Deep Brain Imaging with Multiphoton Microscopy

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Over the last two decades, multiphoton microscopy has created a renaissance in the brain imaging community. It has changed how we visualize neurons by providing high-resolution, non-invasive imaging capability deep within intact brain tissue. Multiphoton imaging will likely play an essential role in understanding how the brain works at the level of neural circuits, which will provide a bridge between microscopic interactions at the neuronal level and the macroscopic structures that perform complex computations.

In this talk, the fundamental challenges of deep tissue, high-resolution optical imaging are discussed. New technologies for in vivo imaging of mouse brain using megawatt femtosecond pulses and three-photon microscopy (3PM) at the 1700 nm spectral window will be presented. We will discuss the requirements for imaging the dynamic neuronal activity at the cellular level over a large area and depth in awake and behaving animals. Finally, we will speculate on the possible future directions to further improve the imaging depth and speed in biological tissues.

Fig 1. left: A sagittal plane of the mouse brain. The dashed red box indicates the area where in vivo imaging was performed. 3PM is capable of reaching the Cornu Ammoni (CA1) of the mouse hippocampus which is lined with the stratum oriens (SO), stratum pyramidale (SP), and stratum radiatum (SR) cell layers. Right: 1700-nm 3PM of the red fluorescent protein labeled mouse brain in vivo, showing neurons in the hippocampus. The scale bar is 50 µm. Third harmonic generation (THG) imaging of the myelinated axons delineates the boundary of the white matter.