926 nm Yb-doped fiber femtosecond laser system for two-photon microscopy

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A Yb-doped fiber femtosecond laser system operating at 926 nm for two-photon microscopy is demonstrated. The laser system includes a master oscillator power-amplifier system, and the output wavelength shifts from 1064 nm to 926 nm by employing the nonlinear effect in photonics crystal fiber. The laser system produces 88 fs pulse train with 3.3 nJ pulse energy, 78 MHz repetition and 253 mW output power. The two-photon fluorescent image of a mouse kidney section proves that our femtosecond Yb-doped fiber laser system is an ideal source for two-photon microscopy. © 2019 The Japan Society of Applied Physics

Two-photon microscopy (TPM) plays a significant role in bio-imaging due to features such as high resolution, deep penetration depth, and the feasibility for in vivo imaging.1) The excitation of two-photon fluorescence signal needs high-power near infrared ultrafast laser. Traditionally, a commercial Ti: sapphire laser is the light source used in the TPM system. However, the large size, high maintenance costs and high requirements of operation environments (i.e., temperature, humidity, vibration isolation) limit its application and development.2) With the development of rare-earth doped fibers, the femtosecond fiber laser becomes a common tool for multi-photon imaging, such as Nd-doped,3) Yb-doped,4) and Er-doped femtosecond fiber lasers.5) It has a lot of advantages, such as low cost, miniaturization, high efficiency, and robustness, as well as its suitability for application in endoscopic instruments.6)

Femtosecond lasers operating at ~920 nm are significant for TPM in biomedical applications because commonly used green fluorophores like Alexa Fluor 488 and fluorescent proteins like green fluorescent proteins (GFP) can all be excited at ~920 nm in two-photon fluorescence imaging. Furthermore, some powerful GFP-based mutant fluorescent proteins have peak absorption wavelengths of ~920 nm, such as enhanced green fluorescent proteins (EGFP) and Ca2+ indicator GCaMP.3) Recently, a TPM system based on Nd-doped fiber femtosecond lasers operating at 930 nm and 910 nm have been demonstrated.8,9) Though Nd-doped fibers can generate ~930 nm based on a three-level transition (F3/2→L3/2) directly, it also endures the undesired competition with four-level transitions at ~1060 nm (F3/2→I12/2). Therefore, operating at a very low temperature or using gain fiber with a special designed structure, such as W-type fiber or photonic bandgap fiber, is necessary for efficient laser emission at ~930 nm.10-12) These conditions limit the development of Nd-doped fiber laser operations at ~930 nm. Yb-doped fibers are more commonly used to gain medium for short pulse fiber lasers owing to its large gain efficiency and broad gain bandwidth.4) Matured Yb-doped fiber femtosecond lasers mainly focused on 1030 nm and 1064 nm.13,14) Recently, Yb-doped fiber femtosecond lasers that can operate from 825 nm to 1210 nm have been demonstrated.15) The narrowband Yb-fiber laser generated the broadband tunable ultrafast laser source by employing the nonlinear effect in a photonics crystal fiber (PCF) to broaden the spectrum and using optical filters to select the required wavelength. The paper focused on how to broaden the spectrum, but did not discuss how to increase the output power. The average output power is only tens of milliwatts when the wavelength is less than 1 micron. The power of the light source is important for two-photon bio-imaging,16) especially in the optimal wavelength for exciting fluorescence.

In this contribution, we demonstrate a Yb-doped fiber femtosecond laser system. In the Yb-doped fiber ultrafast laser system, the seed laser is a narrowband passively mode-locked Yb-doped fiber laser. The multi-stage amplifiers, the stretcher and the grating pair are used to boost the power and compress the pulse. The PCF is used to broaden the spectrum and the filter is used to get the 926 nm light source. The average output power arrives at 253 mW. The pulse-width is 88 fs. The single pulse energy is 3.3 nJ and the repetition rate is 78 MHz.

Figure 1 depicts the experimental setup of the femtosecond laser system. The passively mode-locked cavity consists of a semi-conductor saturable mirror (SESAM) and a fiber Bragg grating (FBG).17,18) The pump of the laser is a 976 nm laser diode (LD) with a maximum power of 400 mW. The output picosecond pulse of this oscillator served as a seed source for further amplification. In order to ensure the stability of the mode-lock, all fibers in the seed source are polarization-maintaining.

