FIBER-OPTIC CONFOCAL MICROSCOPE WITH AN ELECTROTHERMALLY-ACTUATED, LARGE-TUNABLE-RANGE MICROLENS SCANNER FOR DEPTH SCANNING

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ABSTRACT

We present a fiber-optic reflectance confocal microscope using a large-tunable-range MEMS microlens scanner for automatic axial scan. The tunable microlens is actuated by an electrothermal actuator that is capable of scanning up to 0.9 mm at less than 6.3 V. The lateral shift and tilt of the microlens are about 10 µm and 0.4°, respectively. 2-D cross-sectional reflectance confocal images of micro-particles in polymer and rat skin have been obtained with the lateral and axial resolutions of about 1.2 µm and 38 µm, respectively.

INTRODUCTION

Confocal microscopy is an established imaging technique widely used in biomedical imaging. By using a pinhole to eliminate the out-of-focus light reflected back from samples, high-resolution imaging and non-invasive optical sectioning can be obtained [1]. Its optical sectioning capacity and sub-cellular resolution makes it suitable for *in vivo* imaging. However, to extend the confocal microscopy to *in vivo* imaging of internal organs, there exists a major challenge: the optical scanner must be capable of changing the focus point by a few hundred microns and at the same time it must be small enough to fit into a catheter. Therefore, miniature scanners capable of large axial scanning in a half-millimeter range as well as two-dimensional (2D) lateral scanning must be developed to realize 3D *in vivo* imaging.

Miniature fiber-optic confocal microscopes using 2D MEMS mirror scanners for lateral scan have been reported [2-3], but they rely either on external motors for axial scan or are packaged in a handheld probe without axial scan. Liquid or polymer tunable lenses by electrowetting, pneumatic actuation or hydrogels [4-6] can provide large tunable ranges up to a few millimeters but they require large driving voltages (>100 V) and/or have large size and very slow response time (in seconds). Electrostatic MEMS tunable lenses are fast but they also need high driving voltages and their tunable range is limited to only tens of microns [7-8]. We previously demonstrated a lateral-shift-free (LSF) large-vertical-displacement (LVD) electrothermal actuator with over 0.5 mm vertical displacement [9].

In this paper, we demonstrate a fiber-optic confocal microscopic imaging system using an LSF LVD MEMS microlens scanner for large-tunable-range axial scan. This miniature scanner, providing large range depth scanning at low voltage, is especially attractive for *in vivo* 3D confocal imaging.

MEMS-BASED CONFOCAL MICROSCOPE

The schematic diagram of the fiber-optic confocal microscope using an MEMS microlens scanner is shown in Fig. 1. Light from a laser is cleaned up by a spatial filter, collimated by a lens, and split into two paths by a beam splitter. Half of the light is directed into the sample arm, and focused by the microlens scanner into the sample. At the same time, the microlens scans the focal point axially, thereby imaging different depths of the sample. A stage is used to laterally translate the sample. The reflected light from the sample is collected by the microlens, split by the beam splitter, coupled into a single-mode fiber by a fiber collimator, and detected by an avalanche photodiode (APD). The single-mode fiber acts as a pinhole to eliminate the out-of-focus light.

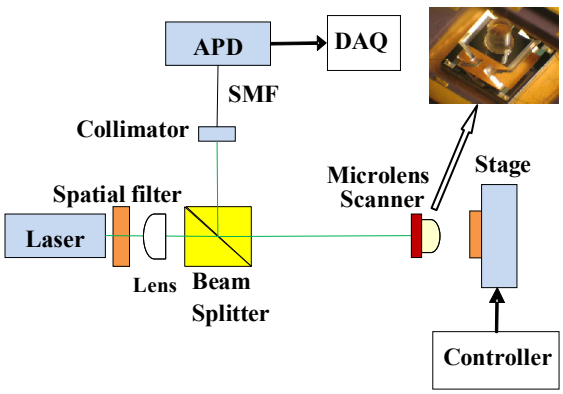

Fig. 1. Schematic diagram of the fiber-optic confocal microscope system.

