High-speed Two-photon Volumetric Endoscopy for Deep Brain Regions

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Neural wiring and neuronal activities are both important components in the brain, and studying them together can play a pivotal role in addressing the mechanism of brain functions. Considering the cm-size and rapid three-dimensional dynamics of brain, deep *in vivo* imaging with high volume-imaging rate is essential for a wide range of brain study. Over the past thirty years, two-photon microscopy (2PM) has emerged as a powerful tool for neuroscience inasmuch as it possesses sub-micrometer spatial resolution, optical sectioning capability, and the ability of deep *in vivo* imaging. However, the penetration depth of 2PM is limited within ~1-mm and volume images taken by most contemporary 2PM largely rely upon slow axial scanning; hence, it is highly desirable to develop high-speed volumetric imaging beyond 1-mm depth. Here, we demonstrate a new method, which significantly improves penetration depth as well as volume-imaging rate, by incorporating two gradient-index (GRIN) lenses into 2PM. One GRIN lens is a thin rod-like lens, which serves as a micro-endoscope and allows penetration depth up to centimeter scale; the other is a tunable acoustic gradient-index (TAG) lens, which furnishes 2PM with 100 kHz-1MHz axial scanning rate, thus enabling high-speed volumetric imaging. We have applied the novel volumetric endoscopic platform to study three-dimensional calcium dynamics in cm-deep brain regions with sub-cellular spatial resolution and sub-second temporal resolution. This pioneering work provides simultaneous examination of structures and activities in deep brain regions and will assist the rapidly growing field of function connectome.