A Portable Two-photon Fluorescence Microendoscope Based on a Two-dimensional Scanning Mirror

Wibool Piyawattanametha[1,2], Eric D. Cocker[1], Robert P. J. Barretto[1], Juergen C. Jung[1], Benjamin A. Flusberg[1]

[1]*James H. Clark Center for Biomedical Engineering & Sciences, Stanford University, Stanford, California 94305*

Hyejun Ra[2] and Olav Solgaard[2]

[2]*Edward L. Ginzton Laboratory, Stanford University, Stanford, California 94305*

Mark J. Schnitzer

James H. Clark Center for Biomedical Engineering & Sciences, Stanford University, Stanford, California 94305

Email: wibool@stanford.edu

Abstract

Towards overcoming the size limitations of conventional two-photon fluorescence microscopy for brain imaging in freely moving mice, we introduce a portable laser-scanning microendoscope based on a microelectromechanical systems (MEMS) two-dimensional (2-D) scanning mirror, compound gradient refractive index (GRIN) micro-lenses, and a photonic bandgap fiber (PBF). The microendoscope achieves fast line scanning acquisition rates up to 3.5 kHz and micron-scale imaging resolution.

Keywords: scanning mirror, scanner, two-photon, 3-D image, fluorescence image

INTRODUCTION

Two-photon fluorescence microscopy has become a widely used technique for three-dimensional imaging of biological specimens due to proven advantages over conventional epi-fluorescence and confocal fluorescence microscopy. Among the most significant of these are inherent optical sectioning without the use of a pinhole due to the non-linear excitation process, reduced photobleaching and phototoxicity, and greater penetration depth into highly scattering tissue. To date, use of two-photon fluorescence microscopy in live subjects has generally been limited to anesthetized animals. Integration of fiber optics [1] and microlens based imaging probes [2], or the two in combination [3, 4] into an imaging system may help to overcome these limitations and provide the ability to image in freely moving subjects. Our goal is to develop a portable two-photon microendoscopy system for brain imaging in freely moving mice. We have chosen to focus on the use of mice, because of the wide availability of transgenic mouse lines with genetically targeted alterations to cellular processes and animal behaviors. Prior miniaturized scanning mechanism for two-photon fluorescence imaging have typically involved resonant scanning of optical fibers [3,4], which limit size reduction, restrict the choice of scanning rates, and are unsuitable for batch fabrication. Recently, we have demonstrated the use of a MEMS scanning mirror in a tabletop two-photon microscope [5]. In this paper we introduce a portable two-photon fluorescence microendoscope based on a MEMS scanning mirror, compound GRIN micro-optics, and a PBF.

MICROENDOSCOPE DESIGN

The 2-D gimbal scanning mirrors are batch fabricated on silicon-on-insulator wafers that have two single-crystalline silicon device layers (30 µm each). The mirror, movable comb teeth, and inner torsional springs reside in the upper device layer. The frame, outer torsional springs, and fixed comb teeth are fabricated within both device layers. Fabrication involves four deep-reactive-ion-etching steps. The first three steps self-align the comb fingers in the device layers by transferring mask features sequentially from upper to lower layers. The last step removes the backside of the substrate behind the mirror, releasing the gimbal for rotation [6]. The scanning mirror is actuated by 6 banks of vertical comb actuators. The total die is 3.2 × 3.0 mm^2 in size and the mirror is 760 × 760 µm^2.

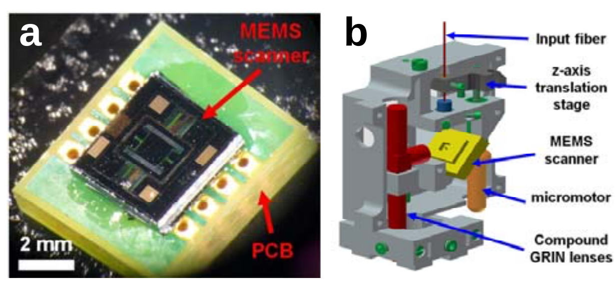

Fig. 1: a) Photograph of a MEMS scanning mirror. b) Schematic drawing of the microendoscope.

