Fast varifocal two-photon microendoscope for imaging neuronal activity in the deep brain

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Abstract: Fluorescence microendoscopy is becoming a promising approach for deep brain imaging, but the current technology for visualizing neurons on a single focal plane limits the experimental efficiency and the pursuit of three-dimensional functional neural circuit architectures. Here we present a novel fast varifocal two-photon microendoscope system equipped with a gradient refractive index (GRIN) lens and an electrically tunable lens (ETL). This microendoscope enables quasi-simultaneous imaging of the neuronal network activity of deep brain areas at multiple focal planes separated by 85–120 µm at a fast scan rate of 7.5–15 frames per second per plane, as demonstrated in calcium imaging of the mouse dorsal CA1 hippocampus and amygdala in vivo.

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1. Introduction

Integrative brain functions such as sensation, perception, cognition, and movement are supported by distributed neuronal networks across different brain areas. Since its introduction into neuroscience in the 1990s, two-photon excitation laser-scanning microscopy [1, 2] has greatly advanced our understanding of the cellular and local circuit bases for these brain functions by visualizing the structure and function of neurons with high resolution in the living brain, particularly the cerebral cortex. However, there is a technical limitation in that a typical two-photon microscope can image only those neurons that lie within a 1-mm depth in vivo. This has prevented us from gaining a more complete picture of how the entire brain works. Indeed, the vast majority of subcortical neurons have not yet been visualized despite the fact that they are involved in a variety of brain functions such as emotion, sociality, instinctive behavior, and homeostasis. Expanding the repertoire of techniques for optical monitoring of deep brain activity would push back the frontiers of neuroscience.

Currently, three major approaches are available for deep brain imaging in vivo, particularly in mice. Firstly, subcortical neurons can be imaged through an imaging window implanted after surgical removal of the overlying cortical tissues [3–6]. This approach can circumvent the problem of strong light scattering by thick brain tissues but at the cost of high invasiveness, and can provide better optical access for widely used conventional two-photon microscopy. Secondly, subcortical neurons can be imaged also through the intact cortex by using a laser with a longer wavelength (> 1000 nm) as a light source for two-photon microscopy [7] or three-photon microscopy [8]. This approach was usable only for imaging neuronal structures, but three-photon calcium imaging of subcortical neurons through the intact cortex at a high scan rate has recently become possible [9]. Finally, microendoscopy based on gradient refractive index (GRIN) lenses or optical fibers visualizes deep brain neurons via a microlens or a thin fiber stuck into the brain [10–14], enabling “ultra-deep” brain imaging beyond the working distance of the microscope objectives used in the first two approaches. In particular, because GRIN lenses generally have a larger field of view and a wider focal range than those of optical fibers, the former are arguably the most promising technique at present for reliable cellular-resolution imaging of neural circuit activity at any depth in the brain. GRIN lenses are often coupled with miniature head-mounted microscopes [15]. However, their epifluorescence imaging has only a limited depth resolution, making it difficult to separate neuronal activity at different depths.
A rapidly adjustable focus function thus should exploit the potential of microendoscopy not only in allowing for studies of three-dimensional architectures of deep brain circuits but also in increasing experimental efficiency. This is because such a function would give experimenters more opportunities to find cells suitable for imaging near the probe implantation site. To fulfill this demand, we have developed a fast varifocal two-photon microendoscope system that consists of a GRIN lens, an electrically tunable lens (ETL), and an upright two-photon microscope. An ETL is an optical fluid-containing polymer lens that changes its curvature by pressure exerted by an electromagnetic actuator. It can achieve movement-free, faster optical focusing compared to moving the microscope objective mechanically with a piezoelectrically-actuated objective mount [16]. The applicability of our system for \textit{in vivo} deep brain imaging is demonstrated in quasi-simultaneous multi-focal-plane imaging of spontaneous network activity in the dorsal CA1 hippocampus and amygdala in mice.

