrix have been introduced in recent years to address complex aberrations in the presence of multiple scattering (19–21). The previous studies usually use confocal detection and discard signals arriving at nonconfocal points (15). On the contrary, full interactions are characterized between a light wave and a complex sample since the reflection matrix approach measures all the backscattered waves, including both confocal and nonconfocal signals, for all the orthogonal input modes. This allows computational compensation of wavefront distortion as if one can conduct hardware-based correction but with a much faster speed and ingenious operations. This has made it possible to realize diffraction-limited imaging through an intact mouse skull obscured by extremely complex aberrations in the near-infrared excitation (22).

The working depth of all the AO microscopy methods is determined by the degree of multiple scattering and the complexity of aberration. Multiple scattering disrupts the fidelity of wavefront sensing and undermines the estimation of image metrics required for the optimization. In this respect, those approaches using temporal or confocal gating or both have achieved deeper depth imaging. We note that the singular value decomposition (SVD) of a reflection matrix can provide a way to attenuate multiple scattering, independent of the existing gating operations. Initially, the SVD of a transmission matrix was used to exploit the wave correlation of multiple-scattered waves to enhance energy transmission through a scattering layer (23–27). According to random matrix theory, specific eigenchannels can have the maximum eigenvalue reaching unity transmittance (28). The underlying physics is that the eigenchannel of the large eigenvalue can induce strong constructive interference (29). In the reflection mode, wave correlation of the ballistic backscattered waves from target particles (30) or multiple-scattered waves reflected from spatially confined targets was used to focus light energy on the targets (31). From an imaging perspective, SVD of a time-gated reflection matrix was used to select the reflection from highly reflecting particles and a resolution target for...
enhanced depth imaging (32). However, these methodologies were difficult to apply for in vivo imaging where complex tissue aberrations undermine image reconstruction.

Here, we propose a label-free deep-tissue imaging technique termed dimensionality reduction adaptive-optical microscopy (DReAM). We established a theoretical framework in which dimensionality reduction of a time-gated reflection matrix can retain a single-scattering signal with a strong wave correlation and attenuate uncorrelated multiple scattering, irrespective of sample-induced aberrations. This enables us to enhance the single scattering–to–multiple scattering ratio (SMR) with minimal impairment to single-scattering signal, which leads to a substantial enhancement of the fidelity of adaptive-optical imaging. We performed in vivo imaging of a mouse brain through an intact skull using a visible-wavelength excitation. Imaging through a skull has been possible only in the near-infrared regime (22) where the scattering mean free path is approximately a couple of times longer than the visible regime; however, the image contrast and resolving power are lower. Despite the strong scattering and aberration at the visible wavelength, we demonstrated a 17-fold increase in SMR and thus visualized the neural fibers in the brain cortex with the diffraction-limited spatial resolution (412 nm) and a 33-fold enhancement of the Strehl ratio. This corresponds to an effective imaging depth increase of 2.4 to 4.2 times the scattering mean free paths. We provided quantitative criteria for the optimal choice of the cutoff eigenchannel for dimensionality reduction through numerical simulation and experimental data analysis. We also proved the inner workings of the proposed method by showing that the choice of eigenchannels with large reflectance increases the focusing of excitation energy to a target by more than 80 times.

RESULTS
Exploiting single-scattering wave correlation

A backscattered wave from an object embedded within a scattering medium is composed of single- and multiple-scattered waves

$$E_R(k; \tau; k_{in}) = E_S(k; \tau; k_{in}) + E_M(k; \tau; k_{in})$$ (1)

where \(k_{in}\) and \(k\) denote the wave vectors of the incident and reflected waves, respectively, and \(\tau\) represents the flight time in the case of time-gated detection. In deep-tissue imaging, a single-scattering signal is much weaker than multiple-scattering backgrounds even after temporal gating (\(|E_S(k; \tau; k_{in})| \ll |E_M(k; \tau; k_{in})|\)). This makes it critical to reduce multiple scattering for enhancing the imaging depth and correcting the sample-induced aberration.

Many biologically interesting structures, including axons, dendrites, and microtubules, have spatially localized fine features. The backscattered waves from these objects provide a strong wave correlation of the single-scattered waves even in the presence of complex tissue-induced aberrations. Let us consider the following single-scattered wave \(E_S\) to clarify this matter

$$E_S(k; \tau; k_{in}) = \sqrt{\alpha(z)} e^{i\phi_z(k)} \tilde{O}(k - k_{in}) e^{i\phi_m(k_{in})}$$ (2)

where \(\tilde{O}(k)\) indicates an object spectrum and \(\alpha(z)\) represents the attenuation of the single-scattered wave reflected from the depth \(z\) due to multiple scattering, \(\phi_m(k)\) and \(\phi_z(k)\) correspond to sample-induced aberrations in the incidence and reflection pathways, respectively. For two representative incident wave vectors, \(k_{in}^{(l)}\) and \(k_{in}^{(m)}\), their respective single-scattered waves are written as follows

$$E_S(k; \tau; k_{in}^{(l)}) = \sqrt{\alpha(z)} e^{i\phi_z(k)} \tilde{O}(k - k_{in}^{(l)}) e^{i\phi_m(k_{in}^{(l)})}$$ (3)

and

$$E_S(k; \tau; k_{in}^{(m)}) = \sqrt{\alpha(z)} e^{i\phi_z(k)} \tilde{O}(k - k_{in}^{(m)}) e^{i\phi_m(k_{in}^{(m)})}$$ (4)

