Fast holographic scattering compensation for deep tissue biological imaging

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Scattering in biological tissues is a major barrier for in vivo optical imaging. The resulting distortion can be corrected by shaping the excitation wavefront to redirect power into a single point in the imaging plane, but finding the optimal correction pattern is nontrivial. Several strategies have been developed to this end, including indirect wavefront sensing techniques which aim to redirect light from a subset of the scattered modes into the focus using an iterative approach. Two indirect wavefront sensing techniques, termed IMPACT and F-SHARP, have recently enabled impressive in-vivo imaging results in various tissues including the mouse brain [1,2]. However, further development is required to increase the imaging depth and match the persistence times of many living tissues.

Here, we introduce a new *dynamic adaptive scattering compensation holography* (DASH) algorithm, which enables significantly faster convergence than IMPACT and F-SHARP by updating the correction pattern using a novel holographic method. In our approach, the optimal measured phase for each test mode is combined with the previous wavefront correction immediately after each interferometry measurement as shown in Fig. 1(a). This leads to rapid, continuous signal growth, in contrast to IMPACT and F-SHARP, which both pursue a stepwise implementation. Experimental evaluation of the DASH algorithm in two-photon excited fluorescence microscopy on a uniform dye sample reveals an order of magnitude higher signal enhancement at the end of the first iteration compared to F-SHARP as shown in Fig. 1(b).

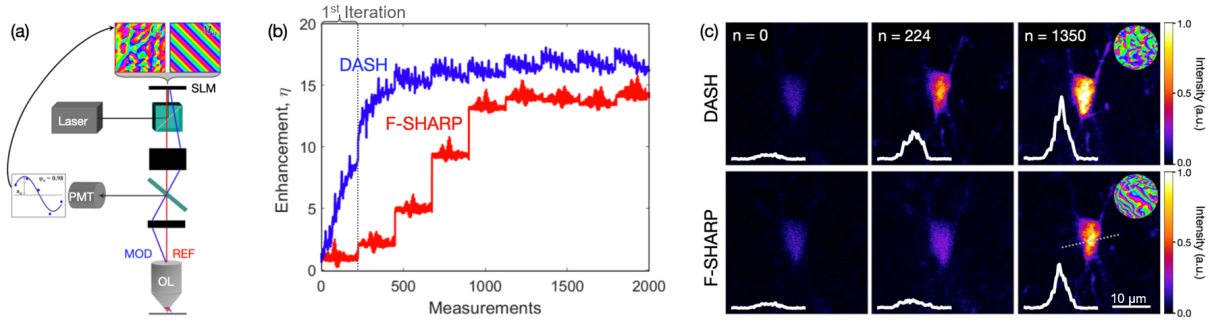

Fig. 1 (a) Experimental implementation of DASH. (b) Signal enhancement after each mode measurement during DASH (blue) and F-SHARP (red) algorithms. (c) Images of a microglia acquired with no correction (n = 0), after the first iteration (n = 224), and after convergence of the DASH and F-SHARP algorithms (n = 1350), with amplitude profiles along the white, dashed line and the resulting correction masks shown in the inset.

The DASH algorithm was further demonstrated by TPEF imaging of microglia in mouse hippocampal tissue. Fig. 1(c) compares images of a single microglia at a depth of 350 µm corrected by the DASH and F-SHARP algorithms. The DASH algorithm produces a well-defined focus that can be used for imaging after just the first iteration through the measurement modes while the F-SHARP correction hardly improves the wavefront distortion at this point. Furthermore, at a depth of 530 µm the F-SHARP algorithm did not converge but the DASH algorithm converged after just three iterations.

Finally, a theoretical framework revealing that IMPACT and F-SHARP are fundamentally equivalent is developed and all results are validated by numerical simulations. DASH is an important advance toward deep, dynamic biological imaging with advanced holographic correction algorithms.

References

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