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

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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.

[1] K.-J. Hsu, Y.-Y. Lin, Y.-Y. Lin, K. Su, K.-L. Feng, S.-C. Wu, Y.-C. Lin, A.-S. Chiang, and S.-W. Chu, "Millisecond two-photon optical ribbon imaging for small-animal functional connectome study," Opt. Lett. **44**(13), 3190–3193 (2019) (**Editor's pick**).