Miniaturized multiphoton microscope with a 24Hz frame-rate

Tzu-Ming Liu[1], Ming-Che Chan[1], I-Hsiu Chen[1], Shih-Hsuan Chia[1], and Chi-Kuang Sun[1,2*]

[1] *Department of Electrical Engineering and Graduate Institute of Photonics and Optoelectronics, National Taiwan University, Taipei 10617, TAIWAN. R.O.C.*
[2] *Research Center for Applied Sciences, Academia Sinica, Taipei 11529, TAIWAN R.O.C.*
**corresponding author: sun@cc.ee.ntu.edu.tw*

Abstract: With miniaturized tube lenses and a micro-electro-mechanical system (MEMS) mirror, we constructed a miniaturized multiphoton microscope system. Through a two-dimensional asynchronous scanning of the MEMS mirror, 24Hz frame rate can be realized. With a high numerical aperture objective, sub-micron resolution can also be achieved at the same time.

OCIS codes: (180.4315) Nonlinear microscopy; (190.4710) Optical nonlinearities in organic materials; (190.3970) Microparticle nonlinear optics.

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1. Introduction

Multi-photon nonlinear optical microscopy has emerged as one of the least-invasive tools to perform three dimensional imaging with a submicron resolution. Physiological and morphological changes of living cells can thus be visualized, providing disease diagnosis at an early stage. To further extend nonlinear optical imaging for hand-held or endoscopic applications, the bulky scanning system should be miniaturized to a compact one. In general, the development of the miniaturization includes two parts: one is the scanning unit and the other is the lens, including tube lenses and objectives. The miniaturization of scanning unit is usually based on the use of micro-electro-mechanical system (MEMS) mirrors [1-3], piezoelectric actuator driven fibers [4], or scanning on a fiber bundle [5]. The size reduction

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of the tube lens and the objective usually employ a gradient-index (GRIN) rod lens [1, 4-8]. These previous miniaturized demonstrations achieved three dimensional sectioning capabilities with a 1~2-micrometer transverse resolution. However most of the previous demonstrations employed a synchronous scanning scheme to acquire the data with a slow frame rate. For studying fast biological processes such as blood flow or neuronal activity, the frame rate should be enhanced. Recent advance in the technology of the MEMS mirror increase the resonant frequency of the mirror plate to several kHz on both axis. High frame-rate image acquisition can thus be achieved using high-resonant-frequency MEMS mirrors. By far, at best 8 frame/sec can be achieved in a MEMS-based fiber-optic confocal microscope system [3]. In this work, we employed a MEMS mirror with a high resonant frequency (9.8 KHz) to perform asynchronous scanning in a miniaturized nonlinear microscope system. Combined with a miniaturized tube lens and a focusing objective, the generated nonlinear signal are detected, sampled, and mapped to a 512×512-pixel reconstructed image. Without severe image distortion, record high 24 frame/sec frame-rate and submicron transverse resolution can be simultaneously achieved for the first time in a miniaturized nonlinear microscope.

2. Methods

The miniaturization of our nonlinear microscope system includes the use of a MEMS mirror and a size-reduced tube lens.

2.1 MEMS scanning scheme

The MEMS mirror is the key component that achieves imaging with a high frame rate (Fraunhofer Institut Photonische Mikrosysteme, 2D scanner). The mirror was resonantly and electrically driven by 2.475 kHz and 19.608 kHz square waves. The corresponding mechanical scanning frequency were f_x=1.2375 kHz and f_y=9.804 kHz, which has an even higher line scanning rate than a previous work [2]. This Lissajous-scanned frequency set were synchronously synthesized from the 50MHz-clock of a Field Programmable Gate Array (FPGA). Cascaded with electrical amplifiers, 0~50V driving voltages were applied to the MEMS mirror. When the MEMS mirror was driven at 50V, the incident laser beam can be sinusoidally deflected by 12 degrees for both axes. Imaged by a tube lens and an objective, the deflected laser beam will be focused on the focal plane of the objective lens and scanned within a square area which is divided into 512×512 pixels. To scan through all the pixels, the highest possible frame rate is 24Hz. Different from the synchronous scanning scheme, the laser beam will not scan line-by-line on the focal plane of the objective lens. Before it scan through a line, the trajectory of the laser beam will move to another line of the 512×512-pixel image. To reconstruct the detected data into an image, we simulate the trajectory of the laser beam and create a mapping table to transform the scanned vector data in a frame period into a 512×512-pixel image. The mapping table is created by $x_n=256+256 \times sin(2\pi f_x n\Delta t)$ and $y_n=256+256 \times sin(2\pi f_y n\Delta t)$, where Δt is the sampling period and x_n and y_n are the pixel indices of the n_{th} sampling, whose value will be round up or down into integer. However, the phase lag between the driving voltage and the mechanical response of the MEMS mirror will cause ghost images. This problem can be solved by cyclically rotating the vector x_n and y_n, by which the phase of the mapping function along x and y axes can by tuned. Ghost images can then be superimposed back to the correct ones.

