Two-Photon Luminescence Imaging Using a MEMS-Based Miniaturized Probe

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Abstract: We present two-photon luminescence (TPL) imaging of cancer cells through a 10 × 15 × 40 mm^3 miniaturized probe employing a two-axis MEMS scanning mirror and an air-core photonic crystal fiber. The combination of TPL imaging with a small probe represents a potential method of distinguishing cancerous cells in tissue for diagnosis.

OCIS codes: (180.4315) Nonlinear microscopy; (170.2150) Endoscopic imaging; (250.5403) Plasmonics;

1. Introduction

Over 1 million new cases of cancer are diagnosed each year in the United States alone, with 85% of cancers originating in epithelial tissue [1]. To increase the rate of early cancer detection in the epithelium, new technologies must be developed, including both new imaging instruments as well as novel contrast agents. Of the new instruments, the development of two-photon endoscopes has created the potential for *in vivo* diagnostics based on multiphoton processes [2]. Because imaging of intrinsic fluorophores is often difficult with these miniaturized devices due to the relatively weak signals, the use of contrast agents is often helpful. Gold nanorods have recently emerged as a useful contrast agent for two-photon imaging due to their strong signal through two photon luminescence (TPL) [3]. Here we demonstrate TPL imaging of gold nanorod-labeled cancer cells using a miniature probe we have constructed for two-photon microscopy and femtosecond laser microsurgery [4].

2. Miniature Two-Photon Microscopy Probe Overview

We have recently developed a small (10 × 15 × 40 mm^3) probe capable of both two-photon microscopy (TPM) and femtosecond laser microsurgery (FLMS) [4], shown in Fig. 1. The probe was built as a first step towards development of a clinical "seek-and-treat" device capable of all-optical diagnosis and treatment of small cancerous lesions. Air-core photonic crystal fiber is used to deliver femtosecond pulses from a Ti:Sapphire oscillator (Mai-Tai, Spectra Physics) at 753 nm center wavelength, which is the minimum dispersion wavelength of the fiber. After collimation by a 0.23-pitch GRIN lens, the laser beam is Lissajous scanned by a two-axis gimbaled 500 × 500 μm^2 MEMS scanning mirror. From there, the beam is imaged to the back aperture of a GRIN objective lens (0.46 NA, 1.8 mm working distance) through a pair of miniature aspherical lenses acting as both relay optics and a beam expander. These relay optics allow for the full scanning range of the MEMS to be used without scanning the laser off of the objective lens and minimize aberrations caused by scanning directly onto the objective lens. By expanding the beam, it also allows the MEMS mirror to be underfilled to maximize energy transmission through the system while also overfilling the back aperture of the objective lens to maximize resolution. Emitted fluorescent light passes through a dichroic mirror and is collected by 1 meter of large-core plastic optical fiber with a 0.51 NA. Collected fluorescent signal was then filtered by a Schott BG38 filter to attenuate any laser light before being detected at a PMT (H7422-40, Hamamatsu). During imaging, the MEMS mirror was scanned at resonance, which occurred at 2.73 kHz and 1.54 kHz for the inner and outer axes, respectively. The driving signals used were sinusoidal with peak voltages between 20 – 80 V, for fields of view between 36 μm and 310 μm. This resulted in a Lissajous scan which was reconstructed by a LabView® program at 10 frames per second. Characterization of the probe found the lateral and axial point spread functions to be 1.64 μm and 16.4 μm, respectively. Detailed discussion about the MEMS scanning and imaging characterization can be found in Hoy *et al.* [4].

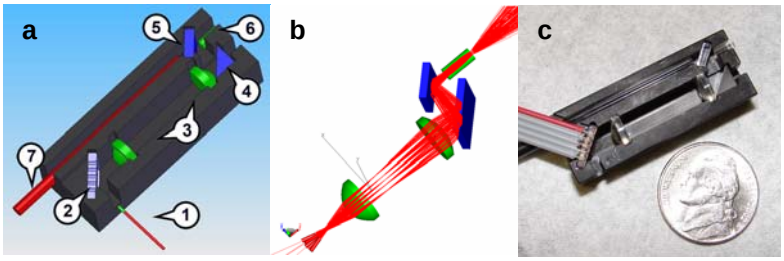

Figure 1. Images of the combined TPM/FLMS probe. **a**, *A spatial model showing 1) air-core fiber, 2) MEMS scanning mirror, 3) relay lenses, 4) mirror, 5) dichroic mirror, 6) GRIN objective lens, and 7) large-core collection fiber.* **b**, *A ray-tracing model (TracePro®, Lambda Research) displaying the excitation pathway through the relay lens system.* **c**, *A photograph of the probe shown without the lid to show detail. The air-core photonic crystal fiber is also not shown in this photograph. A US nickel is provided for scale.*

3. Two-Photon Luminescence Imaging

To demonstrate the imaging capability of the miniature probe, we performed two-photon luminescence imaging of gold nanorod-conjugated breast carcinoma cells (MDMBA468). Gold nanorods with an average aspect ratio of 3.4 (48.1 µm x 14.3 µm) exhibiting a longitudinal resonance centered at 754 nm were synthesized using a seed-mediated, surfactant-assisted growth method. The nanorods were conjugated to antibodies against epidermal growth factor receptor (EGFR) and incubated with the cancer cells for 45 minutes. Excess unbound functionalized nanorods were removed by centrifuging and resuspending the cells in PBS. The cells were then deposited on a tissue culture dish and incubated at 37°C for one hour prior to imaging. Further description of the sample preparation methodology can be found in our previous study [3]. A two-photon image of gold nanorod labeled cells taken with the probe is shown in Fig. 2a. In this image, individual cells are clearly visible and gross morphological features could be distinguished. Due to the elongated axial resolution relative to the thickness of the cells, the cells appear illuminated throughout the entire cell rather than just at the outer membrane as observed using a large-scale two-photon setup (see Fig. 2b) [3]. The use of specifically targeted nanorod luminescence with a miniaturized TPM probe, as demonstrated here, is one of the possible clinical screening technologies that could be developed with this system.

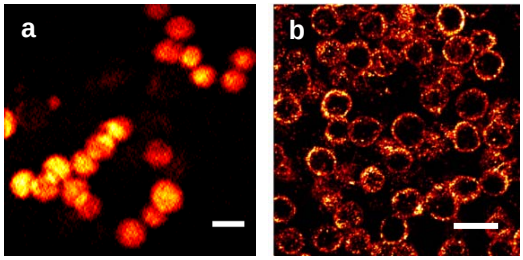

Figure 2. **a.** *TPL image of nanorod-labeled cancer cells taken with the miniature probe. Image shown is an average of 50 frames obtained over 5 second acquisition time using 50 mW average power at the sample.* **b.** *TPL image of a similar sample taken using 0.04 mW in a large scale system as described by Durr et al. [3]. Scale bars are 20 µm.*

4. Conclusion

We have demonstrated TPL imaging of gold-nanorod labeled cancer cells through a miniaturized probe. This combination of emerging technologies, both in imaging devices and in contrast agents, can aid in the early detection of small precancerous lesions in suspicious regions of epithelial tissue. In addition, the use of a fiber-based imaging and microsurgical tool of the design described here, could be used with targeted nanoparticles for plasmonic laser microsurgery [5]. Such a precise and noninvasive imaging and microsurgical modality would find applications in many fields, ranging from dermatology to neurology.

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