Fiber-optic nonlinear endomicroscopy with focus scanning by using shape memory alloy actuation

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Abstract. A miniature fiber optic endomicroscope with built-in dynamic focus scanning capability is developed for the first time for 3-D two-photon fluorescence (TPF) imaging of biological samples. Fast 2-D lateral beam scanning is realized by resonantly vibrating a double-clad fiber cantilever with a tubular piezoeactuator. Slow axial scanning is achieved by moving the distal end of the imaging probe with an extremely compact electrically driven shape memory alloy (SMA). The 10-mm-long SMA allows 150-μm contractions with a driving voltage varying only from 50 to 100 mV. The response of the SMA contract with the applied voltage is nonlinear, but repeatable and can be accurately calibrated. Depth-resolved imaging of acriflavine-stained biological tissues and unstained white paper with the SMA-based scanning fiber-optic endomicroscope is performed, and the results demonstrate the feasibility of 3-D nonlinear optical imaging with the SMA-based scanning fiber-optic endomicroscope.

Keywords: two-photon fluorescence; endomicroscope; focus scanning; shape memory alloy.

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1 Introduction

Two-photon fluorescence (TPF) endomicroscopy is an emerging technology that has the ability to potentially assess 3-D structural and biochemical information of biological tissues with miniaturized scanning fiber optic probes.1,2 This technology is critical for translating powerful nonlinear optical sectioning microscopy3,4 to clinical practice, and can be potentially integrated with a standard clinical endoscopy procedure for diagnosis in situ and real-time guidance of biopsy. Various 2-D scanning mechanisms in a small footprint have been demonstrated, including the use of piezo-based actuators to swing the imaging fiber cantilever, or microelectromechanical system (MEMS)-based micromirrors to scan the imaging beam.1,2,5-7 To achieve accurate depth scanning and maintain a small footprint, a built-in mechanism has to be implemented at the distal end (e.g., for changing the focal plane), which faces many challenges, including system miniaturization, reduction in the actuation voltage, and improvement on focus scanning accuracy and repeatability over a sufficient range.8,9 In this study, we report the use of an electrically driven shape memory alloy (SMA) to achieve slow axial scanning at the distal end by moving the distal end of endomicroscope (including the miniature focusing optics and piezoelectric transducer (PZT)-based beam scanner). By integrating the SMA-based depth scan with a 2-D fiber optic resonant scanner, real-time 3-D two-photon fluorescence imaging becomes feasible for the first time with a compact fiber optic endomicroscope, which is demonstrated by imaging various samples including biological samples.

2 Methods and System

Shape memory alloy (SMA) is a special type of metal that can remember its original cold-forged shape and return to this shape after being deformed by applying heat.10,11 This material has shown promising applications in medical devices over conventional actuators such as hydraulic, pneumatic, and motor-based systems due to its ultralight weight, compact size, and relatively simple operation.12,13 SMA can be fabricated as a small wire that can contract up to 5% of its original length when it is heated above room temperature. With advances in manufacturing technologies, it becomes possible for SMA to operate reliably over tens of millions of cycles. In this study, we employed an SMA wire made of nickel-titanium. The SMA wire had an extraordinary ability to accommodate large strains with a pull force of 80 g while maintaining an ultrasmall diameter (0.003 in.). The heating and corresponding temperature changes were achieved by applying an electrical current through the thin wire of internal resistance of 5 ohms/in. In addition, the SMA had shown physiological and chemical compatibility with the human body.14,15

Figures 1(a) and 1(b) show the schematic and photograph of the SMA-based scanning fiber-optic endomicroscope with a 2.4 mm diam core for single-mode femtosecond laser delivery and a 135-μm diam inner clad for multimode nonlinear optical signals collection, a miniature tubular PZT for resonantly sweeping the DCF cantilever in a spiral pattern, and a gradient-index (GRIN) lens as the focusing unit with a magnification of 0.5 from the DCF tip to the sample. The overall diameter for both tube 1 and tube 2 was 2.4 mm.

When an electrical current is applied to the SMA wire, it contracts through Joule heating and compresses the spring, consequently pulling tube 1 (along with the imaging optics) toward tube 2. When the Joule heating current decreases or stops, the SMA relaxes and the spring pushes the imaging tube back to its original position. Figure 2(a) shows the travel distance of tube 1.
Shape Memory Alloy
SpringTube 1
Movable Imaging Tube
(a)
Tube 2 
Fixed Hollow ... at depths of (d) 0, (e) 32, and (f) 64 μm.

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The details of the endomicroscopy imaging system were described elsewhere. In essence, a home-built Ti:sapphire laser prechirped by a single photonic bandgap fiber was coupled into the endomicroscope through the DCF core for effective excitation. The nonlinear optical signals from the samples were collected by both the core and inner clad of the DCF and directed to a photomultiplier tube (PMT). The fiber optic resonant scanner created a scanning diameter of 150 μm on the sample. Depth-resolved imaging was synchronized with the SMA contraction driven by a step-wise triangle waveform. Each image at each step was averaged over five frames with a total sampling time of ∼1.5 s per averaged image.

3 Results and Discussions

Figure 3 shows representative depth-resolved two-photon fluorescence intensities and images acquired from ex-vivo pig esophageal tissue. Each intensity value is the averaged intensity over the frame at each given depth, while the images are normalized to the maximal intensity of each image. The tissue was stained with 0.01% acriflavine (a dye that predominantly stains nuclei) for one hour, followed by a wash in phosphate-buffered saline (PBS) three times to remove excessive acriflavine molecules. Figure 3(a) shows the TPF intensities as a function of the SMA drive voltage during the course of contraction and expansion. It is noted that the TPF intensities exhibit different voltage dependence during SMA contraction and expansion, indicating the hysteresis effect of the SMA. After calibrating the drive voltage against the scanning depth based on Fig. 2(a), TPF intensities versus the sampling depth are shown in Fig. 3(b). For comparison, Fig. 3(b) also shows the depth-resolved TPF intensities measured with a precision translation stage (of 0.1-μm accuracy) by moving the entire endomicroscope probe with an axial step of 10 μm. In the figure, the 0-μm depth is supposed to be the tissue surface where the fluorescence intensity is maximal, and the positive values along the depth axis refer to imaging within the tissue. After depth
that the performance of the endomicroscope can be further improved by moving only the focusing lens (as opposed to both the lens and the fiber optic scanner) and by developing a drive waveform to produce linear depth scanning.

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References

4 Conclusions
In summary, a flexible fiber optic endomicroscope capable of dynamic depth scanning is developed for 3-D nonlinear optical imaging of biological samples. Depth scanning is achieved with high accuracy and good repeatability by using ultrathin SMA wires that require an extremely low drive voltage. It is expected