2. Materials and methods

2.1 Microendoscope setup

The varifocal two-photon microendoscope system consists of an upright two-photon microscope, an ETL, and a GRIN lens (Fig. 1(a)). The two-photon microscope (Nikon A1MP dual) is equipped with an ETL (Optotune EL-16-40-TC-VIS-5D) and is coupled to a GRIN lens (GoFoton SLW, 1.8 mm diameter, 16.9 mm length) via a 10× dry objective (Nikon Plan Apo λ 10× NA 0.45). The diameter of the entire field of view (FOV) is roughly 1.2 mm. A tunable femtosecond-pulsed Ti:Sapphire laser (Coherent Chameleon Discovery) is used as a light source for two-photon excitation. When \textit{in vivo} imaging is performed, the GRIN lens is inserted into the brain through a pre-implanted stainless-steel guide cannula (Fig. 1(b) and 1(c)). The focal depth of the ETL is controlled by a current control driver (Thorlabs LD1255R). The output current (0–250 mA) is controlled by an external input voltage (0–5 V) generated by a HawkVision stimulator (HawkVision Inc., Fujisawa, Japan). Because the current control driver generates current from a negative power supply, applying the current to the ETL shifts the focus downward, away from the sample end of the GRIN lens. The input voltage is triggered by transistor–transistor logic (TTL) signals that are generated by the microscope controller upon initiation of the scanning of each frame, and lasts during the acquisition of the frame.

2.2 Mice and head plate surgery

All animal experiments were conducted in accordance with the institutional guidelines and protocols approved by the Saitama University Animal Experiments Committee. Male Thy1-G-CaMP7-DsRed2 transgenic mice were used in this study (11–18 weeks old and 23–30 g in body weight at the time of surgery) [17]. Details on the generation and characterization of the transgenic mice will be described elsewhere. Stainless-steel head plates for head fixation were attached surgically to the skull as described previously [5, 6]. Briefly, the mice were anesthetized with isoflurane (3% induction, 1.5% maintenance) and a circular piece of scalp was removed. Custom stainless-steel head plates (25 mm length, 4 mm width, and 1 mm thickness) with an opening (10 mm outer diameter and 7 mm inner diameter) were cemented to the skull using dental acrylic that covered the entire surface of the skull and its anchor screws (Fig. 1(c)). The mice were allowed to recover from anesthesia and were then returned to their home cages.
2.3 Imaging

For point spread function (PSF) measurements and multi-focal-plane imaging of fluorescent beads, green fluorescent microspheres of 0.17 µm diameter (PS-Speck Microscope Point Source Kit, Life Technologies, P7220) and 5 µm diameter (FluoRo-Max Green Fluorescent Polymer Microspheres, Thermo Scientific, G0500) were suspended and used in 1% agarose in water. The PSF was measured by imaging the fluorescent microspheres at a lateral resolution of 0.16 µm/pixel and an axial resolution of 1.0 µm/pixel. Images of 10 beads were normalized separately by the maximum intensity of each image, aligned with each other by the intensity peaks, and then averaged and renormalized. In PSF measurement across the tuning range of the ETL, changes in the FOV size were normalized by imaging a calibration slide.

A ray-tracing simulation of the microendoscope system was performed using a custom simulator written in LabVIEW (National Instruments). The Eikonal equation of rays in the GRIN lens was numerically solved, where the radial index distribution was given by \( n(r) = 1.61 - 0.0905r^2 \) according to the values available from the vendor. The 10× objective was modeled as an ideal lens with a focal length of 20 mm. The beam size and the distance between the focus of the objective and the incident end of the GRIN lens were experimentally determined as 7.5 mm and 0.6 mm, respectively. The resolutions of the focused light for two-photon excitation were obtained as full widths at 0.707 times the peak intensity.
Stability of ETL-mediated fast varifocal imaging was evaluated in 10-min imaging sessions in which fluorescent beads of 5 µm diameter were imaged at a frame rate of 30 frames per second (fps) with 0-V or 1-V voltage pulses applied to the current control driver during acquisition of every other frame. The entire image stacks (18000 frames) were divided into two substacks, each of which contained only odd number frames or even number frames (9000 frames each). Slight in-plane drift during the imaging sessions was corrected by the ImageJ plug-in TurboReg and two-dimensional correlation coefficients were calculated using the first frames of the substacks as reference. The correlation coefficients obtained from the two substacks were averaged to represent the values for the session. The lens temperature of the ETL was measured using Lens Driver Controller v1.9 (Optotune).