The single-scattering wave correlation is then obtained as

$$\left| \left( E_S(k; \tau; k_{in}^{(l)}) E_S^*(k; \tau; k_{in}^{(m)}) \right) \right| = \alpha(z) \left| \tilde{O}^*(k - k_{in}^{(l)}) \tilde{O}(k - k_{in}^{(m)}) \right|$$ (5)

where \(\langle 0 \rangle_k\) indicates the summation of all the possible terms inside the bracket with respect to \(k\). Equation 5 intends to quantify the similarity of the single-scattered waves for different incident wave vectors, where the input aberration serves as an overall phase. It suggests that a single-scattering correlation is determined by the correlation of the object spectrum. We note that this single-scattering correlation is independent of the specimen-induced aberrations because their effects are canceled out during the inner product. If the object function \(O(r)\) has a spatially narrow distribution, then its spectrum \(\tilde{O}(k)\) is a slowly varying function with respect to \(k\). Therefore, the object spectrum in Eq. 5 can have a large correlation, and so does the single-scattering correlation (Fig. 1A). In an extreme case, when the object is a point particle, \(\tilde{O}(k)\) is flat with respect to \(k\), such that the normalized correlation of single scattering can even reach unity. In the case of multiple-scattered waves, their normalized correlation is low because reflected waves for \(k_{in}^{(l)}\) and \(k_{in}^{(m)}\) are different realizations of speckles (Fig. 1B).

We considered the application of SVD of a reflection matrix \(R\) to exploit single-scattering wave correlation. The matrix elements of \(R\) are filled with \(R_{lm} = E_S(k; \tau; k_{in}^{(l)}) E_S^*(k; \tau; k_{in}^{(m)})\) such that the column and row indices of \(R\) correspond to \(k_{in}^{(l)}\) and \(k_{in}^{(m)}\), respectively. Because \(E_S\) is the summation of \(E_S\) and \(E_M\), \(R\) can be considered as the summation of a single-scattering matrix \(S\) made of \(E_S\) and multiple-scattering matrix \(M\) filled with \(E_M\); \(R = S + M\). We then considered the SVD of the reflection matrix \(R = U \Sigma V^H\), where \(\Sigma\) is a rectangular diagonal matrix whose diagonal element \(\Sigma_{jj} = \sigma(j)\) is a non-negative real number called singular value. Singular values are sorted in descending order with respect to the eigenchannel index \(j\) such that \(\sigma(j) \geq \sigma(j+1)\). \(U\) and \(V\) are unitary matrices whose column vectors \(u_j\) and \(v_j\) correspond to the input and output eigenchannels, respectively, for the eigenchannel index \(j\). The square of singular value \(\sigma^2(j)\) is the eigenvalue of \(R^* R\), whose physical meaning is the reflectance when the input wave is coupled to the input eigenchannel \(v_j\).

The SVD decomposes \(R\) into multiple projection matrices, projecting an incident wave to orthogonal output eigenchannels weighted by their eigenvalues. This means that an arbitrary incident wave tends to be projected to eigenchannels with large eigenvalues. Essentially, the output wave has more shared contribution of high-reflection eigenchannels. Single-scattered waves are expected to contribute preferably to the high-reflection eigenchannels considering that the output waves from different \(k_{in}\) values have shared contributions of single-scattered waves due to their wave correlation (33). Our strategy is to select the first few eigenchannels to retain a
single-scattering contribution and eliminate the other eigenchannels to attenuate multiple scattering effectively, which allowed improving the fidelity of aberration correction and increasing the achievable imaging depth.

**Dimensionality reduction to enhance SMR**

We performed a numerical simulation to illustrate the working principle of the proposed method. A spatially sparse target object (Fig. 2K) was numerically prepared. Furthermore, the complex sample-induced aberration (inset in Fig. 2K) was introduced to the single-scattered waves following Eq. 2. After that, we constructed a single-scattering matrix \( S \) (see fig. S1 for details) and performed SVD, \( S = U_S \Sigma_S V_S^T \). The eigenvalue had a narrow distribution due to the spatial sparsity of the target, as shown in Fig. 1C (dashed blue curve). The participation number defined by \( \langle \tau_S(j) \rangle / \langle \tau_S^2(j) \rangle \) was 7, which is approximately the same as the number of diffraction-limit-size pixels constituting the target object. A small participation number indicates the increased correlation because the incident wave is projected to only a few eigenchannels (26). We considered that the eigenvalue distribution of a multiple-scattering matrix is a monotonically decaying function (see a red dashed curve in Fig. 1C), which is typically the case in the experiment. We then constructed a matrix, \( M = U_M \Sigma_M V_M^T \), by multiplying two random unitary matrices, \( U_M \) and \( V_M \), to the diagonal matrix \( \Sigma_M \) whose diagonal elements were filled with the square root of the eigenvalues (see figs. S8 and S9 for the design of \( \Sigma_M \) and the statistics of \( M \), respectively). The participation number of \( M \) was set to 501, close to the total number of orthogonal channels (\( N_{\text{tot}} = 697 \)) given by the effective number of pixels in the view field. This implies that multiple-scattered waves are primarily uncorrelated.

