HISTOLOGY-EQUIVALENT VERTICAL OPTICAL SECTIONING USING A 2-AXIS MAGNETIC CONFOCAL MICROSCANNER

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ABSTRACT

This paper presents a 2-axis confocal scanner for real-time and direct imaging of vertical cross-sections of biological samples. The device incorporated an out-of-plane lens actuator and a transverse optical fiber scanner. Two-dimensional dynamic scanning of samples using this device has been demonstrated by obtaining a scanning range of 145 μm and 190 μm in the X-Z plane (perpendicular to the surface of sample). Optical resolutions of 4.8 μm and 10.7 μm have been demonstrated in X and Z directions respectively. Images obtained by this scanner are analogous to conventional histology images and can facilitate non-invasive clinical diagnosis and evaluation of diseases such as skin cancer.

INTRODUCTION

Scanning a beam of light in the X-Y plane (parallel to the surface of sample) using micromirrors and optical fibers has been previously demonstrated. Micromirror scanners have been developed using electromagnetic [1], electrostatic [2], electrothermal [3] and piezoelectric [4] techniques. Optical fiber scanners are driven using electromagnetic [5] or piezoelectric [6] actuators. These scanning techniques can be used to obtain confocal images from a horizontal cross-section of samples. Vertical cross-sectional images can also be constructed from stacks of horizontal images obtained from various depths within the sample [7]. However, this method of vertical image reconstruction could be time-consuming for high resolution imaging and therefore may be unsuitable for real-time clinical applications. As a result, a confocal scanner capable of providing direct and real-time vertical cross-sectional images is advantageous. Recently, a group has proposed direct vertical imaging by mounting a micromirror on a stage, actuated in the axial direction using piezoelectric unimorph beams [8]. We have previously reported a magnetic actuator for moving a lens in the axial direction and used it for measuring thickness of biological samples and transparent films [9-11].

In this paper, we demonstrate a 2-axis confocal scanner by coupling a magnetic lens actuator with a transverse fiber optic actuator. The device is capable of two-dimensional scanning of the focal spot of a lens in the X-Z plane. The actuators are fabricated by laser micromachining technique and driven by electromagnetic forces. The system requires a low driving voltage of 6 volts and provides a field of view of 190 μm by 145 μm in the axial (Z) and transverse (X) directions respectively with imaging frame rate of 2 fps.

METHODS

A schematic diagram of the optical configuration for light delivery and collection as well as the 2-axis confocal scanner scheme is shown in Fig. 1. A 785 nm laser beam is directed to a beam splitter and then coupled into a single mode optical fiber (SMF) using a fiber coupler. SMF has numerical aperture (NA) of 0.17. The output light from SMF is collimated by lens L1. L1 has diameter of 3 mm, focal length of 4.5 mm and NA of 0.3. The collimated light is then focused into a sample by lens L2. L2 has diameter of 2.4 mm, focal length of 1.45 mm and effective NA of

0.35. Two-dimensional imaging is performed by raster scanning of the laser spot in a vertical plane through the sample. This is achieved by actuating L2 in the axial and SMF in the transverse directions. After scanning the sample, the scattered light is sent into a photomultiplier tube (PMT) using L2, L1 and SMF. The intensity signal is converted into image of cross-section of the sample using a data acquisition (DAQ) card and a computer. Driving signals for fiber and lens actuators are also produced and controlled by the DAQ card.

Figure 1: Schematic diagram of the optical configuration for light delivery and collection and the 2-axis confocal scanning system.

SYSTEM DESIGN AND CONSTRUCTION

Figure 2 shows lens and fiber actuation mechanisms. As shown in Fig. 2(a), L2 is suspended on a nickel flexure that is mounted on an electromagnetic coil (C1). The nickel flexure and L2 are actuated 190 μm at resonant frequency of 378 Hz. Collimating lens L1 is placed inside the hollow core of C1. SMF is mounted on a nickel cantilever beam and actuated 450 μm by coil C2 at frequency of 2 Hz (Fig. 2(b)). The selected lenses provide magnification of 0.32 in transverse direction. As a result, the focal spot into the tissue is moved 145 μm in x-direction.

A commercial laser machining system (QuikLaze 50ST2, New Wave Research Inc.) was used to fabricate the actuators shown in Fig. 3. The selected laser wavelength was green (532 nm) with energy of 3 mJ, pulse rate of 50 Hz and cutting speed of 15 $\mu\text{m/s}$. Thickness of nickel foils (ESPI Metals) used to fabricate lens and fiber actuators were 56 μm and 25.4 μm respectively. The cantilever beam for fiber actuation has width of 2 mm and length of 25 mm.