The picosecond laser is amplified by two single mode fiber (SMF) Yb-doped amplifiers and a double-cladding fiber (DCF) Yb-doped amplifier. The end face of DCF fiber was cut with 8° angle, and the light in the fiber was collimated by a lens (f = 8 mm) to free space. To compress the pulse width, the spectral width should be considered. Temporal and spectral widths have a correlation as that of $\tau = 0.44\lambda_0^2/c\Delta\nu_{WHM}$,19) where $\lambda_0$ is the peak of the

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spectrum (1063.8 nm at this experiment), $c$ is the speed of light in vacuum, and $\lambda_{\text{FWHM}}$ is the spectral width (full wave at half maximum). The spectral width of the seed source is 0.06 nm, which is too narrow for supporting a femtosecond pulse. A segment of a polarization-maintaining (PM) fiber was introduced as the stretching fiber for broadening the spectrum by self-phase modulation (SPM). After the stretching fiber, a grating pair with 1200 grooves per millimeter was used to compress the pulse width to 347 fs.

In order to broaden the output spectrum, the femtosecond pulse was coupled into a segment of PCF (NKT photonics, LMA-PM-5). This PCF has a silica core of 5 μm diameters and is surrounded by hexagonal air holes of 1.22 μm, and the mode field diameter (MFD) is 4.2 μm at 1064 nm. The fiber pitch is 3.25 μm and air hole diameter to pitch ratio is 0.37. The zero dispersion wavelength (ZDW) of the PCF is 1054 nm, and the nonlinear parameter of this PCF is $\gamma \sim 10 \text{ W}^{-1} \text{ km}^{-1}$ at 1064 nm. Free space femtosecond pulse at 1064 nm was coupled into PCF by a coupler lens with 6 mm focal length and 0.21 numerical aperture, and couple efficiency is 54%. The input port of the PCF was spliced to a fiber pigtail of FC/APC connector with a splice loss of 1.6 dB. To avoid the effect of polarization changes on output power, all fibers including the PCF in this system are polarization-maintaining.

According to our previous numerical simulation and experiments results,20) we chose an 80 mm long PCF to broaden the spectrum in this laser system. The wavelengths over 1000 nm were filtered by a dichroic mirror (Thorlabs, DMSP1000) while the wavelengths under 900 nm were blocked by a long pass filter (Thorlabs, FEL0900). Therefore, the wavelengths in the range of 900 nm–1000 nm were left to the output port of this laser system.

Figure 2 shows the performance of the laser system, Fig. 2(a) to Fig. 2(c) are the spectra at different parts, and Fig. 2(d) to Fig. 2(f) are the autocorrelation trace corresponding to the spectra. Figure 2(a) depicts the spectra before and after the stretcher fiber. The red line shows the spectrum of seed pulses whose FWHM is 0.06 nm while the black line shows the broadened spectrum whose 10 dB bandwidth is 17 nm. Figure 2(d) depicts the autocorrelation trace of seed source whose pulse width is 13 ps, the pulse width changes from 13 ps to 20 ps by this stretcher fiber when the repetition rate is 78 MHz and the output power is 12.4 mW. After amplified by the two single mode amplifiers pumped by LD2 and LD3, the output power arrives at 250 mW. With further amplification by the double cladding fiber amplifier, the output power is 2.5 W. The peak power was calculated by $P_{\text{peak}} = \frac{P_{\text{avg}}}{f(T)},$ where $P_{\text{avg}}$ is the average power, $f$ is the repetition rate, $\tau$ is the pulse width. In our case, $P_{\text{avg}}, f \text{ and } \tau$ are 2.5 W, 78 MHz and 20 ps, respectively. So the peak power is about 1.56 kW.

However, such a low peak power is not high enough for deep TPM imaging. The pulse was compressed from 20 ps to 347 fs by two gratings. Figure 2(e) shows the autocorrelation trace. Considering the compression efficiency, the maximum output power decreased from 2.5 W to 1.54 W but the peak power boosted from 1.54 kW to 57 kW. Finally, the laser generates the spectrum at ~920 nm through the nonlinear effect of the 80 mm PCF. The output power is 0.81 W after the 80 nm PCF, the output spectrum is showed as Fig. 2(b). Figure 2(c) depicts the spectrum of the output femtosecond pulse after filtered by a dichroic mirror and a long-pass filter. There is only a wavelength from 900 nm to 1000 nm left. Figure 2(f) shows the autocorrelation trace of the final output pulse, the pulse width is reduced to 88 fs after filtering. The final output power is 253 mW. The single pulse energy is 3.3 nJ. The repetition rate is 78 MHz and the peak power is 41 kW.