The implantation of a guide cannula and imaging of spontaneous neuronal network activity in anesthetized mice were performed as described previously with modifications [5, 6]. Briefly, a mouse with a head plate was anesthetized with isoflurane (3% induction, 1.5% maintenance) supplemented with chlorprothixene (1 mg/kg, i.p.) and was fixed to a stereotaxic frame via the head plate. Atropine (0.3 mg/kg, s.c.) and dexamethasone (2 mg/kg, s.c.) were administered prior to the anesthesia. A 2.5-mm-diameter circular craniotomy was made on the skull overlaying the dorsal hippocampus (centered 2 mm posterior to the bregma and 2 mm lateral to the midline) or amygdala (centered 1 mm posterior to the bregma and 3 mm lateral to the midline) in the left hemisphere. The overlying brain tissue was aspirated using a blunted 25-gauge needle connected to a vacuum pump, with occasional irrigation with cortex buffer (123 mM NaCl, 5 mM KCl, 10 mM glucose, 2 mM CaCl₂, 2 mM MgCl₂, 10 mM HEPES, pH 7.4). Bleeding was treated immediately with a piece of gelatin sponge (Spongell, Astellas Pharma, Tokyo, Japan) wetted with the cortex buffer. A guide cannula that consisted of a stainless-steel cylinder (2.5 mm outer diameter and 2.0 mm inner diameter, 1.0 mm height for hippocampus and 5.5–6.5 mm height for amygdala) with a round coverslip (2.5 mm diameter, 0.12 mm thickness, Matsunami Glass Ind., Osaka, Japan) attached to the bottom using a UV-curable adhesive (NOA81, Norland Products), was then inserted. When the cannula was positioned properly, the hippocampal or amygdalar surface was clearly visible through the bottom coverslip without any trace of bleeding. The outer wall or upper rim of the cannula in contact with the skull was then cemented with dental acrylic (Fig. 1(c)). The GRIN lens was inserted through the cannula before imaging.

We positioned the GRIN lens through a guide cannula, rather than implanting it directly into the brain, because that could prevent contact of the GRIN lens with the tissue and thus facilitate its reuse. Moreover, once a guide cannula is implanted successfully, it is possible to image multiple animals with a single GRIN lens or the same animal using GRIN lenses with different properties, enabling cost-effective and flexible experiment designs, although at the cost of enlarging the diameter of the implants.

G-CaMP7 was excited at 910 nm through the GRIN lens, and time-series fluorescence changes in neurons during spontaneous network activity in the dorsal CA1 hippocampus or amygdala were imaged through the microendoscope using a 506–593-nm bandpass filter and a GaAsP photomultiplier tube. Imaging was performed under 1.0% isoflurane anesthesia on the same day, following the guide cannula implantation. Body temperature was maintained at 37°C with a heating pad throughout the imaging sessions. Images were acquired in 512 × 512 or 512 × 256 pixels using a galvo-resonant scanner at a frame rate of 15–30 fps. For dual-color imaging, DsRed2 was excited simultaneously at 910 nm, and the signal was separated by a 593-nm dichroic mirror and a 604–679-nm bandpass filter.

Image analysis was performed using custom software written in MATLAB (MathWorks) after motion correction using the ImageJ plug-in TurboReg as described previously [5]. The center of a region of interest (ROI) in each cell was determined manually on the time-averaged G-CaMP7 fluorescence image so that the ROI was defined as a circle with a 10–12-µm diameter inscribed within the cell body. The fluorescence intensity values of the pixels within the ROI in each frame of the time-lapse image sequences were then averaged to
represent the cellular signal $F$ for that cell. For each $F$, a baseline value $F_0$ was defined as the mean of the 50th percentile of all the data points. $F_0$ was then used to calculate $\Delta F/F$ as $(F - F_0)/F_0$.

2.4 Histology

To confirm the cannula implantation sites, mice after imaging were perfused transcardially with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in PBS. Brains were removed and further fixed in 4% PFA at 4°C overnight. Coronal sections were cut on a vibratome to a thickness of 100 µm and mounted on a slide glass using Immu-Mount (Thermo Scientific). Fluorescence images were acquired using a Nikon A1R+ confocal microscope using a 4× dry objective. The G-CaMP7 and DsRed2 signals shown in all the images represent their native fluorescence.

