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Opto2P-FCM: A MEMS Based Miniature Two-Photon Microscope with Patterned Optogenetic Stimulation

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Abstract: Miniaturized microscopes for monitoring neural activity are an indispensable tool for neuroscience research. We present a novel MEMS based miniature microscope with patterned optogenetic stimulation capabilities enabling cell-specific 2-photon optogenetics and 2-photon imaging.

1. Introduction

Miniaturized microscopes are valuable tools to measure the activation of neural networks in vivo with behavioral context. Multiphoton miniature microscopes offer greater depth access and better cellular localization than one-photon microscopes although they require a fiber tether to deliver the ultrafast excitation light. Two-photon (2P) miniature microscopes have been engineered by using either a coherent-imaging fiber bundle (CFB) and distal scanning element or an on-board MEMS scanner [1–5]. In order to further develop tools that can not only image but control neural activity, methods to combine imaging and photostimulation into a miniature microscope have gained interest. A recent demonstration used a CFB coupled to a gradient index of refraction (GRIN) lens to perform 2P imaging and photostimulation in vivo [5]. However, 2P imaging through CFBs are intrinsically limited in their resolution due to core-to-core pixelation. In contrast, MEMS based miniature microscopes provide improved resolution and capabilities of larger fields of view. Despite these benefits, to the best of our knowledge, MEMS based miniature microscopes have not been combined with two-photon optogenetic photostimulation. The addition of photostimulation would enable the study of function by precisely activating or inhibiting specific subsets of cells.

Here, we present a 2P MEMS based miniature microscope with a second 2P patterned photostimulation beam path (Opto2P-FCM), enabling combined 2P imaging/photostimulation in an overlapping field of view of 250 × 250 μm^2. The 1030 nm photostimulation laser is phase modified using a spatial light modulator (SLM) allowing arbitrary illumination patterns to be relayed by the CFB to the sample, with capabilities to rapidly switch illumination between regions (or cells) of interest. In addition to photostimulation, the Opto2P-FCM has several novel features including the delivery of the 920 nm imaging laser through a standard, commercially available polarization maintaining (PM) fiber, fabrication of the device housing using a consumer-grade resin 3D printer and using solely off-the-shelf optical components. Head-fixed in-vivo 2P imaging and 2P photostimulation in the somatosensory cortex of a mouse expressing jGCaMP7s and ChRmine is demonstrated.

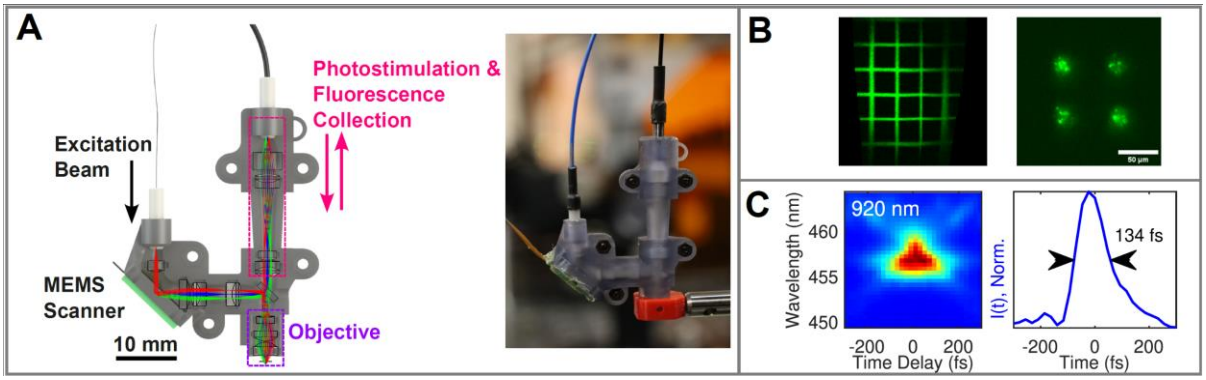

Fig. 1: Opto2P-FCM design and characterization. (A) Left: optical design with excitation at 920 nm through a polarization maintaining fiber, photostimulation at 1030 nm and fluorescent collection at 520 nm though a coherent fiber bundle. Lateral scanning of 250 × 250 μm^2 achieved using a 1.2 mm diameter MEMS mirror scanner. Right: Photo of the assembled device. (B) Scan distortion corrected fluorescence image of 50 μm grid slide and patterned 1030 nm excitation of four 20 μm diameter spots measured at the sample plane. (C) Frequency resolved optical gating (FROG) trace of the 920 nm laser pulse.

