

# BEAM-SCANNING MULTIPHOTON MULTIMODALITY ENDOSCOPY

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**KEY WORDS:** Two-photon-excited fluorescence, second-harmonic generation, endoscopy, biopsy, Cr:forsterite laser, fiber bundle.

Two-photon-excited fluorescence (2PEF) imaging and second-harmonic generation (SHG) microscopy has become well-established techniques for biomedical imaging [1]. Integration of fibers into the multiphoton microscopy system further enables *in vivo* and noninvasive investigation of biological samples. Nonetheless the broadening of ultrashort pulses in fibers has greatly hampered the development of multiphoton endoscopy [2]. Here we introduce the first beam-scanning multiphoton multimodality endoscope which is based on a fiber bundle and uses a femtosecond Cr:forsterite laser as the light source and of which the novel design reduces the rate of pulse broadening in fibers significantly.

The experimental arrangement of the new multiphoton endoscope is shown in Fig. 1. A

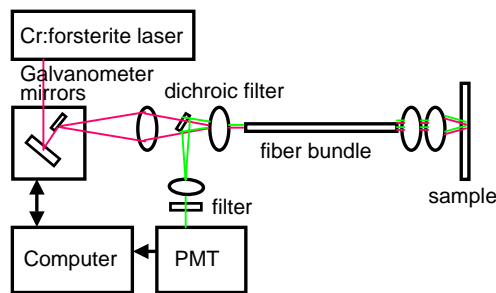


Figure 1: Schematic of the multiphoton endoscope.

homemade Cr:forsterite laser generating 200 fs pulses at the wavelength of 1230 nm and at a repetition rate of 110 MHz was used as the illumination. The beam was scanned across and coupled into every pixel of the fiber bundle (Fujikura FIG-10) via the galvanometer mirrors (Olympus FluoView 300) and a 0.4-N.A. 20X objective (Olympus). Incorporation of the laser with near-zero-dispersion wavelength and the large-pixel-area fiber bundle into the multiphoton endoscope has abated pulse broadening phenomena resulted from dispersion and self-phase modulation.

The subpicosecond output pulses from fiber were then focused on the sample via a pair of aspheric lenses, which also served to collect the emitted 2PEF and SHG from the sample. The signal was delivered through the same fiber bundle and selected by the use of appropriate filters and PMT. Image acquisition was achieved by simply scanning the beam across fiber bundle pixels. Detailed system performance, pulse broadening control, and demonstration of 2PEF and SHG endoscopic imaging in various biological samples will be discussed and presented in the conference.

In summary, we have demonstrated a wholly new multiphoton endoscope capable of performing 2PEF and SHG biopsy. Images of biological specimen will be shown in talk. We sincerely acknowledge National Health Research Institute of Taiwan and National Taiwan University Center for Genomic Medicine for financial support.

[1] S.-W. Chu; I-H. Chen; T.-M. Liu; P. C. Chen; C.-K. Sun, and B.-L. Lin, "Multimodal nonlinear spectral microscopy based on a femtosecond Cr:forsterite laser," *Opt. Lett.*, **26**, 1909-1911 (2001).

[2] D. Bird, and M. Gu, "Two-photon fluorescence endoscopy with a micro-optic scanning head," *Opt. Lett.*, **28**, 1552-1554 (2003).