MICROLENS SCANNER

In this work, the required large depth scan for the confocal microscopy is obtained by axially actuating a lens by a LSF-LVD MEMS actuator. As shown in Fig. 2(a) and 2(b), the LSF-LVD actuator is

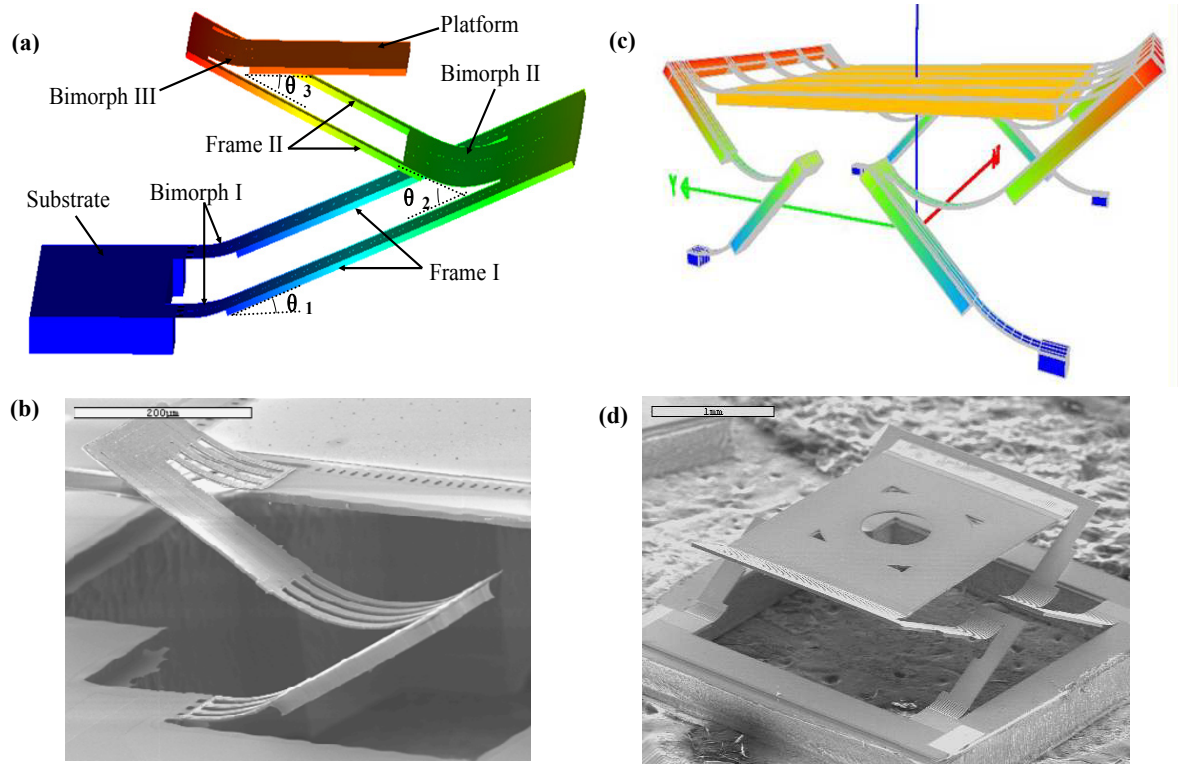

Fig. 2. (a) LSF-LVD actuator design, (b) SEM of fabricated LSF-LVD actuator, (c) lens holder design, (d) SEM of fabricated lens holder.

comprised of three Al/SiO2 bimorphs with two rigid frames connected in between. The lengths of the bimorphs and frames are properly set to compensate lateral shift and tilting [10]. The actuation mechanism is electrothermal actuation, with a thin layer of Pt embedded along the bimorphs as the heater. The large initial elevation due to residual stresses in the bimorph beams provides space for the platform to displace vertically in large range.