We considered a case when SMR at the initial state was 1/80, i.e., \( I_S = I_M/80 \) with \( I_S = \langle |E_S(k, \tau; k_{im})|^2 \rangle = \alpha(z) \langle |\hat{O}(k)|^2 \rangle \) and \( I_M = \langle |E_M(k, \tau; k_{im})|^2 \rangle \), where \( \langle \rangle \) indicates average operation. In the actual experiments, one can only measure the total reflection matrix \( R \) and not the \( S \) and \( M \) constituting \( R \). Therefore, only the eigenvalues and eigenchannels of \( R \) can be obtained from its SVD. The solid black curve in Fig. 1C shows the eigenvalues \( \tau_R(j) \) of \( R \) with respect to \( j \). We investigated the contribution of \( S \) and \( M \) made by each input eigenchannel \( v_i \) to \( R \), which was estimated by \( e_S(j) = \|Sv_i\|_2 \) and \( e_M(j) = \|Mv_i\|_2 \), respectively. Here, \( \|\cdot\|_2 \) indicates L2 norm. They are plotted as blue and red solid curves, respectively (see Fig. 1C). The contribution of single scattering \( e_S(j) \) is predictably high at the first few channels, and its distribution resembled \( \tau_S(j) \) of \( S \). On the contrary, \( e_M(j) \) was reduced with respect to \( \tau_M(j) \) because of the increased contribution of single scattering, while it remained similar to \( \tau_M(j) \) for large \( j \) where multiple scattering was dominant over single scattering.

We selected the first few eigenchannels where a single-scattering contribution was concentrated and discarded the rest to increase the SMR. Specifically, we applied dimensionality reduction to \( R \) by setting \( \tau_R(j) = 0 \) for \( j > N_c \), where \( N_c \) denotes a cutoff eigenchannel index. This modifies \( R \) to a reduced matrix \( R_d \). This operation is identical to that widely used in data science for image compression; however, its purpose in our study was to increase SMR. \( R_d \) can be considered as the summation of modified single- and multiple-scattered waves: \( R_d = S_d + M_d \). The measured backscattered waves were then modified in the matrix element of \( R_d \) as \( E_d^S(k, \tau; k_{im}) = E_S^d(k, \tau; k_{im}) + E_M^d(k, \tau; k_{im}) \), where \( E_d^S \) and \( E_d^M \) denote the modified single- and multiple-scattered waves, respectively. Therefore, the
Dimensionality reduction modifies the SMR from \( I_S / I_M \) to \( I_S / I_M \), where \( I_S = \langle |E_S^d(k, \tau; k_{in})|^2 \rangle \) and \( I_M = \langle |E_M(k, \tau; k_{in})|^2 \rangle \).

The SMR in \( R_d \) depends on the choice of \( N_c \). We computed the cumulative contribution of single scattering up to a cutoff eigenchannel index \( N_c \), \( \sum_{j=1}^{N_c} e_S(j) \), and that of multiple scattering, \( \sum_{j=N_c+1}^{N} e_M(j) \). We then obtained SMR enhancement of the reduced matrix by computing their relative ratio, \( \text{SMR}(N_c) = \sum_{j=1}^{N_c} e_S(j) / \sum_{j=N_c+1}^{N} e_M(j) \), with respect to \( N_c \). (Fig. 1D). SMR\((N_c)\) decreased monotonically because the single-scattering contribution decreased with \( j \). Thus, it may be best to choose only the first eigenchannel (\( N_c = 1 \)) for the maximum SMR. However, it would miss a significant fraction of signals from single-scattered waves, making the image reconstruction incomplete. Hence, it is critical to choose a minimum \( N_c \), up to which single-scattering information is well contained. This would ensure that the single-scattered wave remains intact by the dimensionality reduction \( |E_x^d(k, \tau; k_{in})| \approx |E_x(k, \tau; k_{in})| \), while multiple scattering is effectively reduced \( (I_M < I_S) \). However, it is challenging to make the proper choice because the cutoff of the single-scattering contribution \( e_S(j) \) (solid blue in Fig. 1C) is not as distinctive as that of \( \tau_S(j) \) of \( S \) (dashed blue in Fig. 1C). Because of the presence of multiple scattering, single-scattering contribution is spread over all the eigenchannels, although its contribution is generally lowered with the increasing \( j \). We developed a systematic method to choose the minimum eigenchannel index \( N_m \) based on the coherent accumulation of single scattering (Fig. 1E) (see fig. S2 for details). Essentially, the growth rate of the accumulated single-scattering signal is high at small \( N_c \), but it is attenuated with the increase in \( N_c \) due to the reduced contribution of single scattering (see purple dashed lines in Fig. 1E). We chose \( N_m = 10 \) (black dashed vertical lines in Fig. 1, C to E) was an appropriate value for this specific example.

