Rapid Two-photon Three-dimensional Endoscopy for Deep Tissue Imaging

Yu-Feng Chien¹, Jyun-Yi Lin¹,², Yu-Hsuan Tsai¹,², Kuo-Jen Hsu¹, Po-Ting Yeh³, Shih-Kuo Chen³, and Shi-Wei Chu¹.⁴*

¹ Department of Physics, National Taiwan University, Taipei 10617, Taiwan
² Brain Research Center, National Tsing Hua University, Hsinchu 30013, Taiwan
³ Department of Life Science, National Taiwan University, Taipei 10617, Taiwan
⁴ Molecular Imaging Center, National Taiwan University, Taipei 10617, Taiwan

*Email: swchu@phys.ntu.edu.tw

**Key words:** Multi-photon microscopy, Gradient-index lenses, Endoscopic imaging, Three-dimensional imaging, Functional monitoring and imaging

Studying tissue structures and activities in vivo plays a determining role in addressing mechanisms of animal behavior. For cm-size organs and their transient three-dimensional (3D) dynamics, deep and high-speed in vivo imaging is fundamental for a wide range of biomedical study. Over the past thirty years, two-photon microscopy (2PM) has emerged as a powerful tool for bioscience inasmuch as it possesses sub-micrometer spatial resolution, intrinsic optical sectioning, and deep-tissue penetration capability. However, the penetration depth of state-of-the-art 2PM is limited within ~1-mm. Furthermore, volume imaging acquisition largely relies upon slow axial scanning. Hence, developing rapid 3D imaging beyond 1-mm depth is highly desirable. In this study, two gradient-index (GRIN) lenses are incorporated into 2PM to achieve deep in vivo imaging with high volume rate. One GRIN lens is in the form of a thin rod, that serves as a micro-endoscope and allows cm-deep in vivo imaging. The other one is a thick rod with liquids inside, forming a tunable acoustic gradient-index (TAG) lens, which enables 100 kHz-1 MHz axial scan, and upgrades typical 2PM to rapid 3D imaging [1]. We have applied this novel volumetric endoscopy to study 3D calcium dynamics in cm-deep brain regions with sub-cellular spatial resolution and sub-second temporal resolution. This pioneering work provides simultaneous examination of tissue structures and activities in deep regions and may find applications in basic or translational biomedical research.