MULTI-PHOTON MICROSCOPY AND ADAPTIVE OPTICS FOR *IN-VIVO*, HIGH-RESOLUTION, DEEP BRAIN IMAGING

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Key Words: Multi-photon microscopy, adaptive optics, neuroscience

In order to study dynamic biological processes *in-vivo* in mammalian organisms techniques are required which enable non-invasive imaging at large tissue depth with sub-cellular resolution. However, optical aberrations and scattering in biological tissue lead to signal loss and a degradation of both spatial resolution and penetration depth. We combine two powerful optical techniques, multi-photon microscopy and adaptive optics, to push the depth limit further while retaining diffraction limited resolution. We apply these techniques to open questions at the forefront of biology and neuroscience were we so far have enabled *in-vivo*, high resolution imaging of GFP-labeled neurons up to a depth of 1.1mm in the intact mouse brain.

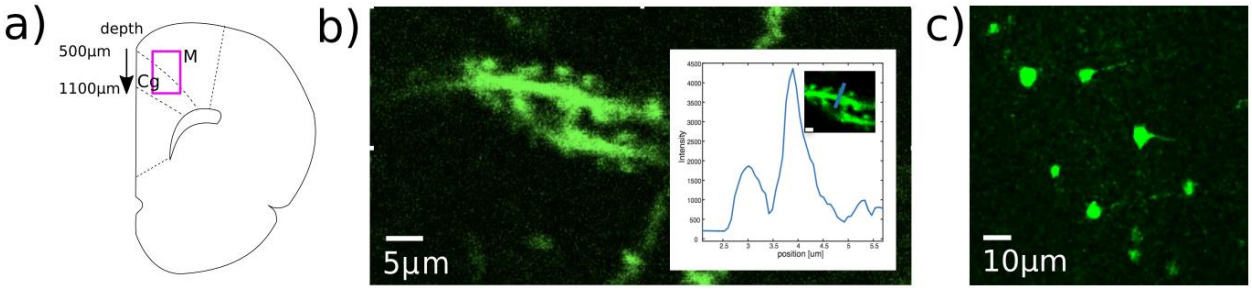

Figure 1: *In-vivo* three-photon images of GFP-labelled prefrontal cortical neurons in a Thy1-GFP mouse brain. a) Schematic representation of imaging area (pink box) in prefrontal cortex. Cg: Cingulate cortex, M: Motor cortex. b) Dendrites and spines at 550-570µm below the cortical surface and intensity profile along the indicated line. c) Cell somata at depth 1000-1100µm below the surface of the brain.

Using three-photon excitation rather than two-photon excitation can be advantage for deep tissue imaging because longer wavelength light is employed, which reduces scattering. Furthermore, a significant improvement in localisation excitation with higher order nonlinearity leads to an increased signal-to-background-ratio and thus enables much larger imaging depth. Here, we demonstrate three-photon excitation of GFP-labeled neurons at the 1300nm spectral excitation window. Fine structures, in particular dendritic spines, could be resolved up to a depth of 550 µm, while larger structures such as somata, were visible up to a depth of 1.1mm. The implementation of adaptive optics into our microscope will allow us to furthermore increase the resolution at these imaging depths so that fine structures can still be reliably resolved. We will present results on three-photon microscopy for studying synaptic turnover during mouse adolescence.