THREE-DIMENSIONAL MICROSCOPY OF BIOPSIES WITH A HANDHELD CONFOCAL MICROSCOPE

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ABSTRACT

Intravital confocal microscopy has provided powerful mechanistic insights into health and disease with three-dimensional imaging capability and has become a common imaging instrument in the modern biological laboratory and clinic. However, the requisite of high numerical aperture, short working distance, and small field of view that enable confocal microscopy limit the ability to investigate hollow organs *in vivo*. Here we introduce a handheld confocal microscope that circumvents the above technical limitations of confocal microscopy and, as a result, provides imaging access to variety tissues *in vivo*. The handheld microscope achieves its miniaturization with micro-optics and microelectromechanical systems scanner technology enabling a small form factor and 3-D imaging performance.

KEYWORDS: Confocal Microscope, MEMS Scanner, in vivo imaging, 3-D imaging

I. INTRODUCTION

Intravital single-axis confocal (SAC) microscope is a versatile tool in disease diagnosis enabling high-resolution three-dimensional (3-D) capability for imaging biological tissues. Its unique features are derived from a high numerical aperture (NA) lens to achieve high lateral resolution, and its optical sectioning property from a pinhole placed in front of the detector to reject out of focus light. Therefore, a high-resolution 3-D image from highly scattering media can be reconstructed by successively scanning each 2-D focal imaging plane. Furthermore, the combination of confocal and fluorescence microscopy, by fusing fluorescent molecules to proteins or enzymes to study cellular or molecular processes, heralds a new *in vivo* imaging era. As a result, *in vivo* 3-D functional images obtained from the combined techniques are unmatched by other imaging modalities. Nonetheless, SAC microscopes have trade-offs among resolution, field of view (FOV), and objective lens size, since a high NA objective is needed for sufficient resolution, and a long focal length is needed for a large FOV and working distance (WD). The dual-axis confocal (DAC) microscope architecture has been proposed utilizing two low NA objectives providing overlapping long working beams circumventing above tradeoffs [1, 2].

II. METHODS

Because of DAC post-objective configuration, it offers several advantages over the SAC architecture. First, the higher NA objective is not required enabling a long WD and larger FOV. An ample space between objectives and focal plane can be accommodated with a microelectromechanical systems (MEMS) scanner for raster or lissajous laser beam scanning. Moreover, a near aberration-free scanning laser beam can also be achieved. Second, in the SAC architecture, the axial resolution is substantially worse than the transverse resolution, while the DAC design provides balanced resolutions in all spatial dimensions. Third, the DAC design has superior optical sectioning because light scattered along the illumination path outside the focal volume couples to the output fiber with very low efficiency, enhancing both detection sensitivity and dynamic range [3]. Previously, MEMS-scanner-based DAC microscopes have been demonstrated in a tabletop setup [4], v-groove mount (unpackaged) [5], and clinical setup (miniaturized package) [6]. In this work, we present a handheld MEMS-scanner-based DAC microscope capable of 3-D real-time imaging for biopsy imaging.

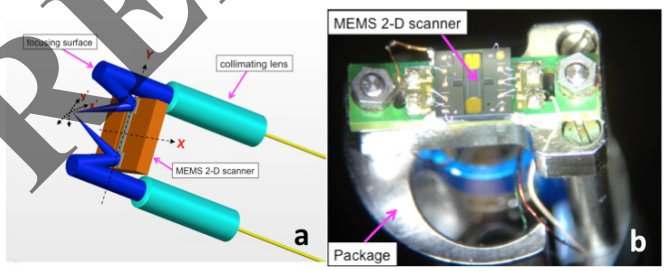

Fig. 1. a) A 3-D schematic drawing of DAC configuration. B) MEMS scanner mounted on a PCB on top a 10-mm package.

Figure 1a shows a 3-D schematic drawing of handheld DAC microscope.
The laser source coupled into an input single-mode optical fiber exiting collimator with a collimated beam. Then, laser gets focused by a parabolic mirror and reflected to out side by MEMS mirror into a sample under investigation. The scattered or fluorescence light from the sample returns to another side of MEMS mirror and coupled back into a collimator connected with a photomultiplier tube (PMT). The photon signal is converted to current by a low noise amplifier and then it is fed to a data acquisition system with real-time display on a computer monitor. Figure 1b shows a photograph of a mounted a two-dimensional MEMS scanner (die size = 3.2 × 3.2 mm^2) on a printed circuit board. The MEMS scanner scans the focused laser beam over an *en face* FOV.