SMR enhancement of the reduced matrix \( R_d \) at \( N_m = 10 \) was approximately 22 times larger than that of the original matrix \( R \) (Fig. 1D), meaning that multiple-scattering intensity is reduced as much. 

Demonstration of the enhanced aberration correction capability

We demonstrated the extended imaging depth in the scattering medium with the enhancement of SMR. As described above, we obtained the reflection matrix \( R \) of the virtual sample (Fig. 2K) subject to strong aberration (inset in Fig. 2K) and multiple scattering. The Strehl ratio set by the aberration map was estimated to be 0.0057 from its PSF (Fig. 2, L and N). We reduced \( R \) to \( R_d \) with varying cutoff eigenchannel index \( N_c \). The target object was invisible due to the strong aberration from the conventional confocal images reconstructed by \( R_d \) regardless of \( N_c \) (Fig. 2, A to E).

Thereafter, we applied a reconstruction algorithm with aberration correction based on the closed-loop accumulation of single scattering (CLASS) (20) to \( R_d \) to obtain an aberration-corrected matrix \( R_d^{cor} \) and an optimal object image. The fidelity of the aberration correction algorithm is determined by the SMR and the complexity of aberration. To elucidate this, we considered the key step of the CLASS algorithm, which is to calculate the correlation of output waves from two representative incident wave vectors \( k_{in}^{(l)} \) and \( k_{in}^{(m)} \) in the momentum difference basis, rather than in the momentum basis. Mathematically, it is expressed as

\[
\langle E^r(k + k_{in}^{(l)}, \tau; k_{in}^{(l)}) E^r(k + k_{in}^{(m)}, \tau; k_{in}^{(m)}) \rangle_k \approx e^{i\phi_o(k_{in}^{(l)})} e^{i\phi_o(k_{in}^{(m)})} \alpha(z) \langle |\tilde{O}(k)|^2 e^{i\phi_o(k_{in}^{(l)})} e^{i\phi_o(k_{in}^{(m)})} \rangle_k \\
+ \langle E^r_M(k + k_{in}^{(l)}, \tau; k_{in}^{(l)}) E_M(k + k_{in}^{(m)}, \tau; k_{in}^{(m)}) \rangle_k
\]
Here, we ignored the cross terms between $E_S$ and $E_M$ because they were much smaller than the second term on the right-hand side of Eq. 6. The first term can be approximated as $e^{i\phi_o(k_o^m) - o_o(k_o^m)} / \xi$ for a spatially narrow target. It contains the input aberration, $\phi_m(k_m^m) - o_m(k_m^m)$, which should be retrieved for aberration correction. The magnitude of the first term is determined by the average single-scattering intensity $I_S \approx a(z) / \xi$ and $\xi \approx \langle e^{i\phi_o(k_o^m) - o_o(k_o^m)} \rangle / \sqrt{N(k)}$, where $\xi$ denotes the normalized cross-correlation of the output aberrations and $N(k)$ represents the number of elements involved in the average over $k$. Essentially, $\xi$ is determined by the degree of aberrations, and its magnitude is reduced with increasing the aberration complexity. This first term competes with the second term from multiple scattering whose magnitude is approximately given as $I_M / \sqrt{N(k)}$. Therefore, the fidelity of aberration correction $\chi$ is determined by the intensity ratio of the first and second terms

$$\chi = \frac{I_S / I_M}{\sqrt{N(k)}}$$

The dimensionality reduction enhances the convergence of the aberration correction algorithm by raising the SMR from $I_S / I_M$ to $I_S / I_M$ in $\chi$. The aberration correction algorithm works when $\chi$ is above a certain threshold $\chi_{th}$

$$\chi_{th} = \text{SMR} \langle N_{ch} \rangle \sqrt{N(k)}$$

The exact value of $\chi_{th}$ varies depending on the system parameters and ingenuity of the algorithm.

The aberration correction algorithm was applied to $R_1$ obtained with varying $N_c$ (see fig. S3 for details of the aberration correction algorithm). When it was applied to the original matrix ($N_c = N_{tot}$, the algorithm failed to reconstruct an object image and an aberration map (Fig. 2J) because SMR was significantly low for $\chi$ to be below the threshold $\chi_{th}$. When $N_c$ was reduced below $N_{ch}$ (Fig. 2F), only a small part of the object was visualized, and the identified aberration map (inset in Fig. 2F) was different from the ground truth (inset in Fig. 2K). This is because $R_1$ contained only a small fraction of the single-scattering signal. On the other hand, the reconstructed image of DREAM clearly visualized the target and precisely identified the original aberration map in the case of minimum cutoff eigenchannel index $N_{ch} = 10$ (Fig. 2G), where SMR was increased by approximately 22 times (Fig. 1D). PSF (Fig. 2M) also validates the precision of aberration correction. With the increase in $N_c$, the central wavelength was set to 675 nm for high spatial resolution and high-contrast imaging. The bandwidth was set to 15 nm, defining the temporal gating window corresponding to the depth gating of 15 μm. We performed in vivo mouse brain imaging through an intact skull introducing strong optical aberrations and scattering. Here, we applied DREAM to the through-skull imaging to visualize myelinated axons that were invisible without enhancing the SMR by dimensionality reduction.