3. Results and discussion

3.1 Basic characterization of the fast varifocal two-photon microendoscope

The resolutions of the microendoscope were measured by imaging fluorescent microspheres smaller than the optical limit (0.17 µm diameter). The full-width half-maximums (FWHMs) of the PSFs of the x and z axes were 2.3 µm and 62.5 µm, respectively (Fig. 2(a) and 2(b)). The resolutions of a two-photon microscope without the GRIN lens that were measured as a control were 1.9 µm and 19.4 µm in the x and z dimensions, respectively (Fig. 2(c) and 2(d)). These results indicate that the GRIN lens stretches the axial resolution noticeably (322% of

![Fig. 2. Spatial resolutions of the two-photon microendoscope. Point spread functions obtained by imaging of fluorescent microspheres of 0.17 µm diameter with the GRIN lens (w/ GRIN) demonstrate the (a) lateral and (b) axial resolutions of the full two-photon microendoscope system, whereas those obtained without the GRIN lens (w/o GRIN) demonstrate resolutions of the two-photon microscope alone (c, d).](image)
control) but only affects the lateral resolution slightly (121% of control). Our ray-tracing simulation indicated that the lateral and axial resolutions of the focused light were 2.1 µm and 21 µm when imaged without the GRIN lens, whereas they were 2.3 µm (110% of without the GRIN lens) and 56 µm (267% of without the GRIN lens) when imaged through the GRIN lens, respectively. These theoretical values are largely consistent with our experimental results and suggest that the spherical aberration of the GRIN lens is a major factor for the degradation of the resolution.

We next characterized the focal adjustability enabled by the ETL. A FOV containing fluorescent beads of 5 µm diameter was imaged at different depths by applying different amounts of current to the ETL. In addition, images of the same FOV at depths corresponding to those obtained with the ETL were acquired subsequently by using a z stepper motor (Fig. 3). The output current from the current driver and the focal shifts exhibited highly linear relationships with the control voltage ($R^2 = 0.999$ and 0.999, respectively) (Fig. 3(b) and 3(c)). Consequently, the focus of the microendoscope could be changed by up to roughly 1.5 mm in the 0–5-V range of the control voltage, demonstrating a highly linear operation across a wide range of focal depth. A linear regression analysis estimated that a focal shift of approximately 340 µm is achieved per 1-V input voltage to the current driver. With an increasing amount of focal depth modulation, the FWHMs of the PSFs of the x and z axes exhibited increasing (43% increase at 5 V) and decreasing (13% decrease at 5 V) tendencies, respectively, (Fig. 3(e)).

Fig. 3. Relationship between control voltage and the focal shift. (a) Changes in the focal depth of the objective by an ETL can elicit focal shifts of the GRIN lens. (b) Relationship between control voltages to the current driver and its current output to the ETL. (c) Relationship between control voltages to the current driver and the focal shift. (d) Comparisons of images acquired at different focal depths using the ETL (ETL, upper panels) and those acquired at the corresponding depths using the z stepper motor (z step, lower panels). (e) The FWHMs of the PSFs of the x (left) and z (right) axes across the whole tuning range of the ETL.
We next examined the precision and stability of ETL-mediated fast varifocal imaging. Images of the fluorescent microspheres were acquired at a rate of 30 fps while rectangular voltage pulses of 1 V were applied to the current driver at every other frame (Fig. 4(a)). This enabled imaging of fluorescent microspheres on two focal planes separated by 340 µm (Fig. 4(b)) in a rapidly alternating manner (Fig. 4(c)). Two-dimensional correlation analysis demonstrated that the correlation coefficients between a reference frame acquired with 0 V
and every even frame were constantly very high (~0.9), whereas those between the reference frame and every odd frame were very low (~0.05). Similar results were obtained when the correlation coefficients were calculated using a reference frame acquired with 1 V (Fig. 4(c)). The stability of fast varifocal imaging during a longer period of time was further evaluated in imaging sessions that lasted 10 min. The two-dimensional correlation coefficients calculated using the first frames of the movies for each depth as reference remained high throughout the session and were comparable to those obtained from the control session that imaged the same focal plane continuously without the operation of the ETL (Fig. 4(d)). The 10-min average of the correlation coefficients of the control session was 0.925, whereas that of the fast varifocal imaging was 0.914 (99% of control). In accordance, the lens temperature of the ETL was also stable under this condition; the temperature fluctuations were at most within 0.21 °C. Together, these results demonstrate that fast and highly reproducible focal adjustment is achievable in this varifocal microendoscope.

