SIMPLE AND ACCURATE CALIBRATION OF AN ADAPTIVE MICROSCOPE WITH PHASE DIVERSITY

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Over the past decade, adaptive optics has proven a powerful tool for improving the quality of images obtained within thick aberrating biological samples, in particular in the case of nonlinear microscopy [1,2]. Nevertheless, setting up an adaptive microscope remains a complicated process, especially as the effect of the adaptive element (usually a deformable mirror (DM)) on the transfer function of the microscope has to be carefully calibrated. Here we present a simple, versatile scheme for characterising the electric field modulation by an active element, such as a DM, in the pupil plane of a high NA microscope. By placing a flat mirror in the vicinity of the focal plane of the objective and recording images of the focal spot on a camera, we show that reliable measurement of the influence function of the DM actuators in the pupil plane of the objective can be obtained using an iterative electric field retrieval algorithm. The setup allows characterisation for a variety of objectives with different NA and pupil size, requires minimal space inside the microscope, and can be used with pulsed sources such as used for multiphoton microscopy. In order to validate our method, we compare our data to the results obtained with a Shack-Hartmann wavefront sensor (SHS), and show that comparable precision is achieved with reduced cost and complexity.

Fig 1. Left, experimental setup for phase diversity-based measurements. Right, measured intensity (top) and influence function of an actuator of a DM (bottom) with electric field reconstruction (first row) and a SHS (second row). Third row, difference between results from each method.