Generation of high-pulse energy, wavelength-tunable, femtosecond pulse at 1600-2520 nm and its second-harmonic for multiphoton imaging

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Abstract: We demonstrate 1600-2520 nm wavelength-tunable, high energy soliton generation using a large-mode-area fiber pumped by a compact fiber source. Using second harmonic generation, we show their applications for *in vivo* multi-photon mouse brain imaging.
OCIS codes: (320.7110) Ultrafast nonlinear optics; (060.2380) Fiber optics sources and detectors; (110.0180) Microscopy

Multiphoton laser scanning microscopy (MPM) enables non-invasive, 3-D imaging. It is a valuable tool for biological and medical imaging applications [1]. A unique advantage of MPM is imaging deep into scattering tissues, particularly for *in vivo* applications. The imaging depth of MPM critically depends on the pulsed excitation source, such as wavelength, pulse energy and repetition rate.

Soliton self-frequency shift (SSFS) in optical fiber or waveguide with anomalous dispersion provides a convenient and cost-effective approach to obtain the high-energy, femtosecond pulses [2]. Recently, SSRS has been explored to 2000-3000 nm based on a small-core, germanium-doped fiber [3]. However, these solitons suffer from low pulse energy so that they are unsuitable for deep tissue imaging. A simple way to improve the pulse energy is using large-mode-area (LMA) fibers or photonic crystal (PC) rods, because the soliton energy is proportional to the effective mode area (A_{eff}) of the optical fiber. The longest wavelength of solitons generated from LMA fiber (LMA-35, A_{eff} ~530 μm^2) so far is 2130 nm [2]. Here we explore the SSFS in LMA fiber (LMA-40, A_{eff} ~760 μm^2) and generate solitons up to 2520 nm, with a pulse energy up to 73 nJ. We also perform second harmonic generation (SHG) of the solitons (data shown from 900-1156 nm), with pulse energy up to 21 nJ. Finally, *in vivo* fluorescence imaging of blood vessels in a mouse brain is demonstrated by using pulses at 1150 nm as excitation source.

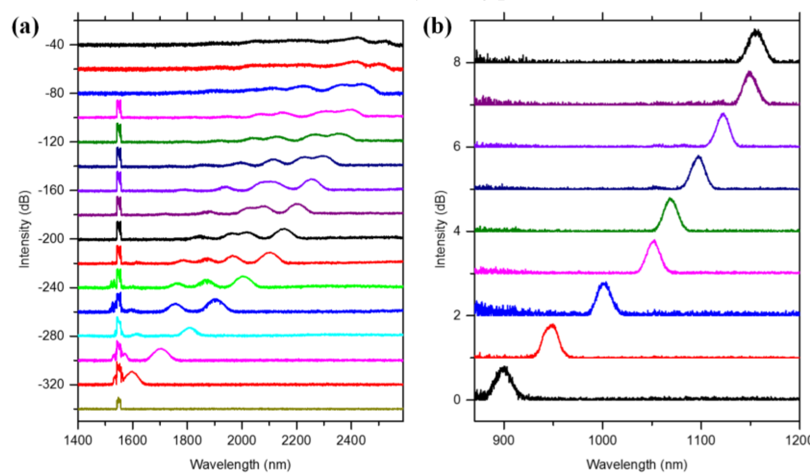

Fig. 1 (a) Measured output spectra of the LMA fiber at various launch powers, a 1720 long-pass filter is used for the last three spectra measurement. (b) Measured output spectra of SHG pulses from 900 to 1156 nm.

The pump source in our experiment is a fiber-based femtosecond laser (Calmar, FLCPA-02C), delivering 412-fs and linearly polarized pulses with 0.66-MHz repetition rate at 1550 nm. SSFS is performed in a 2-m LMA fiber (LMA-40) with an A_{eff} of ~760 μm^2 at 1550 nm. The coupling efficiency is measured to be ~64% (measured at low input power of ~100 mW). With the increase of the input pump power, the soliton forms and continuously shift from 1580 nm to 2520 nm, as shown in Fig. 1(a). To separate the longest wavelength soliton from the residual input and other solitons, a long-pass or band-pass filter is used. Pulses at shorter wavelength can be obtained by SHG of the solitons. Pulses at 775-950 nm have been demonstrated in [4]. Here we demonstrate pulse generation at 900-1156 nm. Pulses at those wavelength with high pulse energy are difficult to generate by a conventional mode-locked Ti: sapphire (Ti:S) laser or optical parametric oscillator (OPO). For example, pulses at 1150 nm with a pulse energy of 21 nJ is generated and used for *in vivo* two photon fluorescence imaging of blood vessels in a mouse brain.