2.2 Tube lens design

A tube lens was employed to image the laser beam spot on the MEMS mirror into the back aperture of the objectives. When the MEMS mirror scan, all of the deflected beams will be first collimated and then converged again onto the back aperture of the objective. Then these deflected beams were focused and scanned on the focal plane of the objective. To minimize

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the coma and the spherical aberration caused by the oblique incidence of the deflected laser beams, the radius of curvature (ROC) of both lenses were carefully designed and simulated by the ZEMAX software. In simulation and design, for different incident angles, the wavefront distortion resulted from aberration and coma was controlled within 4/λ. The focused spot size after objective lens was also controlled to be within 1μm. Under these design constraints, the image resolution and quality won't be affected much by oblique incidence. The first lens is made of SF11 glass with 10.13mm ROC on the incident side and -8.813mm ROC on the other side. The effective focal length on the incident side of the first lens is 5mm. The second lens is made of BK7 with 38.24mm ROC on the incident side and -45.27mm ROC on the other side. Both lenses have 10mm diameter and 7mm thickness. The outer diameter of the whole tube lens module (including the mounting) is 3cm and the resulted magnification factor is 5.7. All the lenses are antireflection coated for the high transmission around 1250nm.

3. Experimental setup

Our high frame rate nonlinear optical imaging system is composed of a home build femtosecond Cr:forsterite laser source, the 2D MEMS scanning unit, the miniaturized tube lens pair, an objective, and a computer control system with a FPGA core (Fig. 1). The Cr:forsterite laser can typically deliver 500mW output power of 60fs pulses with 83MHz pulse repetition rate. Its 1250nm operating wavelength falls in the optical penetration window of most biological tissue. The mode locking of the laser system is kept stable with the help of a semiconductor saturable absorber mirror. Before delivering the laser beam to the MEMS mirror, we shrink the size of the laser beam to fit the 1.2×1.2mm area of the MEMS mirror plate.

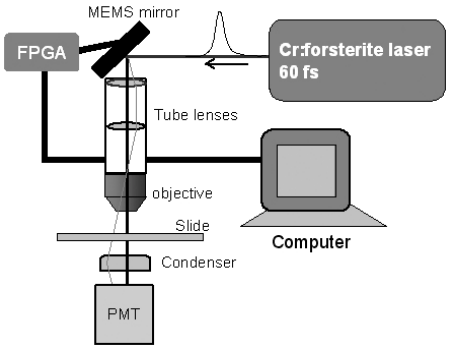

Fig. 1. Schematic diagram of our experimental setup. FPGA: Field Programmable Gate Array. PMT: photomultiplier tube.

The reflecting area of the MEMS mirror is a silicon plate suspended by two torsional springs. Gimbal mounting of the mirror plate is used and the reflectivity of the mirror plate is enhanced by a thin layer of aluminum. For high transmission of the incident and reflected laser beam, the glass window covering on the MEMS mirror is anti-reflection coated around the wavelength of 1250nm.

After the MEMS mirror, we placed the designed miniaturizing tube-lens set. The scanned laser beam was imaged onto the back aperture of the objective and focused on the sample by a 60× water-immersion objective (NA=0.9) with 3mm effective focal length. The sample under test is placed on the slide. As the sample moved to the focal plane, the generated two-photon-excited fluorescence (TPEF) or second harmonic generation (SHG) signals were collected by a condenser and detected by a thermal electric (TE)-cooled PMT tube. The detected analog voltage signals were sampled by a data acquisition card with an 8.33MHz sampling rate synchronous with the FPGA system. Within each frame period, the acquired

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data vector will be reconstructed into a 512×512-pixel image according to the method described above. Thus reconstructed image or movie will be displayed and saved on the computer.

4. Two-photon fluorescence and second harmonic generation images

We first tested our TPEF image quality by the fluorescent beads (λ=625~640nm) with a 15-μm diameter. These beads were drop on the slide with a cover glass. As shown in the Fig. 2, the 512×512-pixel image has a 76μm×70μm field of view. All the fluorescent beads appear with a shape close to a sphere. There is no shape distortion on the boundary of the image.

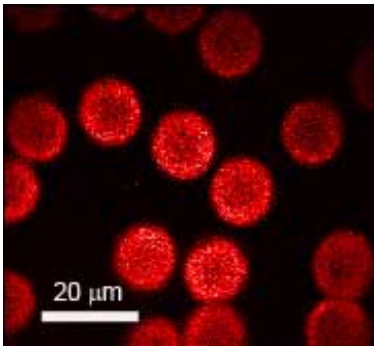

Fig. 2. Two-photon-excited fluorescent microscopic imaging of 15-μm fluorescent beads.