In order to demonstrate the performance of our laser system, we imaged a section of a mouse kidney labeled with Alexa Fluor 488. Figure 3(a) depicts the schematic of the homemade two-photon laser scanning microscope system. In this system, the output femtosecond pulses firstly
pass through a MEMS mirror (Changzhou Micro Innovation Technology, CW1015) to get scanning in two orthogonal directions, then through two relay lenses to expand the beam for matching the back aperture of objective lens (Olympus, LUMPlanFL 60 × 1NA). Fluorescence is excited at the focus of the objective lens, the back-scattered fluorescence is reflected to the collection path by a dichroic mirror (Edmund, #87-061). In the collection path, the signal is filtered by a band-pass filter (Thorlabs, FBH520-40) and collected to a PMT (Hamamatsu, H7422-20) by a collection lens. The scanning of the MEMS mirror is controlled by a Labview program through a drive circuit board. The MEMS mirror has fast axis and slow axis for the X and Y direction scanning. The frequency of fast axis is 2.3 kHz and the frequency of slow axis is set to 2 Hz to get images with appropriate size. A data digitizer (National Instruments, NI-5220) is used as the signal capture of PMT. The sampling frequency of the digitizer is set to 8 MHz. The trigger signals of the MEMS mirror and sampling signals of digitizer are all synchronized. We got the image of 512 × 512 pixels with a rate of 2 frames per second.

A section of mouse kidney (Invitrogen, FluoCell Prepared Slide #3) was used for TPM imaging. This slide is labeled by Alexa Fluor 488, it is a typical fluorescence used in biomedical experiments, whose absorption peak is about 490 nm for single photon imaging, and near 920 nm for TPM imaging. Figure 3(b) shows the two-photon fluorescent image of the mouse kidney. The structure of nephric tubules could be observed clearly. The field of view (FOV) is about 300 μm × 300 μm and the scale bar is 30 μm. The experimental results demonstrated the capability of this TPM system for two-photon fluorescent imaging.

The Yb-fiber laser system with PCF can generate broadband tunable ultrashort pulse output, which are suitable for the TPM system operating at ∼920 nm. In our case, 1.54 W power is coupled into the PCF to get 253 mW output power at ∼920 nm.

Fig. 2. (Color online) Characterizations of the laser system. (a) Spectra before and after broadening by the stretcher fiber. (b) The output spectrum after the PCF. (c) Spectrum of final output. (d) Autocorrelation trace of the seed pulse. (e) Autocorrelation trace after compressed by gratings. (f) Autocorrelation trace of final output.

Fig. 3. (Color online) (a) Schematic of TPM system. L, lens; DM, dichroic mirror; BP, band pass filter; CL, collect lens; PMT, photon multiplier tuber. (b) TPM imaging of mouse kidney slice labeled by Alexa 488.
As far as we know, this is the first time to build a Yb-doped femtosecond laser focused on ∼920 nm with few hundred average power for two-photon imaging. In fact, our multi-stage amplifiers can boost the maximum power to 10 W before PCF, only 1.54 W power is coupled into the PCF because the peak of output spectrum will move to short wavelength when increasing the power. It is the limitation of PCF parameters, such as the size of microstructure and dispersion. Therefore, the output power of this femtosecond laser could be further increased by using a customized PCF. Besides that, the length of PCF also affects the coupled power, for example, when using half the length PCF, the coupled power should be twice the size before to get the same output spectrum. It means that we can get about 500 mW output power at ∼920 nm when using a 40 mm PCF, but it will be difficult to manipulate such a short fiber and the FWHM will be broadened. Both the output spectrum and power could be optimized by using a well-designed PCF. It has great potential to get a much higher output power at ∼920 nm by Yb-doped fiber laser combined with PCF. Further experiments would need to be conducted to verify the performance of our TPM system in deep tissue imaging.

In conclusion, we have demonstrated a Yb-doped fiber laser source generates femtosecond pulse at 926 nm by the nonlinear effect of PCF and the filter. The multi-stage amplifiers are used to boost the power; the stretcher and gratings are used to compress the pulse and increase the peak power. The final performance of this laser source is the following: 253 mW output power, 3.3 nJ pulse energy, 78 MHz repetition rate, 88 fs duration and 41 kW peak power. The two-photon microscopy gets the image of the mouse kidney slice labeled by Alexa Fluor 488. The image has 512 × 512 pixels and FOV of 300 μm × 300 μm. The experimental performance proves the great potential of this system for deep two-photon fluorescence imaging.

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