In-vivo two-photon fluorescence endoscope with 475µm×475µm field of view

Hongchun Bao¹, John Allen², Rober Pattie², Rod Vance², and Min Gu¹,

¹ Center for Micro-Photonics, Faculty of Engineering & Industrial Sciences, Swinburne University of Technology, Hawthorn, Victoria 3122, Australia
² Optiscan Pty. Ltd. 15-17 Normanby Rd, Notting Hill, Victoria 3168, Australia
hbao@swin.edu.au

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1 Introduction: A two-photon endoscope has been newly developed for dynamic and thorough investigation of cellular detail in 3D tissue structures in-vivo without the need to sacrifice animals or remove tissues from the body, which is advantageous for early diagnosis of disease, and understanding complex mechanism of disease and bio-phenomenal in living animal for biological research [1, 2]. The developed handheld probe of a two-photon endoscope is based on the standard Optiscan Pty Ltd commercialized confocal endoscope FIVE1® probe which is simply placed into contact with the target tissue to reveal clear surface and sub-surface histological images without biopsy. It has been proven that the two-photon endoscope could image animal tissue with a large field of view as 475×475µm and with images taken at operator selected depths into the tissue.

2. Two-photon fluorescence endoscope setup

The schematic setup of the two-photon fluorescence endoscope is shown in Figure 1. A Ti: Sapphire laser which emits 80 MHz 70 fs short pulses is used as an excitation laser source. The laser is pre-chirped by 400 g/mm grating pairs before coupling into the fibre. A 3 m long commercially available double-clad fibre (DCF) is used for delivery of excitation light and fluorescence collection. The custom design lens (NA 0.35) which consists of multiple elements accurately corrects the image aberration within the field of view. A micro-scanner designed for confocal fluorescence in-vivo endomicroscopy (Optiscan Pty. Ltd.) is used for scanning the DCF and moving the fibre and lenses in axial direction for focusing. An insert at the right bottom of Figure 1 shows the photo of the probe, which is a modified commercialized confocal endoscope FIVE1® probe (Optiscan Pty. Ltd).

3 Two-photon fluorescence image

A male rat Sprague-Dawley (Approximate 780g body weight) was injected 0.3-0.4ml of the fluorescein intravenously (1% solution diluted in saline). Figure 2 shows the two photon fluorescence image of the rat kidney. The image size is 475×475µm. The image is a single 1s scan at 1024×1024 pixel resolution. Figure 2 demonstrates that the developed two-photon endoscope is able to image of subcellular structures of animal tissue.

References: