# Three Photon Adaptive Optics for in-vivo Mouse Brain Imaging.

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Multiphoton fluorescence microscopy is a well-established technique for deep-tissue imaging with subcellular resolution [1]. Three-photon fluorescence microscopy (3PM), when combined with long wavelength excitation was shown [2,3] to allow deeper imaging than two-photon fluorescence microscopy (2PM) in biological tissues, such as mouse brain, because out-of-focus background light can be further reduced due to the strong localization of the higher order nonlinear excitation. As was demonstrated in 2PM systems, imaging depth and resolution can be by applying adaptive optics (AO) techniques which are based on shaping the scanning beam using a spatial light modulator (SLM). In this way, it is possible to compensate for tissue low order aberration and to some extent, to compensate for tissue scattering.

It was already demonstrated that the compensation for signal degradation due to aberrations will be much more significant in higher order nonlinear imaging such as 3PM than in 2PM [4,5]. Therefore, the impact of adding AO to three photon fluorescence system should be more significant and will show stronger signal improvement resulting in deeper imaging depth and higher resolution in the mouse brain.

In this work, we present a 3PM AO system for in-vivo mice brain imaging. We use a femtosecond source at 1300 nm to generate 3-photon response in YFP mouse brain and a microelectromechanical (MEMS) SLM to apply different Zernike phase patterns. The nonlinearity of the 3PM fluorescence signal is used as a feedback to calculate the amount of phase correction without direct phase measurement, allowing fast convergence towards stronger signal.

Our results show that after applying correction we achieve signal improvement in the cortex and the hippocampus beneath 1-mm depth inside the brain and demonstrate diffraction limited imaging in the cortical layers of the brain, including imaging of dendritic spines.

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