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Miniature near-infrared dual-axes confocal microscope utilizing a two-dimensional microelectromechanical systems scanner

Jonathan T. C. Liu, Michael J. Mandella, Hyejun Ra, Larry K. Wong, Olav Solgaard, and Gordon S. Kino

Edward L. Ginzton Laboratory, Stanford University, Stanford, California 94305, USA

Wibool Piyawattanametha, Christopher H. Contag, and Thomas D. Wang

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

Abstract

The first, to our knowledge, miniature dual-axes confocal microscope has been developed, with an outer diameter of 10 mm, for subsurface imaging of biological tissues with 5–7 μm resolution. Depth-resolved *en face* images are obtained at 30 frames per second, with a field of view of $800 \times 100 \mu\text{m}$, by employing a two-dimensional scanning microelectromechanical systems mirror. Reflectance and fluorescence images are obtained with a laser source at 785 nm, demonstrating the ability to perform real-time optical biopsy.

Conventional single-axis confocal microscopes perform optical sectioning with an axial resolution that scales with NA according to an inverse square law. For high-resolution imaging, a large NA is required, which implies a short working distance and small field of view (FOV) unless a large-diameter objective is employed. Such objectives are difficult to miniaturize, often requiring multiple elements to mitigate the effects of aberrations due to high-NA focusing and preobjective beam scanning.¹ Therefore a design contradiction exists in building a miniature single-axis confocal that images deeply over a large FOV with high axial resolution. The dual-axes confocal architecture utilizes two low-NA beams, with intersecting focal volumes, for illumination and light collection.^{2–4} Unlike single-axis confocals, where transverse resolution is much superior to axial resolution, the dual-axes confocal design provides a resolution that is relatively balanced in all spatial dimensions. In addition, it has been shown that the dual-axes configuration provides superior optical sectioning and rejection of out-of-focus scattered light compared with a single-axis design.⁵ Low-NA lenses enable long working distances, which, along with the superior rejection of scattered light afforded by the dual-axes configuration, enable deep subsurface imaging in tissues. The long working distance also provides room for an aberration-free postobjective scanner to image over a large field of view, which in this case is accomplished by a two-dimensional (2D) microelectromechanical systems (MEMS) scanner.

Figure 1 shows a simplified layout of the miniature dual-axes confocal microscope. The design incorporates a parabolic reflector to ensure that any two collimated beams aligned parallel to one another will intersect at their focus. The 10 mm diameter parabolic reflector (Anteryon BV Eindhoven, The Netherlands) has a focal length of 4.6 mm and a 1.9 mm diameter hole at the center where an index-matching hemisphere is placed. The fused-silica hemisphere enhances the resolution by $1/n$, where n is the index of refraction, and also acts to minimize various aberrations.⁵ A custom 2D MEMS mirror,⁶ which scans the illumination and collection beams in tandem as they are focused by the parabolic mirror, directs the beams through the hemispherical lens and into the specimen.

The angles θ and α , shown in Fig. 1, correspond to the intersection half-angle of the beams and the free-space NA of the individual beams, respectively. The theoretical resolution (FWHM)

may be calculated,⁵ in micrometers, assuming Gaussian beams truncated to allow 99% power transmission, $\lambda=0.785\text{ }\mu\text{m}$, $\alpha=0.128$, $\theta=24^\circ$, and $n=1.4$:

$$\Delta x_d = \frac{0.466\lambda}{na \cos \theta} = 2.2, \quad \Delta y_d = \frac{0.466\lambda}{na} = 2.0,$$

$$\Delta z_d = \frac{0.466\lambda}{na \sin \theta} = 5.0. \quad (1)$$

Fiber-pigtailed collimators (Lightpath Technologies), designed for single mode use at telecom wavelengths (SMF-28 fiber), are used in this preliminary prototype. Although the Lightpath collimators and fibers are not designed for single mode transmission at 785 nm, we have observed single mode illumination due to negligible mode mixing along the short fiber lengths used (several meters). In addition, the use of the 9 μm core SMF-28 fiber improves the collection efficiency for fluorescence imaging (larger pinhole), albeit at the cost of a slight degradation in resolution compared with the calculation in Eq. (1) for purely single mode illumination and collection.

Figure 2 depicts various elements of the miniature design. The collimators are placed in precision wire electrical discharge machined v-grooves with centers separated by 3.7 mm. A pair of 1° wedges (Risley prisms) are positioned after each collimator. These are rotated to provide angular adjustment of the beams, aligning them parallel to each other as well as parallel to the central axis of the parabolic mirror. The MEMS mirror chip (3.2 mm $\times$ 2.9 mm) is mounted at the center of a rectangular printed circuit board (PCB), which is mounted at the end of an axial sliding mechanism to enable imaging at various depths within the sample. The circuit board acts as a junction between external electrical wires from a high-voltage amplifier (AgilOptics) and the MEMS device itself, which is connected through wire bonds onto bonding pads on the PCB. Oversized mounting holes in the PCB allow for lateral adjustment of the MEMS mirror to center the illumination and collection beams on the mirror surfaces. Driving signals are generated with LabVIEW on National Instruments wave form generators. Each dimension of the MEMS mirror is actuated with two wave forms that are equal but 180° out of phase.^{6–9}