A supercontinuum laser was used as a light source to control the central wavelength and spectral bandwidth (see fig. S5 for the detailed experimental setup). The wavelength was tunable in the visible range, and the central wavelength was set to 675 nm for high spatial resolution and high-contrast imaging. The bandwidth was set to 15 nm, defining the temporal gating window corresponding to the depth gating of 15 μm. We prepared a live mouse (4-week-old C57BL/6 mice) and observed the brain cortex through an intact skull whose thickness was approximately 70 μm (see Materials and Methods for sample preparation). First, a volume of a mouse brain was observed by confocal reflectance imaging (Fig. 3, A to E) as a point of reference. Figure 3 (A and B) visualizes osteocytes inside the skull of the mouse at the dorsal view. In general, osteocytes flourished between bone layers and dura maters a couple of weeks after fertilization. The mineralized bone matrix induces both a strong aberration and multiple light scattering. This makes it challenging for conventional confocal reflectance imaging to access the brain cortex. As shown in Fig. 3 (C to E), it could not visualize any neural fiber in the brain cortex.

The same volume was investigated by the time-gated reflection matrix microscope developed earlier (34). Holograms formed by the interference between backscattered waves from sample and reference waves were recorded by a camera for multiple illumination angles for each objective focus targeting a specific depth. The illumination angle was scanned sequentially to follow the spiral trajectory at the pupil plane to cover the entire numerical aperture. The number of illumination angles was determined depending on the optical property of the skull. One set of holograms was recorded with the camera at a frame rate of 160 Hz by scanning the incident waves of $N_{inh} = 1369$ angles for the view field of 55 μm by 55 μm. The recorded images were used to construct $R$ at the depth set by the objective focus. We recorded $R$ at a 10-μm-depth interval by scanning objective focus from the top of the skull to the brain cortex layer. Object image can be reconstructed from $R$ within the depth range set by the coherent length of 15 μm centered by the objective focus.

Aberration correction was applied to the recorded $R$ by the following procedure. The full field of view (55 μm by 55 μm) was
divided into six-by-six segments for the aberration correction. The local sample-induced aberration was corrected by independently examining the submatrix for each segment. The image was then reconstructed from the diagonal elements of the time-gated reflection matrix in the space domain (see figs. S3 and S6 for details of image reconstruction with aberration correction). The size of each segment was determined by the isoplanatic patch, where pupil aberration has invariance at the given area. The size of the isoplanatic patch becomes smaller with the increase in local aberration, and thus, the segment size is required to be adjusted accordingly. The reconstructed images were acquired (Fig. 3, F to J) from the aberration correction of the measured matrix $R$. At shallow depths from the surface, distinct osteocytes were visualized with the improved contrast (Fig. 3, F and G), where $N_c$ was 30 of the total channel number $N_{\text{tot}} = 1369$. $N_{\text{tot}}$ was given by the size of each segment (14 $\mu$m by 14 $\mu$m) and the diffraction-limited resolution of 412 nm. The fine structure of neural fibers of myelinated axons in the brain cortex was revealed at increasing depths of 110, 130, and 150 $\mu$m (Fig. 3, M to O). Those depths were estimated to be 2.75, 3.25, and 3.75 times the scattering mean free path. The periodic brightness variation is observed along the resolved neural fibers, which is due to the interference at the multilayer structures in the myelin (35, 36). The aberration maps obtained by DReAM are shown in Fig. 3 (P to T). The degree of the

DReAM was applied for the same time-gated reflection matrix to verify the benefits of enhancing SMR. The dimensionality reduction was applied to obtain the reduced matrix $R_d$ based on the SVD of $R$ for each segment. The cutoff eigenchannel index $N_c$ was chosen to optimize the contrast of the reconstructed image. The reconstructed images of DReAM were retrieved (Fig. 3, K to O) from the aberration-corrected matrix $R_d^{\text{cor}}$, which was obtained by applying aberration correction to the reduced matrix $R_d$. At shallow depths from the surface, distinct osteocytes were visualized with the improved contrast (Fig. 3, K and L), where $N_c$ was 30 of the total channel number $N_{\text{tot}} = 1369$. $N_{\text{tot}}$ was given by the size of each segment (14 $\mu$m by 14 $\mu$m) and the diffraction-limited resolution of 412 nm. The fine structure of neural fibers of myelinated axons in the brain cortex was revealed at increasing depths of 110, 130, and 150 $\mu$m (Fig. 3, M to O). Those depths were estimated to be 2.75, 3.25, and 3.75 times the scattering mean free path. The periodic brightness variation is observed along the resolved neural fibers, which is due to the interference at the multilayer structures in the myelin (35, 36). The aberration maps obtained by DReAM are shown in Fig. 3 (P to T). The degree of the
skull-induced aberrations was so severe that the Strehl ratios estimated from the acquired aberration maps in Fig. 3 (P to T) were between 0.2 and 0.03. Both the multiple light scattering and aberration attenuate the peak PSF intensity. We can define the optical thickness of the imaging depth using the effective scattering mean free path accounting for both scattering and aberration. In this context, our analysis shows that the imaging depth of 150 μm is equivalent to 7.4 times the scattering mean free path (see fig. S10). We derived $N_c^N$ and $N_c^N$ of this mouse brain imaging in the following section.

