Photonic Crystal Fiber Based Two-Photon-Fluorescence Microscopy Systems at 0.8μm and 1.3μm Wavelength Regimes

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Abstract--- We demonstrate hollow-core and large-core photonic-crystal-fiber-based two-photon fluorescence microscopes at 0.8μm and 1.3μm. The two-photon excitation efficiencies and image performances are comparable to or better than those acquired by the conventional fiber-free system.

OCIS codes: (110.2350) Fiber optics imaging; (170.2520) Fluorescence microscopy; (180.6900) Three-dimensional microscopy

Since the first demonstration in 1990, two-photon fluorescence microscopy (TPFM) has made great impacts on biomedical researches due to its high penetration ability, low out-of-focus photodamage, and intrinsic three-dimensional(3D) sectioning capability [1]. For future biomedical or even clinical applications, fiber-based TPFM systems are required due to their high flexibility, high reliability, re-alignment-free capability, and vibration-isolation from surrounding devices [2]. An ideal fiber-based TPFM system should maintain efficient two-photon excitation where both high peak power and short pulse width are required. Besides, single-mode propagation is necessary to preserve a spatial profile suitable for diffraction-limited focusing as well as to avoid additional temporal broadening as a result of intermodal dispersion. However, due to self-phase modulation and dispersion effects inside conventional single mode fibers, femtosecond laser pulses are severely broadened when propagating through them. Thus, the two-photon excitation efficiencies of fiber-based TPFM systems are strongly reduced when compared with the conventional one. The reduced two-photon excitation efficiencies in fiber-based TPFM systems cause severe image degradations [3]. In this presentation, we report photonic-crystal-fiber- (PCF-) based TPFM systems at both 0.8μm and 1.3μm wavelength regimes. Without use of any extra dispersion compensation optics, much improvement of two-photon excitation efficiency is achieved.

In our systems, hollow-core PCFs and large-core PCFs were utilized in the fiber-based TPFM systems excited by a mode-locked Ti:Sapphire laser and a modelocked Cr:Forsterite laser respectively. In hollow-core PCFs, the air guidance eliminates nonlinear effects produced by high energy ultrashort pulses. If the excitation wavelength is properly selected to be near dispersion-zero wavelength and within the transmission window of the hollow-core PCFs, it will thus minimize the pulsewidth broadening effect for the delivery of femtosecond laser pulses in a fiber-based TPFM system to maintain comparable two-photon excitation efficiency and image quality. On the other hand, in large-core single-mode PCFs, due to enlarged mode-field area, the nonlinear effects are also reduced compared with the conventional single mode fibers. With a zero-dispersion wavelength shifted to be less than 1200nm, the large-core PCFs can thus provide weak negative dispersion for 1200-1300nm excitation pulses for positive chirp compensation to actually "enhance" the TPFM image quality even compared with a free space TPFM system.

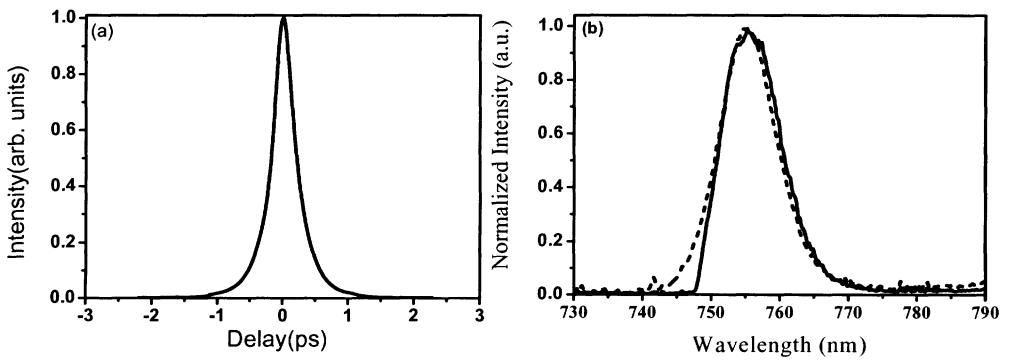

Figure 1: (a) Autocorrelation trace and (b) spectra generated by a mode-locked Ti:sapphire laser (dotted line) and after delivered through a hollow-core photonic crystal fiber (solid lines).

Due to the fact that external pre-chirp units are required in the delivery of mode-locked Ti:Sapphire laser pulses through large-core PCFs [4], in our system, a piece of 1m-long hollow-core PCF is selected in fiber-based TPFM excited by mode-locked Ti:Sapphire laser pulses at 755nm with 200fs pulsewidth. Figure 1(a) shows autocorrelation trace at the fiber exit end. At output end of the hollow-core fiber, pulses were with 80mW average power and 253fs pulse-width. Figure 1(b) show the spectra of laser pulses at fiber entrance end and exit end. The results show that inside hollow-core fibers, output pulses were temporally broadened from 200fs to 253fs due to the weak dispersion effect and no significant spectral broadening occurred.

