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Two-photon microscopy with an 1μm-band wavelength-selectable picosecond light pulse source based on a gain-switched laser diode

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INTRODUCTION Two-photon fluorescent microscopy (TPM) has had profound implications for investigations of complex biological samples. For TPM, the development of a compact, highly functional optical pulse source is thus highly desirable. We have developed a high-peak-power picosecond light pulse source based on a 1060nm-band gain-switched laser diode (GSLD). This enabled deep-site observations of neuron cells of a mouse brain expressing enhanced yellow fluorescent proteins (eYFP).[1] Although our previous 1060nm-band light pulse source was a wavelength-fixed one, a wavelength tunable (selectable) feature is desired to efficiently excite fluorescent materials.[2] We here report the results for extending the light pulse source feature to wavelength-tunable around 1μm band and for utilizing it to demonstrate the wavelength-dependence of two-photon imaging of neuron cells expressing eYFP.

EXPERIMENTAL The 774nm kilowatt-peak power picosecond optical pulses were generated from the light pulse source involving a GSLD,[3] and were injected into a highly nonlinear optical fiber to produce supercontinuum (SC) light. The SC light generated was filtered by a long-wavelength pass filter to remove the incident light pulses centered at 774nm wavelength and visible light components of the SC light. Figure 1 shows the spectrum of the filtered pulses. The 3dB bandwidth is 290 nm, covering 1μm-band wavelength range which is essential for two-photon excitation of common visible fluorescent proteins.
We amplified the filtered pulses by an ytterbium-doped fiber amplifier (YDFA) and picked up the wavelength components at 1030, 1060 and 1080 nm by using 2nm-bandwidth optical band-pass filters. After this, these pulses were amplified to sub-watt average power by an additional stage YDFA and coupled into a laser scanning microscope. Figure 2 shows the two-photon fluorescent images of a brain slice expressing eYFP from a Thy1-eYFP-H transgenic mouse illuminated under the same average power of 8.6 mW (measured after the objective lens) at 1030 and 1060 nm. Using 1030nm pulses resulted in a clearer image with high signal to noise ratio in comparison with using 1060nm pulses, while the neuron cells cannot be revealed by 1080nm pulses. Our present results indicate that the two-photon-excitation efficiency of neuron cells expressing eYFP is enhanced by using 1030nm pulses. As shown here, a GSLD-base wavelength-tunable picosecond light source could be potentially used to effectively excite a variety of fluorescent materials for TPM applications.

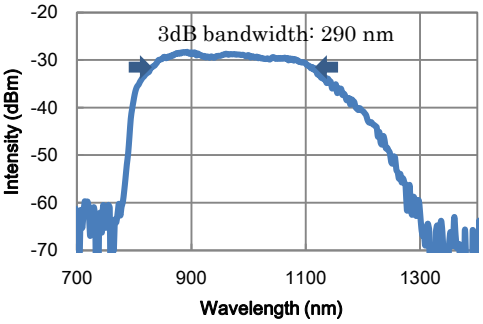

Fig. 1. Spectrum of supercontinuum light filtered by a long-wavelength pass filter.

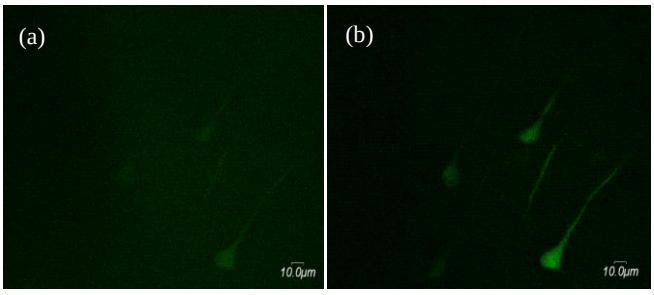

Fig. 2. Two-photon fluorescent images of a brain slice expressing eYFP from a Thy1-eYFP-H transgenic mouse illuminated by picosecond light pulses at (a) 1060nm and (b) 1030nm wavelength.

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