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Fiber-based microendoscopic multiphoton imaging

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Abstract

We developed a multiphoton microscope which integrates an all normal dispersion fiber laser, a double cladding photonic crystal fiber and a MEMS mirror scanner based hand-held probe. The fiber laser has a central wavelength of 1.06 μ m, a repetition rate of 76MHz and maximum average output power of more than 1W. The MEMS mirror based probe is compact and Second harmonic generation and two photon excited fluorescence images of biological sample were demonstrated.

Keywords: Multiphoton microscope, Ultrafast fiber laser, Endoscope, Photonic crystal fiber

1. Introduction

Multiphoton microscopy (MPM), including two-photon excited fluorescence (TPEF) and second harmonic generation (SHG), has become an important tool for high-resolution, non-invasive imaging of thick biological tissues. Since the first demonstration of MPM in 1990 [1], MPM has been widely used to image morphology and function of various cells and tissues [2]. For the past a few years, deep tissue and in vivo MPM imaging has been reported [3, 4]. MPM has found wide applications in neurobiology and developmental biology to monitor calcium dynamics, image neuronal plasticity, and evaluate neurodegenerative disease in animal models. MPM has also been shown to be a valuable tool to study angiogenesis and metastasis and to characterize cell/extracellular matrix interactions in cancer research [2].

The clinical MPM system requires an ultrafast laser source that is low-cost, compact, and portable. Currently, commercially available Ti:sapphire laser systems are the most popular laser sources for MPM system. Ti:sapphire laser offers short pulse width, high power, high repetition rate and wavelength tunability from 710nm to 1000nm. However, it also has the disadvantages of bulky, expensive and not maintenance-free. Recent advance in the development fiber laser has made cheap, portable and maintenance-free laser source available. We have reported the development of all normal dispersion (ANDi) fiber lasers [5-8]. ANDi fiber laser uses dissipative soliton and spectral filtering to achieve high pulse energy. It offers a combination of excellent performance and simplicity. We have demonstrate 80fs pulse with average power of more than 2 W [6]. ANDi lasers also show wavelength tuning ability from 1.0 μ m to 1.3 μ m by soliton self-frequency shift technology [8].

In this manuscript, we demonstrate a fiber based MPM system using a 120 fs compact ANDi fiber laser as light source. In addition, clinical applications also require compact and miniature probes capable of 2-D scanning. We report a fiber based MPM system that incorporates a compact ANDi fiber laser as light source, a double clad photonic crystal fiber (DCPCF) for femtosecond beam transportation and signal collection, and a MEMS scanning probe for imaging formation. Second harmonic generation and two photon excited fluorescence imaging of biological tissue were demonstrated with the system.

2. System design

The schematic of the fiber based MPM system is shown in Figure 1. Output from the ANDi laser is sent to a dispersion compensation system. Then the beam is then coupled into the core of a DCPCF fiber (DC-165-65,

Crystal fiber) with a 4X objective. The multiphoton signal is collected by the high NA large diameter clad of the DCPCF. The collected signal is reflected by a dichroic beam splitter and sent to the PMT after passing through a filter. The PMT converted the optical signal to electrical signal and the electrical signal was further amplified by a low noise amplifier. The amplified signal was acquired and digitized by a multifunction card in a personal computer.

Figure 1. Schematic of the MPM system. G:Grating;M:Mirror;DM:Dichroic Mirror; L:Lens; F:Filter; PMT: Photon multiplier tube; A: Amplifier; GW: MEMS mirror control wire; DCPCF: double clad photonic crystal fiber; MEMP: MEMS mirror base probe; PC: Personal computer;

Fig.2 (a) Schematic of the fiber laser. PBS: Polarization beam splitter

Fig.2 (b) Photography of inside of the fiber laser.

Fig. 2(a) shows the schematic of the fiber laser and Fig.2 (b) shows the photography of inside of the fiber laser. The home-built cladding pumped ANDi fiber laser use nonlinear polarization rotation for passive mode locking[6]. It produces high energy, clear and short duration pulse. The pump has a wavelength of 976nm. The pump beam is delivered into the clad of a double clad Yb:doped fiber via a home-built pump/signal combiner. The double clad Yb:doped fiber is fused with a single mode fiber (SMF) with diameter of 10 μ m. The SMF is terminated with a FC/APC connector and is connected to a collimator. After passing through several free space, mode-locking elements, the collimated beam is focused into the SMF at another end of the pump/signal combiner. In the ANDi laser, several stable mode locking conditions can be achieved by tuning the waveplates. The output pulse from the polarization beam splitter is dechirped by one grating pair. Typically, the dechirped output pulse has a pulse width of around 125 fs (fig.3 (a)) and wavelength bandwidth of 40 nm (fig.3 (b)). The average power of the dechirped pulse is more than 1W when pump power is around 7.5W. The ANDi fiber laser occupies a space of 60cm $\times$ 45cm $\times$ 24cm, and it can be condensed into a much smaller space.

Fig.3 (a) Interference autocorrelation trace of the output pulse (b) Output spectrum of the fiber laser.

3. MEMS scanning mirror based miniature probe

Schematic of the MEMS based miniature probe is shown in Figure 4(a). The photograph of the packaged probe is shown in Fig.4 (b). The DCPCF is glued with a Grin index lens which acts as a collimator. The collimated beam is reflected by the MEMS mirror into the lens series. The miniature lens series include a scan lens, a relay lens and a focusing lens. In our previous design [9], a 6mm diameter aspheric lens was used as focusing lens after the MEMS mirror. While the MEMS mirror only has a diameter of 2 mm, we cannot make full use of the large NA of the aspheric lens and larger beam size was required. In the new design, the scan lens and tube lens are configured as a telescope serving as a beam expander which expands the beam around 3 times. Both of the scan lens and the tube lens have a diameter of 3mm. An aspheric lens with a diameter of 3mm and NA of 0.5 is used as focusing lens. Taking into consideration of the laser beam size, the effective NA in the new design is much higher. Because these lenses are smaller in diameter, the diameter of the packaged probe tip is much smaller than previous design. The diameter of the packaged lens series is 4.6mm and the length of the total packaged lens series is 33.5mm. The MEMS mirror has a diameter of 2mm. The X and Y axes resonant frequencies for the MEMS mirror are respectively 1260 Hz and 780 Hz. Both X and Y axes of the MEMS mirror provide a maximum deflection angle of 7 degree.

Fig.4 (a) schematic of the MEMS mirror scanner based probe (b) photography of the packaged MEMS probe.

4. Results

To demonstrate performance of the MPM system, we took multiphoton images of biological sample with the fiber laser source, MEMS mirror probe based system. Fig. 5(a) shows the two photon excited fluorescent (TPEF) image of rabbit ear. The samples were prepared as standard histology sample and stained with hematoxylin and eosin (H&E). Fig. 5(b) shows second harmonic generation (SHG) images of a fish scale. The scale bar is 15 μ m.

Fig.5 MPM images obtained with the system. (a) TPEF images of the rabbit ear (b) SHG image of fish scale.

5. Conclusion

We have developed a multiphoton microscope. It which integrates a fiber laser and double cladding photonic crystal fiber, a MEMS mirror scanner based hand-held probe. The system is compact. The fiber laser occupies a space of 60cm×45cm×24cm, and it can be condensed into a much smaller space. The MEMS based probe has a dip diameter of 4.6mm. Second harmonic generation and two photon excited fluorescence images of biological sample were demonstrated.

6. Acknowledgement

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