The lens holder uses two sets of LSF-LVD actuators symmetrically located at two sides as shown in Fig. 2(c) and Fig. 2(d). The last bimorphs of both actuators are line-anchored to the edge of the platform to ensure a stable actuation. The platform has a central hole and is used as a lens holder. The central hole allows light to pass through. The device is fabricated using a combined surface- and bulk-micromachining process that is reported in [10]. The device footprint is 3 mm by 3 mm. The initial elevation of the lens holder is 0.9 μm.

The microlens scanner is assembled by adhesively bonding a miniature glass lens to the lens holder of an LSF LVD MEMS device. The glass lens has a length of 1 mm, a diameter of 1 mm, and a focal length of 3 mm. The lens is placed on the microactutor's platform which has a central opening for passing the light. An assembled microlens scanner is shown in Fig. 3.

A 0.9 mm vertical actuation range of the glass lens has been obtained at only 6.3 V dc as shown in Fig. 4. The frequency response of the device is shown in Fig. 5 and a mechanical resonance of 79 Hz is observed. There are small tilting and lateral shift during axial actuation, mainly due to some small differences among the bimorph actuators caused by fabrication variations. The tilting and lateral shift could be minimized to below 0.4° and 10 μm, respectively, by controlling the driving voltages ratios of the two actuators.

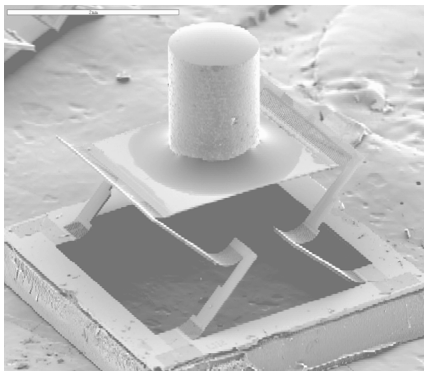

Fig. 3. SEM of an assembled microlens scanner.

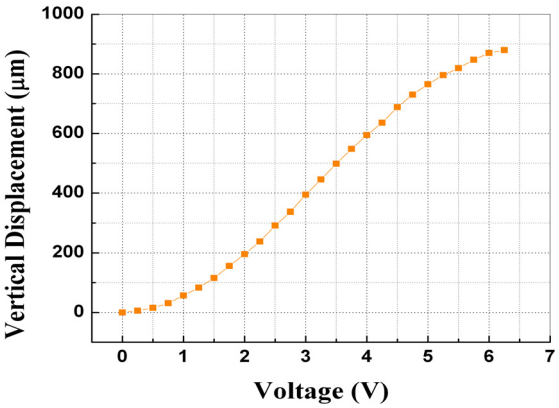

Fig. 4. Vertical displacement versus voltage.

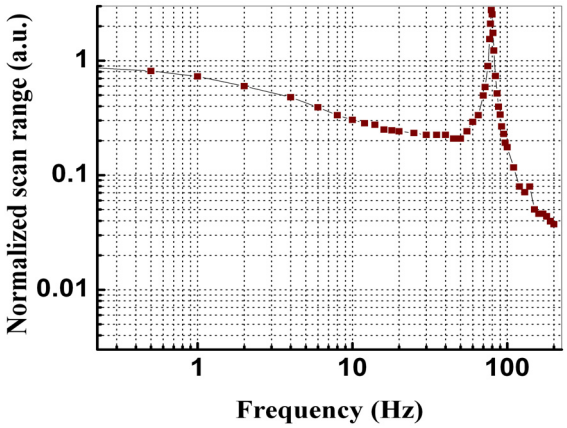

Fig. 5. Frequency response of the microlens scanner.

IMAGE SYSTEM AND RESULTS

The optical system is illustrated in Fig. 1. The light source is an argon ion laser with a center wavelength of 514.5 nm. The single-mode fiber has a mode-field diameter of 3.3 μm and N.A. of 0.13, which is also used to provide the small aperture required by confocal microscopy. Light transmitted by the single mode fiber is detected by a high-sensitivity avalanche photodiode (APD) with an integrated amplifier (Hamamatsu, C5460-01). The amplified signal is acquired into a PC using Labview through a MCC data acquisition card. Matlab is used to process the data.