3.2 Fast multifocal deep brain imaging of neuronal population activity in mice

We tested the applicability of this fast varifocal two-photon microendoscope for in vivo deep brain imaging in mice. Firstly, we imaged spontaneous activity of hippocampal CA1 neuronal networks under isoflurane anesthesia. A guide cannula was implanted in the overlying cortex of Thy1-G-CaMP7-DsRed2 transgenic mice and the GRIN lens was inserted through the cannula to the dorsal hippocampus (Fig. 5(a)). The hippocampal surface covered with alveus axons was readily visualized by two-photon microendoscopic imaging through the GRIN lens (Fig. 5(b)), and a population of pyramidal neurons labeled with G-CaMP7 and DsRed2 were then imaged by lowering the objective by 140 µm using a z stepper motor (Fig. 5(b) and 5(c), see Visualization 1). The G-CaMP-expressing neurons are usually imaged as ring-like structures that comprise G-CaMP-positive cytoplasm and G-CaMP-negative cell nuclei in high resolution two-photon microscopy [5]. However, this characteristic morphology appeared less clear in our microendoscopy, most likely due to the fact that the resolution of the microendoscope, particularly that along the z axis, is not as high as in typical two-photon microscopy using a high NA objective lens (Fig. 2(a) and 2(b)). Time-lapse imaging at 15 fps visualized time-series changes in G-CaMP7 fluorescence intensity that reflected spontaneous activity of CA1 neuronal networks on a single focal plane (Fig. 5(d), see Visualization 2). Consistent with our previous findings [5], activation of CA1 pyramidal neurons occurred asynchronously, as verified by there being no discernible fluorescence changes in a large ROI covering the full FOV. We next imaged the activity of neurons at two different depths in alternating frames at an overall scan rate of 30 fps by applying 0.25-V voltage pulses (~85 µm focal shift) to the ETL current driver during the acquisition of every other frame (Fig. 5(e)). The acquired frames were then sorted for each focal plane to achieve quasi-simultaneous imaging of neuronal activity on two focal planes at 15 fps per plane. In the example shown in Fig. 5(e)–5(g), the ROI for cell 3 detected G-CaMP7 fluorescence changes on the focal plane for 0-V control voltage but not on that for 0.25-V control voltage (Fig. 5(g), see Visualization 3), demonstrating that the varifocal microendoscope imaged neuronal activity in a depth-specific manner.

Finally, to demonstrate its potential for “ultra-deep” functional neural circuit imaging, we performed microendoscopic imaging of spontaneous activity of amygdalar circuits in anesthetized mice. Thy1-G-CaMP7-DsRed2 transgenic mice strongly express G-CaMP7 and DsRed2 in their amygdalar neurons, which are located ~4 mm deep from the brain surface and are therefore difficult to image without the aid of GRIN lenses or optic fibers [18]. To visualize amygdalar neurons, we implanted a guide cannula in the surface of the amygdala.
Fig. 5. Spontaneous activity of mouse hippocampal CA1 neuronal circuits imaged using fast varifocal two-photon microendoscope. (a) A coronal brain section of a Thy1-G-CaMP7- DsRed2 transgenic mouse that was implanted with a guide cannula for hippocampal imaging (HP: hippocampus; scale bar = 1 mm). (b) Dorsal hippocampal CA1 area of the transgenic mouse was imaged using the microendoscope at the surface (left, 0 µm) and 140 µm below (right, 140 µm). The red dotted box delineates the area imaged in (c) and (d) (A: anterior; L: lateral; scale bar = 200 µm). (c) G-CaMP7 (left, green) and DsRed2 (right, red) fluorescence images of hippocampal CA1 pyramidal neurons imaged 140 µm from the hippocampal surface. Arrowheads indicate two representative neurons [cells 6 and 7 in (d)] (scale bar = 50 µm). (d) Positions of 10 active neurons are indicated by red boxes in an average G-CaMP7 fluorescence image (left). Baseline-normalized G-CaMP7 fluorescence time traces for the 10 cells (right). In addition to each cellular signal, the average fluorescence signal across the whole field (Field) is shown. (e) Average G-CaMP7 fluorescence images acquired in the CA1 hippocampus at different focal depths using the ETL controlled by 0 V (left) and 0.25 V (right) voltages. The positions of representative neurons on each focal plane are indicated by red boxes (scale bar = 100 µm). (f) Baseline-normalized G-CaMP7 fluorescence time traces for the four cells shown in (e). Statistically significant fluorescence changes that are larger than five times the standard deviation of the baseline signal are indicated by asterisks. (g) Example time-lapse images of G-CaMP7 fluorescence during spontaneous activity of cell 3 are shown from top to bottom. Series of images acquired quasi-simultaneously at different depths are shown in left (0 V) and right (0.25 V) panels. Red arrows indicate cell 3 in the left panel and the corresponding position in the right panel. Their magnified views are shown in the right-hand side of each panel.
Fig. 6. Fast varifocal microendoscopic imaging of spontaneous activity in amygdalar neuronal circuits in mice. (a) A coronal brain section of a Thy1-G-CaMP7-DsRed2 transgenic mouse implanted with a guide cannula for amygdalar imaging (AMY: amygdala; scale bar = 1 mm). (b) G-CaMP7 (top, green) and DsRed2 (middle, red) fluorescence images of the amygdalar surface. A merged image of the two fluorescence signals is shown at the bottom. The white dashed box delineates the area imaged in (c) (scale bar = 100 µm). (c) Average G-CaMP7 fluorescence images acquired in amygdala using the ETL controlled by 0 V (left) and 0.35 V (right) control voltages. The positions of neurons on each focal plane are indicated by red boxes (scale bar = 50 µm). (d) Baseline-normalized G-CaMP7 fluorescence time traces for the seven cells shown in (c). In addition to each cellular signal, average fluorescence signals across the whole field (Field) are shown for each plane. Statistically significant fluorescence changes that are larger than five times the standard deviation of the baseline signal are indicated by asterisks. Shaded bars represent significant field activity detected at depths of 0 V (green), 0.35 V (red), or both (gray).

