Reflection-matrix microscopy for aberration-free imaging through intact mouse skull

Seokchan Yoon, Hojun Lee, Jin Hee Hong, and Wonshik Choi
Center for Molecular Spectroscopy and Dynamics (CMSD)
Institute for Basic Science
KU R&D Center, Korea University, Seoul 02841, Korea
sc_yoon@korea.ac.kr

KEY WORDS: Adaptive optics, Deep tissue imaging, Reflection matrix, Through-skull imaging.

ABSTRACT

Optical microscopy suffers from sample-induced aberrations and multiple light scattering when it comes to imaging within thick scattering tissues [1]. In this presentation, we introduce a label-free imaging modality termed laser scanning reflection-matrix microscopy (LS-RMM) [2] capable of correcting spatially varying sample-induced aberrations for up to 10,000 angular modes, despite the presence of strong multiple scattering. In contrast to conventional confocal laser scanning microscopy, the LS-RMM records non-confocal signals as well as confocal signals and exploits them to find aberrations and an object image. Specifically, electric-field images of backscattered waves from the sample are recorded by means of off-axis low coherence digital holography while a focused illumination is scanned on the sample. We construct a time-gated reflection matrix from the recorded images and apply an aberration correction algorithm to the matrix for the reconstruction of the aberration-free object image. Our aberration correction algorithm is unique in that an extended object and aberrations in the illumination and detection pathways are jointly identified with no need of guide stars [3,4].

We demonstrate reflectance imaging of myelinated axons in the mouse brain through the intact skull with a diffraction-limited resolution of 450 nm. The LS-RMM is readily integrated with an adaptive optics multiphoton microscopy which offers deeper imaging depths and higher molecular specificity. We demonstrate aberration correction in two-photon fluorescence imaging of neuronal dendrites and spines through the intact skull, which is made possible by displaying a correction map for the identified aberration on a spatial light modulator in the excitation beam path. The two-photon fluorescence image with aberration correction shows up to a 19-fold increase in fluorescence intensity, which enables us to see fine structures of dendritic spines with a spatial resolution of 500 nm, close to the diffraction-limited resolution of the imaging system, 380 nm.

[1] S. Yoon, M. Kim, M. Jang, Y. Choi, W. Choi, S. Kang, and W. Choi, "Deep optical imaging within complex scattering media", *Nat. Rev. Phys.* **2**, 141-158 (2020).

[2] S. Yoon, H. Lee, J. H. Hong, Y.-S. Lim, and W. Choi, "Laser scanning reflection-matrix microscopy for aberration-free imaging through intact mouse skull", *Nat. Commun*, **11**, 5721 (2020).

[3] S. Kang, P. Kang, S. Jeong, Y. Kwon, T. D. Yang, J. H. Hong, M. Kim, K.–D. Song, J. H. Park, J. H. Lee, M. J. Kim, K. H. Kim, and W. Choi, "High-resolution adaptive optical imaging within thick scattering media using closed-loop accumulation of single scattering", *Nat. Commun*, **8**, 2157 (2017).

[4] M. Kim, Y. Jo, J. H. Hong, S. Kim, S. Yoon, K.-D. Song, S. Kang, B. Lee, G. H. Kim, H.-C. Park, and W. Choi, "Label-free neuroimaging in vivo using synchronous angular scanning microscopy with single-scattering accumulation algorithm", *Nat. Commun*, **10**, 3152 (2019).