2. Methods

The optical design for our device was carried out in Optics Studio (Zemax) constrained to only commercially available optics. The optical design and ray schematic are shown in Fig 1A. The excitation light is delivered through a single mode PM fiber and collimated using an aspheric lens and reflected from a MEMS scanner (Mirrorcle A3I12.2-1200AL) mounted on a custom designed PCB with flex cable. The numerical aperture (NA) from the simulation is 0.4 across the scanning field of 250 × 250 µm^2. The design has a working distance of ~1 mm enabling imaging access to a depth of 300 µm below the coverslip while maintaining a Strehl ratio of ~0.8. A clamshell housing was designed in Solidworks to simplify the installation of the optics as depicted in Fig. 1A. The housing was fabricated by using a resin 3D printer (Anycubic Photon M3 premium). A custom dichroic (T ≥ 90% 330-870, 975-1200 nm – R ≥ 90% 895-935nm) separates the excitation and photostimulation/fluorescent beam paths. The photostimulation/fluorescent path is configured to be chromatically corrected and focuses the 1030 nm light from the CFB (Fujikura FIGH-15-600N) to the same imaging plane as the 920 nm light. The 920 nm laser (MaiTai HP DeepSee) is coupled to a 15 cm PM fiber (Thorlabs PM780-HP) to broaden the spectrum, sent through a grating compressor to compensate for ~100,000 fs^2 dispersion and focused into a 2 m PM fiber (Thorlabs PM780-HP) to deliver the light into the Opto2P-FCM. The 1030 nm photostimulation laser (Spirit 70-1030) is sent through a grating compressor to compensate ~92,000 fs^2 dispersion, sent to an SLM (Phasor, Intelligent Imaging Innovations) and coupled into the CFB to deliver the patterned light to the miniature microscope. The fluorescence emission from GCaMP is collected by the CFB, spectrally filtered and detected with a PMT (Hamamatsu H7422P-40). Animals were prepared by injecting pGP-AAV9-syn-jGCaMP7s-WPRE and pAAV8-hSyn-ChRmine-mScarlet-Kv2.1-WPRE at a 2:1 ratio and 10x dilution into somatosensory cortex region S1Tr of mice and installing cranial windows and head bar.

3. Results & Discussion

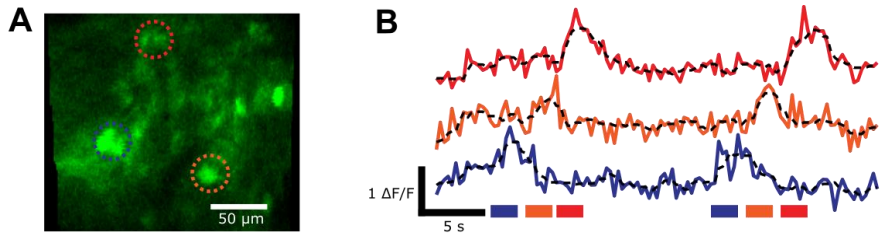

Fig. 2: In vivo Opto2P-FCM data. (A) Timelapse projection of GCaMP imaging captured at 3.5 Hz in somatosensory cortex in a head-fixed mouse. Stimulated ROIs are outlined, and color matched to ΔF/F traces shown in B. (B) ΔF/F traces (solid lines) of ROIs outlined in A – dashed lines show a moving median of 6 frames. Timing of photostimulation of different ROIs are indicated as bars. Stimulation was applied for 5 ms at 4 Hz for 2 s duration.

Table 1: Device Characteristics

Field of View	Working Distance	Imaging PSF FHWM (Lateral/Axial)	Photostimulation Axial FWHM (20 µm spot)	Photostimulation Axial FWHM (40 µm spot)	NA (Sim. / Measured)	Weight
250 × 250 µm^2	1.0 mm	1.2 µm / 14 µm	65 µm	67 µm	0.4 /0.29	~ 5 grams

Results of in-vivo 2P imaging and photostimulation in a head-fixed mouse using the Opto2P-FCM are shown in Figure 2 demonstrating the capabilities of photostimulating sequences of cells and simultaneous GCaMP recording. The properties of the Opto2P-FCM were characterized experimentally and are summarized in Table 1. Fig. 1B shows 2P images of a fluorescent grid target and 1030 nm photostimulation pattern recorded in the sample plane. Additionally, the temporal profile of the 920 nm laser pulse after propagating through the fiber was characterized, showing a sub-150 fs pulse for efficient 2P excitation. The Opto2P-FCM will allow novel studies of neural circuits with cell-specific optogenetic control to study the neural basis of behavior.

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