and imaged through the GRIN lens inserted through it. The microendoscope imaged a deep brain region with strong labeling with G-CaMP7 and DsRed2 that is characteristic of the amygdala of the transgenic mice (Fig. 6(a) and 6(b)). Subsequently, activity of amygdalar
neurons at two different depths was imaged in alternating frames at an overall scan rate of 15 fps by applying 0.35 V (~120 µm focal shift) to the ETL current driver during the acquisition of every other frame (Fig. 6(c) and 6(d), see Visualization 4). Significant fluorescence changes in neurons were imaged quasi-simultaneously at the two separated focal planes at 7.5 fps per plane, which is high enough for fluorescence kinetics of G-CaMP genetically-encoded calcium indicators [19]. Remarkably, unlike hippocampal neurons, amygdalar neurons repeatedly exhibited synchronous activity similar to that recorded electrophysiologically in vitro [20, 21], as supported by significant fluorescence changes in the full-field ROI (Fig. 6(d)). Moreover, this synchronous activity occurred across different depths, indicating that it involves a group of three-dimensionally distributed neurons. Although whether this pattern of activity reflects normal physiology such as that associated with slow-wave oscillations under anesthesia or lesion-induced pathology remains to be investigated in future in-depth studies, this observation demonstrates that the microendoscope can visualize a three-dimensional pattern of “ultra-deep” brain activity and that the mode of spontaneous activity of amygdalar circuits may differ from that of the hippocampus.

Overall, our ETL-mediated fast varifocal two-photon microendoscope provides a simple and effective solution to an existing technical problem in microendoscopy, namely that only neurons very close to the end of a GRIN lens or optical fiber can be imaged. The addition of this fast varifocal feature will thus greatly enhance imaging studies of three-dimensional neuronal network function in the deep brain, as demonstrated in our hippocampal and amygdalar imaging. The GRIN lens with a relatively large diameter used in this study is advantageous for successful targeting of guide cannula implantation and identification of the region to be imaged, although it can cause more severe damage to the brain. The present microendoscopic imaging can visualize activity of neuronal circuits essentially as deep as the length of the GRIN lens permits. Therefore, its application is not limited to rodents but extends to the brains of larger animals such as marmosets [22]. In principle, the ETL-mediated focal adjustment that we have demonstrated here could be applied to any GRIN lens. In particular, the use of ones with a higher NA [12] or a smaller diameter [13] would allow for multi-focal-plane imaging of deep brain activity at a higher resolution or with less invasion.

4. Conclusions

We have developed a fast varifocal two-photon microendoscope system that enables quasi-simultaneous imaging of neuronal activity at different depths in the deep brain at a high scan rate. The FOV is sufficiently wide for easily locating an area to be imaged, and the focal adjustment achieved by an ETL is fast and reliable. We proved its utility for in vivo imaging by presenting distinct multi-planar patterns of neuronal network activity in the hippocampus and amygdala in mice. This varifocal microendoscope system thus adds a new dimension to current single-plane approaches to deep brain imaging.

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Disclosures

The authors declare that there are no conflicts of interest related to this article.