On the other hand, in PCF-based TPFM excited by a femtosecond Cr:Forsterite laser, a piece of 65cm-long large-core PCF was selected due to the fact that its dispersion zero wavelength was shifted to be below 1200nm. The 1230nm excitation pulses directly generated from a compact prismless laser oscillator with weak positive chirp can be compressed to enhance two-photon excitation efficiency. Figure 2(a) and 2(b) show the measured autocorrelation traces and spectra of the 1230 nm excitation pulses before fiber entrance end and after its exit end. No significant spectral broadening was observed even with an average power of 260mW. Excitation pulses with 320fs pulsewidth were compressed down to 200fs transform-limited Gaussian pulses with a 0.44 time-bandwidth product.

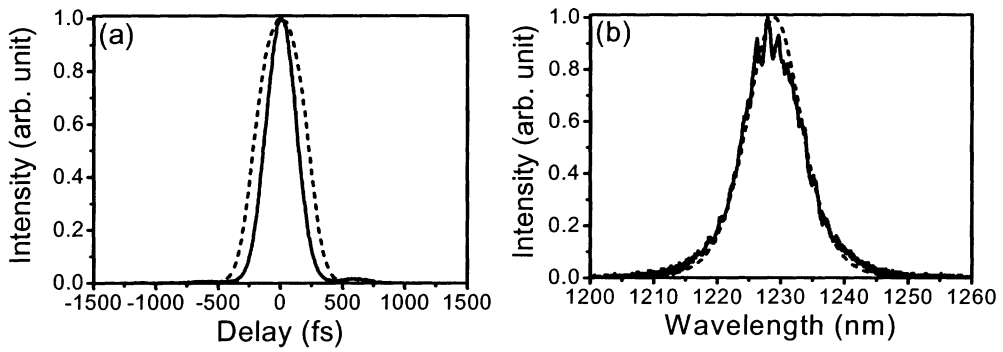

Figure 2: (a) Autocorrelation traces and (b) spectra generated by a compact prismless Cr:forsterite laser (dotted lines) and after delivered through a large-mode-area photonic crystal fiber (solid lines). The average power after the photonic crystal fiber was 260mW.

With a negligible pulse broadening effect or even with a compressed optical pulse, great improvement in the image quality can thus be achieved compared with a regular-fiber-based TPFM. With 755nm excitation pulses, figure 3 shows examples of the acquired two-photon fluorescence (chlorophyll) images with and without the PCF in the mesophyll tissues of a fresh *Rhaphidophora aurea* leaf where chloroplast distribution inside the mesophyll cells can be identified with a sub-micron resolution. Negligible imaging degradation can be found; indicating efficient pulsewidth control with the PCF based system. By moving the central wavelength of the optical excitation and compare the wavelength-dependent TPFM image quality, we can find a strong correlation between the pulsewidth broadening effect and the image quality degradation, indicating the pulsewidth broadening is the primary issue in a fiber-based TPFM system. On the other hand, in large-core PCF based TPFM system with a compact prismless Cr:forsterite laser, the temporal broadening ratio is actually less than 1 (0.64), meaning the image quality is actually enhanced after introducing the large-core PCF into the TPFM systems.[5] Figure 3(c) shows an example two-photon fluorescence (chlorophyll) image of the mesophyll tissues acquired also in the fresh leaf of *Rhaphidophora aurea*, with the large-core PCF-based TPFM. Enhanced image quality can be obtained due to the pulsewidth shortening effect. Please notice that this is the first fiber-based nonlinear microscope where the optical fiber can actually enhance the imaging performance by compressing the pulsewidth, without the aid of other pre-compensation elements. More discussions on wavelength-dependent image-degradation ratios in both PCF-based TPFM systems will be presented in the conference.

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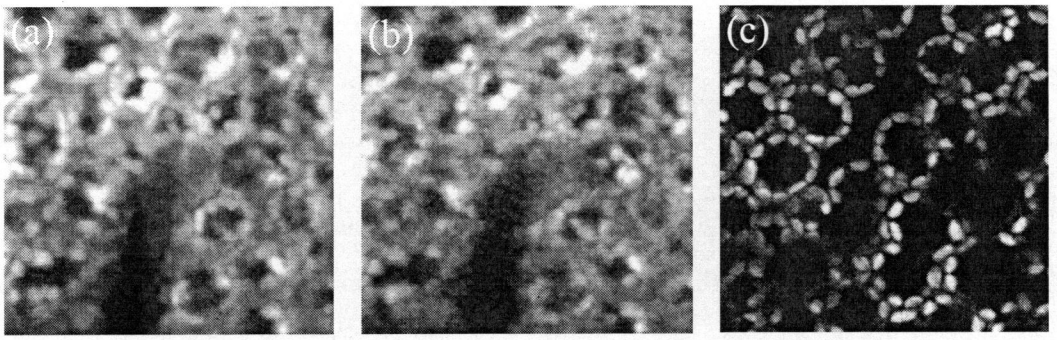

Figure 3: Two-photon fluorescence images of mesophyll tissue in the leaf of *Rhaphidophora aurea* acquired by (a) a free-space TPFM at 755nm, (b) a hollow-core PCF-based TPFM at 755nm, and (c) a large-core PCF-based TPFM at 1230nm. Image size: 160×160μm.

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