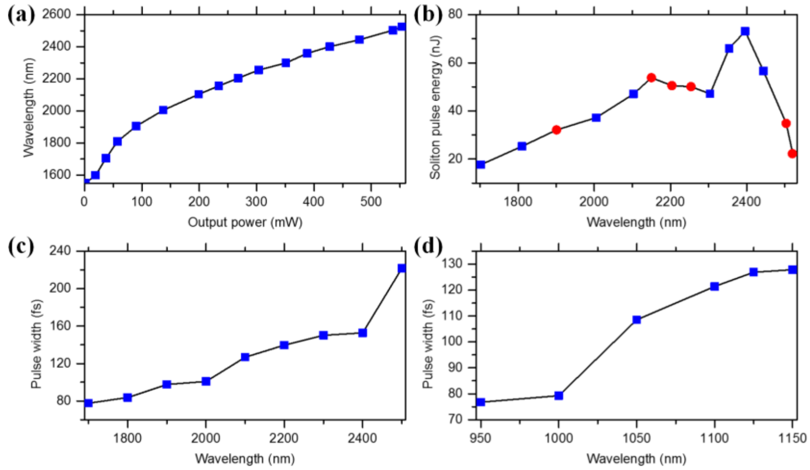

Fig. 2 (a) The center wavelength of the longest wavelength soliton as a function of the output power from the LMA fiber. (b) Measured energies for the longest wavelength solitons at various soliton wavelengths. (c) Measured pulse widths for the longest wavelength solitons at various soliton wavelengths. (d) Measured pulse widths for the SHG pulses at various wavelengths.

The center wavelength (defined as the peak position of the soliton spectrum) of the longest wavelength soliton as a function of the output power from the fiber is shown in Fig. 2(a). A tuning range of more than 900 nm, i.e., from 1600 nm to 2520 nm, is achieved. The pulse energy of the longest wavelength soliton are measured by using long-pass (LP) or band-pass (BP) filters (1630 nm LP, 1720 nm LP, 1950 nm LP, 2000-2250 nm BP, 2250-2500 nm BP, 2500-2750 nm BP) and a power meter, taking into account the transmission of the filters. The measured soliton energies as a function of the soliton wavelength are shown in Fig. 2(b). Two methods are used to measure the soliton pulse energy because of the availability of LP and BP filters. The blue squares are directly measured by using filters and power meters; while for the red circles, we measured the power of the filtered spectra, and then calculated the power of the longest wavelength soliton by integrating the soliton spectrum. We performed second-order interferometric autocorrelation for pulse width characterization. The long-pass and band-pass filters were used to isolate the soliton at the longest wavelength. The measured pulse widths as a function of the soliton wavelength and the SHG pulse wavelength are shown in Fig. 2(c) and Fig. 2(d), respectively. The soliton pulse width increases at longer wavelengths due to increased dispersion values of the LMA fiber at the longer wavelengths. Fiber propagation loss or absorption might also play a role for the decreasing pulse energy and increasing pulse width at the long wavelength tuning range beyond 2400 nm.

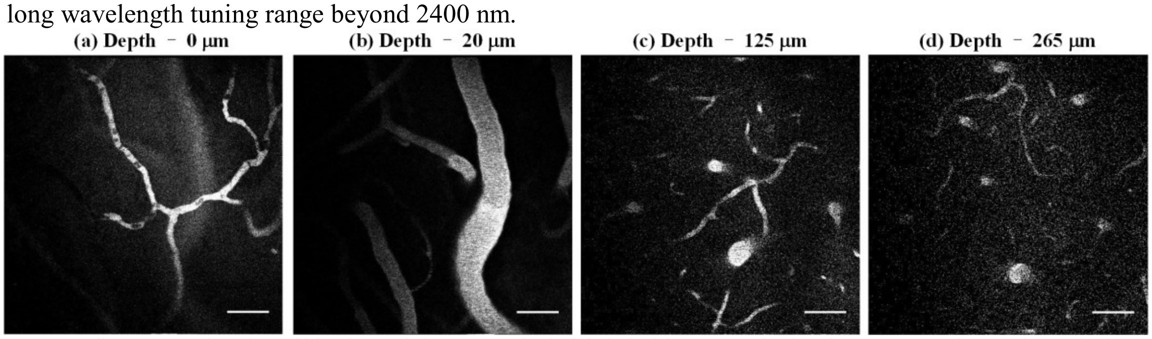

Fig. 3 *In vivo* fluorescence imaging of blood vessels in a mouse brain labeled with Texas-red using the 1150 nm pulses as the excitation source. Scale bar: 50 um, pixel size: 512 × 512, frame time: 4s, no average.

To demonstrate the applications of our source, we imaged Texas-red labeled vasculature in a mouse (3-month old) brain *in vivo*. SHG pulses at 1150 nm were used to excite the 2-photon fluorescence (Fig. 3).

In summary, we demonstrate SHG of 1600-2520 nm wavelength-tunable, high-energy solitons for *in vivo* 2-photon microscopy. With further optimization of the SSFS and SHG process, the demonstrated method has the potential to provide wavelength-tunable, energetic femtosecond pulses over the entire spectral range of 800 to 1250 nm in a low-cost, robust, and compact setup.

References

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