The lateral and axial resolutions can be respectively estimated from the following equations [11]:

$$x_{FWHM} = \frac{0.37}{n \sin \alpha} \quad (1)$$

$$z_{FWHM} = \frac{0.89}{n(1-\cos\alpha)} \quad (2)$$

where λ is the laser wavelength, n is the refractive index, α is the acceptance angle of the microlens. So, the calculated lateral resolution and axial resolution are about 1.1 μm and 32.9 μm, respectively.

The axial resolution can be measured by using a mirror as the sample and moving the mirror's surface through the microlens' focal plane. The detected signals at various mirror positions are plotted in Fig. 6(a), which indicates that the FWHM of the axial resolution is 38.0 μm. The lateral resolution can be obtained by placing a sample with strip patterns at the focal plane and moving the sample laterally. Fig. 6(b) shows the detected signals when a sharp edge on a USAF resolution test target passes the focal point of the microlens. Using the 10%-90% edge width, the measured lateral resolution is about 1.25 μm. The experiments show that the measured axial and lateral resolutions are in good agreement with the calculated values.

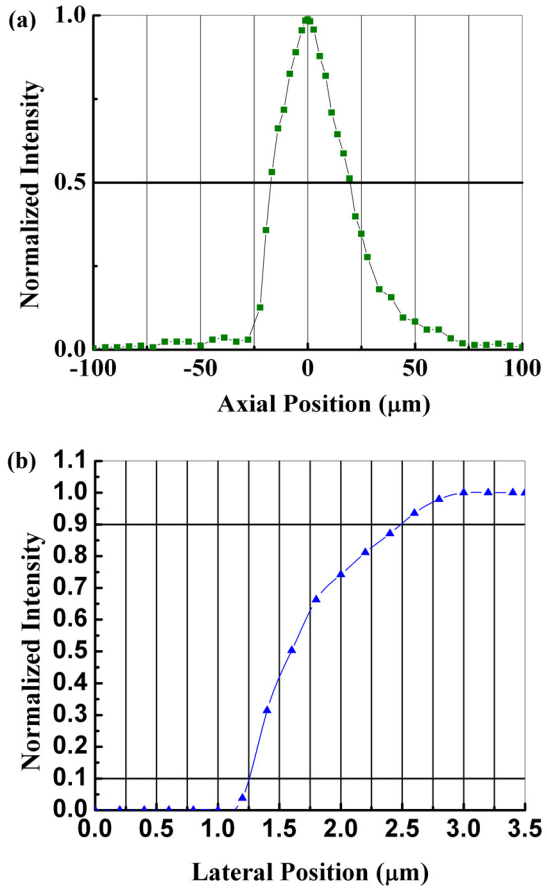

Fig. 6. (a) Axial resolution measurement. FWHM=38 μm, (b) Lateral resolution measurement. 10%-90% rise distance is about 1.25 μm.

Confocal imaging experiments have been performed on various samples including alloy micro-particles embedded in polymer and rat skin. A 2D cross-

sectional image of the micro-particles in polymer is shown in Fig. 7. The image size is 200 µm by 200 µm. The diameters of the micro-particles are around 25 µm. It is obvious that the depth (vertical) resolution is much higher than the lateral (horizontal) resolution. A 2D image of a piece of rat skin is shown in Fig. 8.

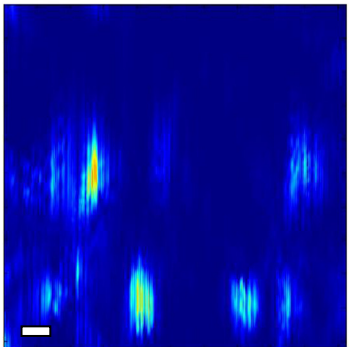

Fig.7. Confocal image of micro-particles embedded in polymer. 200-µm depth scan by microlens scanner with a driving voltage of 3 Vp-p at 2Hz. 200-µm lateral scan by stage. Scale bar is 20 µm.