The input and output bases of the recorded $R$ were $k_m$ and $r$, respectively. The output basis was converted into $k$ by taking the Fourier transform with respect to $r$ for the application of DReAM. It is noteworthy that one needs to cover only a fraction of $k_m$ channels because aberrations can be found for all the orthogonal angular channels from the fully covered $k$. This can substantially reduce the matrix recording time. Theoretically, one can choose any orthogonal bases in recording $R$ as long as they can be converted into $(k_m, k)$. For example, earlier studies chose $(r_m, r)$ (22) and $(r_m, k)$ (7).

### Quantification of SMR enhancement in imaging myelinated axons under the intact skull

We analyzed the eigenchannels of the time-gated reflection matrix $R$ recorded for the brain cortex imaging to quantify the effect of dimensionality reduction to increasing SMR. As a representative example, a local 14 μm–by–14 μm area at a depth of 130 μm (white square box in Fig. 3N) was selected for the detailed analysis. A black curve in Fig. 4A shows the eigenvalue distribution $\tau_k(j)$, which was measured $R$ at the selected area. It is necessary to know $S$ and $M$ to obtain the single-scattering contribution $e_s(j) = |S|v_j|^2$ and multiple-scattering contribution $e_M(j) = |M|v_j|^2$. However, these were not directly accessible in the experiment. Instead, we obtained approximate experimental single- and multiple-scattering matrices, $S_0$ and $M_0$, as follows (see fig. S6 for details of identifying single- and multiple-scattered waves). We obtained the aberration-corrected matrix $R_{d}^{\text{cor}}$ from the measured matrix $R$ by the reconstruction process. The basis of $R_{d}^{\text{cor}}$ was converted from $(k_m, k)$ to $(r_m, r)$ using inverse Fourier transform along the columns and rows of $R_{d}^{\text{cor}}$. After that, we retained only the diagonal elements and set all the off-diagonal elements to zero. This corresponds to applying digital confocal gating with the pinhole size equivalent to the diffraction-limited resolution of our imaging system. This operation provided reasonable quantification of single scattering because diagonal elements in the aberration–corrected matrix $R_{d}^{\text{cor}}$ were primarily composed of single scattering. $S_0$ was retrieved by converting the basis of this diagonal matrix back to $(k_m, k)$ and applying original skull-induced aberrations. $M_0$ was retrieved from $R_{d}^{\text{cor}}$ by the common procedure, but only off-diagonal elements were retained instead of the diagonal elements.

Blue and red curves in Fig. 4A show $e_s(j)$ and $e_M(j)$ obtained from $S_0$ and $M_0$, respectively. SMR of the original matrix was measured to be $\langle \tau_s(j)/\tau_M(j) \rangle = 1/2200$. Single-scattering contribution was concentrated mainly on the eigenchannels with small indices as predicted by the numerical simulation in Fig. 1C, and hence, this enabled the enhancement of SMR by dimensionality reduction. We plotted the SMR enhancement of the reduced matrix (Fig. 4B) depending on the cutoff eigenchannel index $N_c$ given by $\text{SMR}(N_c) = \sum_{j=1}^{N_c} e_s(j)/\sum_{j=1}^{N} e_M(j)$, after normalizing it with $\text{SMR}(N_c = N_{c0} = 1369)$ of the original matrix to quantify the enhancement compared to the initial state. There was 17 times the enhancement of SMR at the minimum cutoff eigenchannel index $N_c^N = 7$. The minimum cutoff index was determined by analyzing the single-scattering coherent accumulation method (Fig. 4C), which is the same procedure as analyzing the numerical simulation shown in Fig. 1E. We displayed reconstructed images for various $N_c$ values (Fig. 4D) after aberration correction. For $N_c < N_c^N$, only a fraction of the myelinated axon was visible because of the loss of the single-scattering contribution. For $N_c \geq N_c^N$, full myelination structure was recovered, whereas there was a gradual decrease in image contrast due to decreasing SMR, e.g., $N_c = 100$. We analyzed the fidelity of the aberration correction and image contrast with respect to $N_c$ (see fig. S7 for details). Aberration correction worked up to $N_c^N = 100$, where SMR enhancement decreased to 2.9. Further increase in $N_c$ made it impossible to reconstruct the object information.

