3D Fibre-optical Nonlinear Optical Microscopy Imaging

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Abstract: The recent development of fibre-optical nonlinear optical microscopy for 3D endoscope tissue imaging is reported. The new compact probe is designed with double-clad photonic crystal fibre components and a microelectromechanical system (MEMS) mirror.

OCIS codes: (110.0180) Microscopy; (110.2350) Fiber optics imaging; (180.4315) Nonlinear microscopy; (180.4315) Three-dimensional microscopy

1. Introduction

The invention of nonlinear optical microscopy based on multiphoton fluorescence and harmonic generation has provided a powerful tool for 3D imaging through tissue. The development of compact nonlinear optical microscopy or endoscopy is motivated by three novel applications. First, it provides high-resolution *in vivo* cellular imaging under conditions in which conventional bulk microscopy cannot be used. The examples of these conditions include imaging hollow tissue, imaging within solid organs, imaging freely-moving animals, and remote delivery and collection in a minimally invasive manner. Second, nonlinear optical endoscopy is a critical tool for long term imaging studies such as the investigation of the cellular effect of aging and the development of new *in vivo* assays for testing of drugs and therapeutics. Third, it facilitates the development of minimally invasive clinical diagnostics and surgical procedures.

One of the implementations toward a compact nonlinear optical microscope is the adoption of fibre optics. Various efforts have been made to produce a compact probe based on single mode fibre optics [1-3]. It has been shown that the use of a single-mode fibre does not efficiently facilitate both the delivery of near-infrared pulsed laser light and the collection of visible nonlinear optical signal [2, 4-6]. Here we report on the new development of a fibre-optical nonlinear optical endoscope probe towards 3D high-resolution tissue imaging. The signal of this new probe is increased by two orders of magnitude.

2. Nonlinear optical micro-probe

The probe comprises of a double-clad photonic crystal fibre (PCF), a micro-prism, a two-dimensional (2D) microelectromechanical system (MEMS) mirror, and a gradient-index (GRIN) lens [3, 7-11]. A pulsed laser beam of wavelength 800 nm generated from a turnkey Ti:Sapphire (Spectra Physics, MaiTai) is launched into the double-clad PCF through a prechirp unit consisting of a grating pair. The endoscope probe allows side-view imaging at a working distance of approximately 200 μm. The back-scattering nonlinear optical signals propagating through the fibre and the MEMS mirror are collected by a photomultiplier tube.

The imaging capability of the nonlinear optical endoscope is demonstrated by the two-photon fluorescence imaging with 10 μm diameter fluorescent microspheres. It shows that the 2D MEMS mirror enables the efficient delivery of the light beam over the broadband wavelength range and the smooth response for image acquisition. *In vitro* three-dimensional (3D) images of internal organ tissue and cancer tissue have been achieved with a penetration depth of up to 100 μm and axial resolution of approximately 10 μm. Epithelial cells and intestinal crypts in 3D images from rat large intestine tissue are clearly identified. Morphological details in the breast cancer tissue (human u-87 MG glioblastoma cells) are also provided by the visualisation of cell nuclei (Fig. 1) [11].

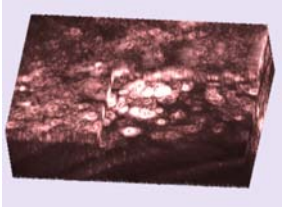

Fig. 1 Three-dimensional visualization of *in vitro* images of the humanu-87 MG glioblastoma tissue.

3. Discussion

The nonlinear endoscopic probe can be further developed to have 3D functional imaging. This can be achieved by the use of supercontinuum generation in nonlinear photonic fibre. In the case of imaging with coherent nonlinear signal, the combination of the digital holography microscopy technique [12] with such a probe may facilitate the reconstruction of new 3D phase information from tissue.

4. References

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