Then we used 1-μm fluorescent beads to test the transverse resolution of our imaging system. The solution of fluorescent beads was dropped on the slide and we took images on the fixed beads at the solution-glass interface. Figure 3(a) shows the TPEF image of two fluorescent beads close to the boundary of the image with a magnified field of view. To improve the signal-to-noise ratio for this study, we first obtained a 512×512-pixel image by averaging 12 frames of images (corresponding to a 0.5-second integration time). We further reduce the size of the image to 256×256 pixel by averaging every 4 pixels from the 512×512-pixel image. Taking a cross section along a line from A to A', the corresponding full width of half maximum (FWHM) of the intensity profile is 1.33 μm (See Fig. 3(b)). Since the size of the fluorescent beads are close to the focused spot size, there is a plateau region around the maximum of the convolved trace. Considering the 2D convolution of a Gaussian point spread function (1-μm spot diameter) with a 1-μm fluorescent bead (a hat function with 1-μm diameter), the FWHM of the cross sectional trace is 1.35 μm. With a smaller FWHM, our result indicated a submicron transverse resolution of the studied imaging system.

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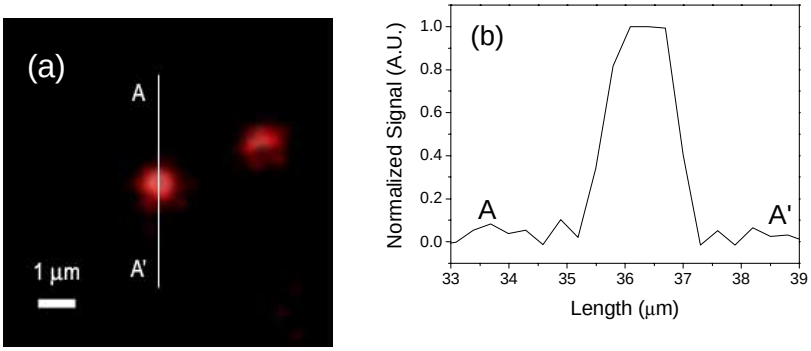

Fig. 3. (a) Two-photon-excited fluorescence image of 1-μm fluorescent beads. (b) Intensity distribution of a fluorescent bead along a line crossing the peak intensity in Fig. 3(a).

After studying the spatial resolution performance, we examined the temporal capability of our MEMS-based miniaturized nonlinear microscope system. In order to test the frame-rate of our system, we recorded the Brownian motion of these 1-μm fluorescent beads suspended in the solution. Figure 4 shows the recorded movie with a 24Hz frame rate and 512×512-pixel images. The corresponding field of view is 76μm×70μm.

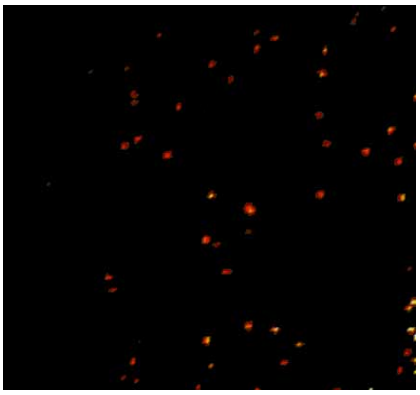

Fig. 4. (2.15 MB) The two-photon-excited fluorescence movie showing the Brownian motion of the 1-μm fluorescent beads. (10.5 MB version). These images were taken with a 24Hz frame rate and 512×512-pixel resolution. Image size: 76μm×70μm

Finally, this miniaturized system was also be applied to the second harmonic generation microscopy. Figure 5 shows a SHG microscopic imaging taken inside a frozen bovine tendon with the constructed microscopic system. This specific image was also taken by averaging 12 frames, corresponding to a 0.5-second integration time. With a sub-micrometer transverse resolution, the wavy type-I collagen fibers can be clearly identified in the sectioned SHG image [9-11].

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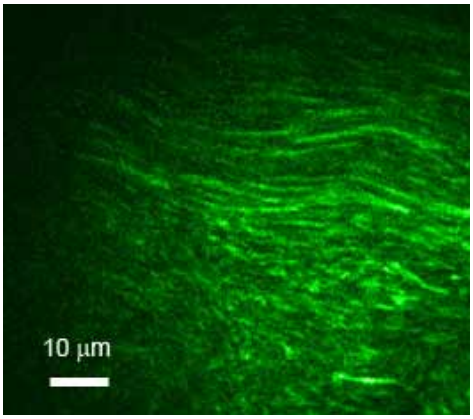

Fig. 5. Second harmonic generation image of a bovine tendon.

5. Summary

In summary, we demonstrate a high frame-rate (24Hz) miniaturized TPEF/SHG microscope system. This was achieved by asynchronous scanning of a MEMS mirror with a 9.8KHz resonant frequency. With a careful design on the radius of curvature of two miniaturized tube lenses, no image distortion was found on the boundary of the acquired 2D image and the corresponding transverse resolution can be down to sub-micron, through a regular high NA microscope objective.

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