Figure 3 shows a scanning electron micrograph of the MEMS device. Vertical electrostatic comb actuators provide gimbaled torsional motion in two orthogonal dimensions to allow a 2D raster scan. The inner axis possesses a mechanical resonance frequency of 3.5 kHz whereas the outer axis exhibits a resonance at 1.25 kHz. The inner axis of the scanner is driven sinusoidally at its resonance frequency, enabling a large deflection angle to be obtained with a reduced-amplitude driving wave form. The scan range of this fast inner axis is 800 μm in our prototype. The slow outer axis is driven with a sawtooth wave form, modified with a smooth turn around to prevent mirror ringing. The slow axis scan range of the MEMS mirror used here is 100 μm , corresponding to an optical deflection of $\pm 1.0^\circ$ at a voltage of 110 V. Additional details on the MEMS fabrication and operation have been described elsewhere.^{6–9}

Axial resolution is measured to be 7.5 μm by translating a plane mirror in the z-direction and recording the FWHM. A reflectance image of a U.S. Air Force (USAF) target is shown in Fig. 4, in which the lines in element 4 of group 7 are resolvable (with a 5.5 μm line-space period). For these studies, the maximum laser intensity on the sample is 1 mW at 785 nm. The edges of the field of view are dark since the image-section plane is curved, with the center of the field of view imaging 25 μm deeper than at the edge.

For fluorescence images, a near-infrared dye from Li-Cor Biosciences is used (IRDye 800CW) with an absorption maximum at 774 nm and an emission maximum at 789 nm in water. The dye is dissolved in saline at a concentration of 0.1 $\mu\text{g}/\mu\text{L}$ (90 μM). Fresh colon biopsy samples are obtained from the Palo Alto Veterans Affairs Hospital with informed patient consent. Colon

specimens are soaked in the dye solution for 1 min before being irrigated with water to remove excess dye. An emission filter from Semrock (LP02-785RU-25), with a passband from 795.2 to 1771 nm, is used to remove the laser background prior to detecting the fluorescence signal with a Hamamatsu PMT (H7422-50).

A proof-of-principle fluorescence image of a normal human colon, at a depth of 20 μm beneath the mucosal surface, is shown in Fig. 4(b). The image is an average of five frames acquired at 30 frames/s. Note that small colonocytes surrounding the three circular crypt lumen are resolvable.

Several improvements are planned for future prototypes. Optimized MEMS scanners will be used, with larger deflection angles, enabling increased fields of view. Future mirrors will be evaporatively coated with a layer of aluminum to improve the reflectivity of the two mirror surfaces.⁹ In addition, custom gradient-index collimators will be utilized with larger beam diameters than our current collimators. As a result, the NA of the beams will be increased, providing an improvement in resolution according to Eq. (1), as well as an improvement in collection efficiency (scales with the square of NA). These improvements in optical efficiency will enable improved imaging at greater tissue depths in the future.

We have demonstrated what we believe to be the first design, construction, alignment, and utilization of a miniature dual-axes confocal reflectance and fluorescence microscope at 785 nm. While the outer diameter of this device is 10 mm, all of the essential optics and optical paths lie within a central 5 mm diameter. A future 5 mm device will be attained by utilizing alternative alignment techniques. The eventual goal is to perform *in vivo* confocal microendoscopy with a device that may be conveniently deployed through the instrument channel of an endoscope. Together with the use of small fluorescent molecules targeted against biomarkers of disease, such a device could revolutionize the practice of endoscopy by enabling real-time optical biopsy for the early and accurate detection of cancer and for guiding therapeutic procedures.

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Fig. 1. Miniature dual-axes confocal scan head optics. (1) Collimated illumination beam. (2) Collimated collection beam. (3) MEMS 2D scanner. (4) Parabolic reflecting surface. (5) Index-matching hemisphere.

Fig. 2.

Miniature dual-axes microscope package design drawings. The outer diameter for this device is 10 mm, with all essential optics and optical paths contained within the central 5 mm diameter. (1) Fibered collimators. (2) Risley alignment prisms. (3) MEMS PCB. (4) Parabolic reflector. (5) Axial sliding mechanism to control imaging depth. (6) Removable end cap holding the parabolic reflector.

Fig. 3. Scanning electron microscopy image of the 2D scanner. Dimensions of the MEMS chip are $3.2 \text{ mm} \times 2.9 \text{ mm}$, with each mirror surface measuring $650 \mu\text{m} \times 600 \mu\text{m}$. (1) Outer torsional spring. (2) Outer axis electrostatic comb actuators. (3) Inner torsional spring. (4) Inner axis electrostatic comb actuators.

Fig. 4.

(a) Dual-axes reflectance image of a USAF target, obtained at 30 frames/s, demonstrating resolvable lines in element 4 of group 7 ($5.5\ \mu\text{m}$ period). FOV= $350\ \mu\text{m} \times 100\ \mu\text{m}$. (b) Dual-axes fluorescence image of normal colonic mucosa at 785 nm, showing circular crypts and surrounding colonocytes. The image is an average of five frames acquired at 30 frames per second. Imaging depth $\approx 20\ \mu\text{m}$ at the image center. FOV= $800\ \mu\text{m} \times 100\ \mu\text{m}$.