The effective enhancement of the imaging depth by DReAM can be deduced from the attenuation of single- and multiple-scattered waves depending on imaging depth. In the reflection-mode imaging, the intensity of single-scattered waves $I_s$ and that of multiple-scattered waves $I_M$ can be described as $I_s(z) = e^{-2zl}$ and $I_M(z) = e^{-2zl^2}$, respectively. Here, $l_s$ corresponds to the mean free path of single-scattered waves, and $l_m$ represents the attenuation length of multiple scattering. $l_m$ depends on various factors such as the numerical aperture, field of view, and the types of gating operations. It is longer than $l_s$ in general, e.g., typically, $l_m = 1.5l_s$ to $2.5l_s$ when the temporal gating is applied (19). The SMR was attenuated with the increase in the imaging depth by $\text{SMR}(z) = e^{-l_s/2}$, where $l_s = l_m/l_s$ corresponds to the attenuation length of SMR. The achievable imaging depth $z_0$ without the dimensionality reduction was set when $\text{SMR}(z_0) = \text{SMR}_{d0}$, Assuming that the SMR is enhanced by a factor of $\beta$ by DReAM, the achievable imaging depth $z_d$ is given by the condition, $\text{SMR}(z_d) = \beta e^{-2z_d/l_s} = \text{SMR}_{d0}$. Therefore, imaging depth enhancement by DReAM was obtained as

$$z_d - z_0 = \frac{l_m l_s}{l_m - l_s} \ln \beta$$

(9)

The effective imaging depth enhancement was 2.4 to 4.2 $l_s$ when the enhancement factor was $\beta = 17$. This enhancement is substantial considering that the imaging depth of high-resolution optical microscopy ranges around 5 to 10 $l_s$.

### Wave focusing of input eigenchannels to myelinated axons

This section elucidates the origin of SMR enhancement when eigenchannels with large eigenvalues (or small eigenchannel indices) were used for image reconstruction. The certain eigenchannels have larger reflectance than the average, meaning that these eigenchannels lead to focusing of incident waves to the target with higher reflectance than the surrounding area (30, 31). It is expected that the eigenchannels with small $j$ tend to focus on the myelinated axons in the brain cortex because they have a higher refractive index than the background tissues (35, 36). This was validated by analyzing the space-domain representation of each input eigenchannel $v_j$. The electric field map of the incident wave of each eigenchannel consists of the coefficient $c_j(k_m)$ of each incident planar wave

$$v_j(r_m) = \sum_j c_j(k_m) e^{ik_mr_m}$$

(10)
A few representative images of $\mathbf{v}_j(\mathbf{r}_{in})$ are shown in Fig. 5A for various $j$ values. Regardless of $j$, all the images showed speckles without providing any signature of wave focusing. This is because the eigenchannels send input waves compensating the sample-induced aberrations. Therefore, we applied the correction of input aberration to $\mathbf{v}_j(\mathbf{r}_{in})$ to obtain an incident wave that has gone through the input aberration

$$
\mathbf{v}_j'(\mathbf{r}_{in}) = \sum_i c_j(\mathbf{k}_{in}) e^{i \mathbf{k}_a \cdot \mathbf{r}_{in} + \phi_0(\mathbf{k}_{in})}
$$

(11)

Figure 5B shows $\mathbf{v}_j'(\mathbf{r}_{in})$ for the same $j$ in Fig. 5A. Notably, incident waves were focused on the myelinated axons for small $j$ up to approximately $j = N_c^m$, and a further increase in $j$ led to the gradual loss of the focusing. Figure 5C shows the line profiles of $\mathbf{v}_j'(\mathbf{r}_{in})$ across the neural fiber for the representative $j$. It is clearly shown that the incident waves were focused on the myelinated axons for small $j$ up to $j = N_c^m$ or so, and a further increase in $j$ led to the gradual loss of the focusing. This tendency is clarified by estimating the focused energy enhancement of $\mathbf{v}_j'(\mathbf{r}_{in})$, which is retrieved from the ratio of the localized energy at the target region of the neural fiber over the dispersed energy at the background (Fig. 5D). The focused energy enhancement is larger up to two orders of magnitude for $j < N_c^m = 7$. Its maximum value is about 80, and it converges to unity at $j \geq 30$. To verify whether the focused wave is directly related to the object, we compared the object function $O(\mathbf{r}_{in})$ retrieved in Fig. 4D for $N_c = 30$ with $\mathbf{v}_j'(\mathbf{r}_{in})$ by computing their correlation with respect to $j$ (Fig. 5E). The correlation was higher than the baseline for eigenchannels up to $j = 30$, indicating that these eigenchannels focused the incident wave on the myelinated axons better than the random inputs. This goes well with the observation in Fig. 4D where image quality was the best around $N_c = 30$, indicating that these eigenchannels focused the incident wave on the myelinated axons for small $j = 30$, and a further increase in $j$ led to the gradual loss of the focusing. This tendency is clarified by estimating the focused energy enhancement of $\mathbf{v}_j'(\mathbf{r}_{in})$ without aberration correction (Fig. 5F) cannot provide any information at all. All these results support that the input eigenchannels with large eigenvalues retrieved by DReAM were preferentially focused on the neural fiber in the brain cortex under the mouse skull.