Each image is acquired by the MEMS scanners performing a 2-D raster scan on the image plane. Both the inner axis (slow-

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axis) and the outer axis (fast-axis) have their opposing comb actuator banks driven 180° out of phase. This is done to maximize the linear region of the angular deflection [6, 8]. However, small image distortions can still be observed on acquired images because higher order voltage dependent terms of the actuators still remain. The voltage on each axis is tuned to have similar deflection angles. The fast-axis is driven at resonance by a unipolar sinusoidal waveform. The slow-axis is driven in a DC driving mode (1-5 Hz depending on required imaging frame rate) with smooth-turn-around saw-tooth waveforms to avoid MEMS scanner oscillating [7].

III. RESULTS

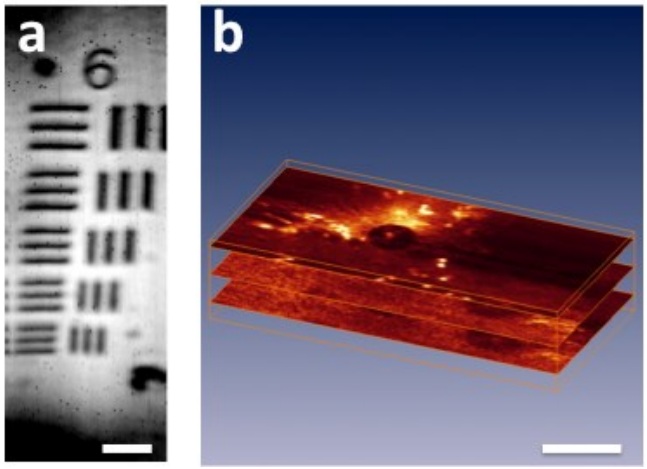

Figure 2: a) A cropped 7-6 of USAF resolution target image with no frame averaging (scale bar 5 μm). b) A 3-D rendered image of a human colon biopsy tissue (scale bar 100 μm).

All images are captured at 5-10 Hz (single-sided data acquisition from MEMS raster scan) with approximate maximum FOV of 700 × 260 μm^2. The acquired image in Figure 2a shows a US Air Force (USAF) resolution target of Group 6. It is also used as a sample to measure the image resolutions and FOV. The full width at half maximum (FWHM) transverse resolution is 5 μm. The FWHM axial resolution measured by translating a plane mirror in the *z*-direction is 7 μm. Figure 2b show a 3-D rendered image of a human colon biopsy.

IV. CONCLUSION

We demonstrated a 3-D microscopy with a handheld confocal microscope in 10 mm diameter package for biopsy imaging. When coupled with fluorescence markers targeted against a variety of diseases, the handheld microscope will enable *in vivo* optical biopsy for early and accurate detection of cancer and for precise surgical resection.

V. ACKNOWLEDGMENT

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VI. REFERNCES

[1] T. D. Wang, M. J. Mandella, C. H. Contag, and G. S. Kino, "Dual-axis confocal microscope for high-resolution *in vivo* imaging," *Opt. Lett.,* vol. 28, pp. 414-416, 2003.

[2] T. D. Wang, C. H. Contag, M. J. Mandella, N. Y. Chan, and G. S. Kino, "Dual-Axes confocal microscopy with post-objective scanning and low coherence heterodyne detection," *Opt. Lett.,* vol. 28, pp. 1915-1917, 2003.

[3] J T.C. Liu, M. J. Mandella , S. Friedland, R. Soetikno, J. M. Crawford, C. H. Contag, G. S. Kino, and T. D. Wang, *J. Biomed. Opt. 2006*;11:054019-1-10.

[4] H. Ra, Y. Taguchi, D. Lee, W. Piyawattanametha, and O. Solgaard, "Two-Dimensional MEMS scanner for Dual-Axes Confocal *In Vivo* Microscopy," *MEMS 2006, IEEE Int. Conf. on Micro Electro Mechanical Systems*, Turkey, 2006, pp. 862-865.

[5] W. Piyawattanametha, J. T. C. Liu, M. J. Mandella, H. Ra, L. K. Wong, P. Hsiung, T. D. Wang, G. S. Kino, and O. Solgaard, "MEMS Based Dual-Axes Confocal Reflectance Handheld Microscope for *in vivo* Imaging," in *IEEE Int. Conf. on Opt. Micro. Electro. Mech. Sys.*, Montana, Aug 21-24, 2006, pp.164-165.

[6] W. Piyawattanametha, H. Ra, M. J. Mandella, K. Loewke, T. D. Wang, G. S. Kino, O. Solgaard, and C. H. Contag, "3-D Near Infrared Fluorescence Imaging using a MEMS-based Miniature Dual-Axes Confocal Microscope," IEEE JSTQE, vol. 15, no. 5, pp. 1344-1350, 2009.

[7] N. Khemthongcharoen, S. Rattanavarin, R. Jolivot, and W. Piyawattanametha, "Advances in imaging probes and optical microendoscopic imaging techniques for early in vivo cancer assessment (invited paper)," J. ADDR, July 30, 2014, Vol. 74, pp. 53-74.