Figure 2: Cross-sectional drawing of 2-axis confocal scanner showing (a) axial scanning and (b) transverse scanning mechanisms.

Figure 3: Photo of (a) nickel cantilever beam and (b) nickel flexure, fabricated by laser micromachining.

OPTICAL PERFORMANCE

Optical resolutions of the scanner in both axial and lateral directions will affect the image quality. The laser beam distribution at the focal point of a lens is shown in Fig. 4. $w(z)$ is beam radius that varies along the z -axis and is calculated using Eq. (1) [12]:

$$w(z) = w_0 \sqrt{1 + \left(\frac{z}{z_R}\right)^2} \quad (1)$$

where w_0 is the beam waist radius and z_R is the Rayleigh range that is related to the beam waist according to:

$$z_R = \frac{\pi w_0^2}{\lambda} \quad (2)$$

In Eq. (2), λ is wavelength of the laser beam. Optical performance of the system in axial and lateral directions can be assessed by $2z_R$ and $2w_0$ respectively. In our system, from Zemax optical simulation software, the Rayleigh range is 1.63 μm and on-axis beam waist is 0.64 μm .

Figure 4: Laser beam profile for focusing a Gaussian beam.

Figure 5: Experimental measurement of (a) axial and (b) lateral resolutions of confocal scanner.

Axial and lateral resolutions of the scanner were experimentally evaluated from intensity signals obtained by scanning of a flat mirror and a blade-edge respectively [13]. Figure 5(a) is generated by plotting the intensity signal detected by PMT while keeping SMF stationary and actuating L2 toward a flat mirror. The axial resolution can be approximated from the full width at half maximum (FWHM) of the intensity signal:

$$R_{axial} = Z_{2(\%50)} - Z_{1(\%50)} = 10.68 \mu m \quad (3)$$

Figure 5(b) is an intensity signal obtained from scanning edge of a sharp blade. From this graph, lateral resolution can be calculated according to Eq. 4:

$$R_{lateral} = 0.78 (X_{\%90} - X_{\%10}) = 4.84 \mu m \quad (4)$$

IMAGING RESULTS

Functionality of the confocal scanner is demonstrated by obtaining vertical cross-sectional images of a photoresist feature on a silicon wafer and an onion epidermal peel. SU-8 photoresist (MicroChem Corp.) was used to fabricate a feature with rectangular cross-section on a silicon wafer. The width and height of the feature are 100 μm by 33 μm . First, an optical profile image of this feature was obtained by Wyko optical profiler (Veeco Instruments Inc.) for comparison (Fig. 6(a)). Cross-section of the feature was then scanned by the confocal scanner (Fig. 6(b)). Confocal image clearly shows surfaces of the photoresist and silicon wafer. However, the sidewalls of the rectangular feature were not detected. This is due to the large incident angle of illumination beam to the side walls.

Figure 6: (a) Optical profile of a SU-8 feature with rectangular cross-section measured by an optical profiler; and (b) Confocal image of same feature.

Confocal scanner was also used to image cross-section of an onion epidermal peel layer obtained from a yellow onion. Figure

7(a) shows image of an onion cross-section stained with Alcian blue. This image was obtained using traditional histology imaging techniques. Figure 7(b) is an image obtained by our confocal scanner. Confocal image shows onion epidermal cells and provide information regarding the cell size and position of walls. Image quality of the system has much room for improvement by performing optical optimization to enhance image resolution.

Figure 7: (a) Microscopy image of onion cross-section stained with Alcian blue, and (b) confocal vertical optical section image of onion peel obtained using the 2-axis confocal scanner.

CONCLUSION

A 2-axis confocal scanner for vertical optical sectioning of samples has been developed and demonstrated by obtaining confocal images of polymer features and onion peel cells. The device has optical resolution of 10.7 μm and 4.8 μm in axial and transverse directions respectively. Scanning range and speed are 190 μm and 378 Hz in axial and 145 μm and 2 Hz in transverse directions. The device described in this paper has several advantages that make it attractive for further development. The scanner is driven by electromagnetic forces only, hence requires low driving voltages that is desired in clinical applications. The optical configuration of the scanning head has only one optical axis which makes alignment of the system easier. The confocal scanner can potentially be packaged and be used as a hand-held device for various clinical applications such as skin or oral cancer diagnosis. Finally, the scanner design can be further miniaturized using Microelectromechanical Systems (MEMS) technology and micro-electromagnets to form a catheter for endoscopic applications.

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