Figure 1a and 1b show a photograph of a scanning mirror mounted on a printed circuit board (size = 6.0 × 5.0 mm^2) and a schematic drawing of a microendoscope (size = 2.0 × 1.9 × 1.1 cm^3), respectively. Figure 2 shows a photograph of the microendoscope with overlay drawing of optical paths. The optical circuit can be described as follows. An ultrashort pulsed Ti:sapphire laser (λ_0=800 nm) provides an excitation beam (red arrow) through a PBF. The beam is collimated by a collimator before it reflects off the scanning mirror. The beam is then re-expanded, passes through a dichroic prism, and fills the back aperture of a GRIN objective, which focuses the light at the specimen plane. Fluorescence (green arrow) returns back through the GRIN objective, reflects off the dichroic prism, and is detected by a photomultiplier tube. The excitation and collection numerical aperture (NA) values of the system are 0.46 and 0.61, respectively. The microendoscope working distance from the GRIN objective is 400 μm. The power output at the specimen can reach up to 60 mW.

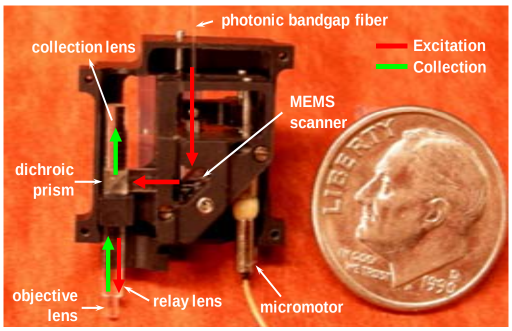

Fig. 2: Photograph of the microendoscope.

RESULTS & DISCUSSIONS

Table 1: Summary of scanning mirror performance

	Inner-axis	Outer-axis
Mirror radius of curvature	> 1 m	
Mirror surface avg. roughness (*RMS*)	< 20 nm	
Resonant freq. (kHz)	1.6 to 1.8	0.6 to 0.8
Max. optical DC scan angle (°)	± 4.0° to ± 8.0°	± 1° to ± 3°
Voltage at Max. DC angle (V)	70 V to 100 V	120 V to 180 V

The general performance range of non-metalized 2-D scanning mirrors is summarized in Table 1. To image with the scanning mirror, both inner- (fast-axis) and outer- (slow-axis) axes have their opposing comb actuator banks driven 180° out of phase with unipolar sinusoidal waveforms. This is done to maximize the linear region of the angular deflection [5]. The fast-axis is driven at resonance while the slow-axis is driven in DC mode (5 Hz). All images are 402 pixels × 162 pixels taken at 5 frames per second with double-sided fast-axis acquisition. The maximum field of view is 80 × 20 μm^2. No frame averaging is performed on the images. Figure 3a and 3b show parts of a spiky-shape and a clover-leaf shape pollen grains, respectively. The maximum power to obtain these auto-fluorescence images is 30-40 mW. These power levels were needed because of the fast acquisition rate. The scale bars are 10 μm.

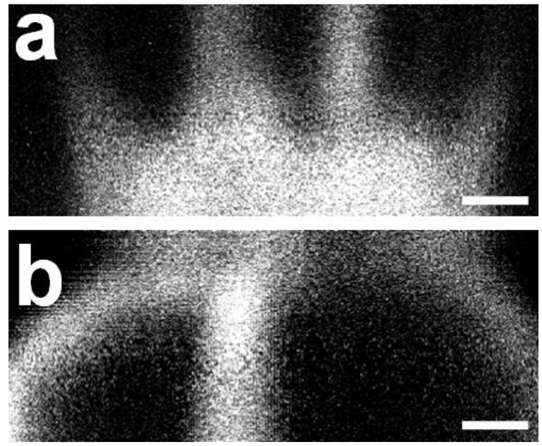

Fig.3: Fluorescence images of pollen grains (a) spiky shape (b) clover-leaf shape.

CONCLUSION

We have built the first MEMS based miniaturized and portable two-photon fluorescence microendoscope with micrometer-scale resolution. We anticipate a broad set of future imaging applications for this microendoscope including freely moving mice brain imaging.

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