**DISCUSSION**

In imaging deep within the scattering medium such as an intact mouse skull, it is critical to remove complex sample-induced aberrations for finding fine structures embedded underneath. However, strong multiple-scattering backgrounds pose a fundamental limitation for identifying and correcting the aberrations. This study presents a framework that attenuates the contribution of multiple scattering with minimal changes to a single-scattering signal and tissue-induced aberration, the critical information required for image reconstruction. This was realized by the dimensionality reduction of the reflection matrix, exploiting the wave correlation of single scattering from fine structures, such as myelinated axons, for refining a single-scattering signal. We applied the proposed method for visible-wavelength imaging of a mouse brain cortex through an intact mouse skull. The accessible imaging depth at visible wavelengths is much shallower than the that in previous systems using near-infrared wavelengths due to the increased degree of scattering and aberration, which undermines its benefits in providing higher image contrast and resolution than that with the near-infrared light. We demonstrated the $17$-fold enhancement of SMR, which led to the reconstruction of fine myelinated axons under an intact skull that could not be visualized by the original reflection matrix measured in the visible wavelength. This SMR enhancement corresponds to increasing imaging depth by $2.4$ to $4.2$ $\mu m$, which is substantial considering that the imaging depth of high-resolution microscopy is approximately...
We showed by analyzing the experimentally recorded reflection matrix that high-reflection eigenchannels led to the focusing of the incidence waves on myelinated axons, directly supporting the inner workings of the dimensionality reduction. Multiple-scattered waves that our time-gated interferometric microscope records are almost time invariant. Because of the nature of in vivo imaging, motion-induced perturbation exists. However, high-speed reflection matrix recording along with anesthetization of the mouse makes the recorded multiple scattering almost static. Note that many important previous studies demonstrated wave focusing by controlling the time-invariant components of multiple scattering (37, 38). While multiple scattering can be considered as a signal in these studies, it should also be noted that all these studies demonstrated a wave focusing on the opposite side of a scattering layer, not within a thick scattering medium. The systematic way of focusing multiple scattering at an arbitrary position within a scattering medium is yet to be realized. Our proposed method reconstructs an object embedded within a scattering medium with the ideal diffraction-limited spatial resolution by removing the perturbation of single-scattered waves on their roundtrip through the medium. This is an essential step toward the full use of multiple scattering for the microscopic image formation of an embedded target.

One of the prerequisites of the proposed method was the spatial confinement of the object of interest. Point particles are ideal targets, but that is seldom the case in most biological specimens. Filament structures, such as the myelinated axons, satisfy the requirement, as demonstrated in the present study. In particular, the analysis of small sub-areas makes it possible to apply the proposed method for the complex network of filament structures that can undermine the validity of dimensionality reduction. The proposed method also requires the target object to have higher reflectance contrast than the surrounding area, which is the case with any reflectance-based label-free imaging. Targets with weak reflectance contrast would set the initial SMR small, making it more difficult to reconstruct the image. The initial SMR determines the operating range of dimensionality reduction in enhancing the SMR. If the initial SMR is too low, then the single-scattering signal is not concentrated on the high-reflection eigenchannels of the total reflection matrix anymore (33). The minimum initial SMR that the dimensionality reduction works depends on the degree of sparsity, not on the complexity of aberrations. The proposed method can be readily applied to any matrix-based AO imaging to enhance their effective imaging depth because the method requires no additional data acquisition.

Fig. 5. Focusing of high-reflection eigenchannels on the myelinated axon. (A) Intensity map of each input eigenchannel $v_i$ of the original reflection matrix $R$ for various eigenchannel indices. (B) Input eigenchannel $v_i^c$ after correcting the input aberration to each $v_i$ in (A). (C) Line profile averaged along the direction indicated by an arrow in a dashed box in (B). (D) Focused energy enhancement defined by the ratio of the wave energy at the myelinated axon relative to that at the background for each $v_i^c$. (E) Degree of wave focusing of each input eigenchannel estimated by the correlation between $v_i^c$ and aberration-corrected image in Fig. 4D ($N_c = 30$). Black dashed line indicates the baseline determined by the average of the correlation between multiple-scattered waves and aberration-corrected image. (F) Summation of the intensity images of $v_i$. (G) Summation of the intensity images of $v_i^c$. Each figure panel was normalized by its maximum intensity. Scale bar, 5 µm.
Preparation of intact skull window for in vivo imaging

All animal experiments were approved by the Korea University Institutional Animal Care and Use Committee (KUIAUC-C-2019-0024). Four-week-old C57BL/6 mice [postnatal day 28 (P28) to P34, 14 to 18 g] were anesthetized with isoflurane (4% in oxygen for induction and 1.5 to 1.8% in oxygen for maintenance). A homeothermic blanket maintained the body temperature at 37°C, and the eyes were covered with an ointment during surgery and imaging. Dexamethasone (1 mg/kg) was administered intramuscularly on the two consecutive days after the surgery to minimize inflammatory responses at the surgery site. The hair in the scalp was removed with scissors and Nair hair removal cream. Subsequently, the scalp was removed to expose the bregma, lambda, and both parietal plates. Sterile saline was applied to the skull, and the remaining connective tissue on the skull was gently removed with sterilized forceps and wet cotton swabs. A custom-made metal plate was attached to the skull with cyanoacrylate for head fixation, and the exposed part of the skull was covered with dental cement (Dentsply DeTrey GmbH, Germany). For imaging, mice (P28 to P34) were anesthetized with isoflurane (1.2 to 1.5% in oxygen to maintain a breathing frequency of approximately 1.5 Hz) and placed on a three-dimensional motorized stage heated by a heat blanket at 37°C.

SUPPLEMENTARY MATERIALS

Supplemental material for this article is available at https://science.sciencemag.org/content/10.1126/sciadv.abc04366

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