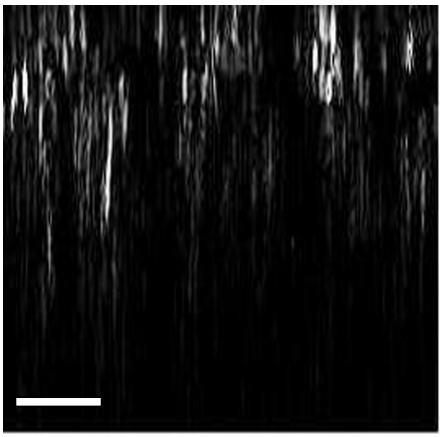

Fig. 8. Confocal image of rat skin. 250- µm depth scan by microlens scanner with a driving voltage of 3.5 Vp-p at 2Hz. Scale bar is 50 µm. The image size is 250 µm × 250 µm.

CONCLUSION

We have demonstrated a fiber-optic reflectance confocal microscope using a large-tunable-range MEMS microlens scanner for automatic depth scan. Confocal images of particles in polymer and rat skin have been obtained with more than 200-µm depth scan. More imaging experiments and more compact confocal microscope design are ongoing. Combining with 2D MEMS scanning mirrors, this microlens scanner is very promising to enable truly 3D *in vivo* confocal microscopy.

ACKNOWLEDGEMENTS

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REFERENCES

[1] D. M. Shotton, "Confocal scanning optical microscopy and its applications for biological specimens," J. Cell. Sci., vol. 94, pp. 175-206, 1989.

[2] H. Ra, W. Piyawattanametha, M. J. Mandella, P. L. Hsiung, J. Hardy, T. D. Wang, C. H. Contag, G. S. Kino and O. Solgaard, "Three-dimensional in vivo imaging by a handheld dual-axes confocal microscope," Optics Express, vol. 16, pp. 7224-7232, 2008.

[3] H. J. Shin, M. C. Pierce, D. Lee, H. Ra, O. Solgaard and R. Richards-Kortum, "Fiber-optic confocal microscope using a MEMS scanner and miniature objective lens," Opt.Express, vol. 15, pp. 9113-9122, 2007.

[4] T. Krupenkin, S. Yang, and P. Mach, "Tunable liquid microlens," Appl. Phys. Lett., vol. 82, pp. 316-318, 2003.

[5] K. Hoshino and I. Shimoyama, "An elastic thin-film microlens array with a pneumatic actuator," in MEMS 2001. pp. 321-324.

[6] X. Zeng and H. Jiang, "Tunable liquid microlens actuated by infrared light-responsive hydrogel," Appl. Phys. Lett., vol. 93, pp. 151101, 2008.

[7] S. Kwon, V. Milanovic and L. P. Lee, "Large-displacement vertical microlens scanner with low driving voltage," IEEE Photonics Technology Letters, vol. 14, pp.1572-1574, 2002.

[8] C. Gorecki, L. Nieradko, S. Bargiel, J. Dziuban, D. Henis, J. A. Sylvestre, K. Alkowska, G. Soto-Romero, J. Thevenet and R. Yahiaoui, "On-chip scanning confocal microscope with 3D MEMS scanner and VCSEL feedback detection," in TRANSDUCERS 2007, pp. 2561-2564.

[9] L. Wu and H. Xie, "A tunable microlens with 0.9 mm scan range and small lateral shift," Proc. of IEEE/LEOS Optical MEMS and Nanophotonics, Clearwater, Florida, United States, Aug. 17-20, 2009, pp. 69-70.

[10] L. Wu and H. Xie, "A large vertical displacement electrothermal bimorph micro-actuator with very small lateral shift," Sensors & Actuators: A., vol. 145, pp. 371-379, 2008.

[11] T. R. Corle and G. S. Kino, Confocal Scanning Optical Microscopy and Related Imaging Systems, Boston: Academic press, 1996.