An adaptive excitation source for multiphoton imaging

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Abstract: We demonstrate an adaptive femtosecond laser source that improves the imaging speed by >10 times for multiphoton imaging of brain activity in awake mouse, achieving 30 frames/s, 734x734 µm field-of-view (FOV) at 700 µm depth. © 2018 The Author(s)
OCIS codes: (320.7110) Ultrafast nonlinear optics; (060.2380) Fiber optics sources and detectors; (110.0180) Microscopy

High imaging speed is necessary for recording of a large number of neurons. Because typical multiphoton microscopes (MPM) operate at the photon shot-noise limited regime, the maximum number of neurons that can be imaged at high spatial and temporal resolution is fundamentally limited by the maximum permissible average and peak power in biological specimens. Increasing the scanning speed cannot overcome the limit imposed by the "photon budget", i.e., a certain number of signal photons per second is needed in order to assess the neuronal activity with a high confidence level. An effective approach to increase the imaging speed is to only image the region of interest (ROI). Since neurons consist of only ~ 10% of the volume in the mouse brain, an order of magnitude improvement in imaging speed can be achieved by only illuminating the ROI (i.e., neurons) when compared to conventional raster scanning. 3D random access MPM (RAMP) using acousto optic deflectors (AOD) [1] is currently the fastest method for imaging large number of neurons simultaneously in scattering tissue. Nevertheless, RAMP suffers several significant shortcomings, such as a small FOV, expensive and complex optical setup (e.g., need 2 or more large-aperture AODs and relay telescopes), and low deflection efficiency of the AOD (e.g., about 70% for each AOD at ~ 800 nm, even lower for longer wavelengths due to material limitations).

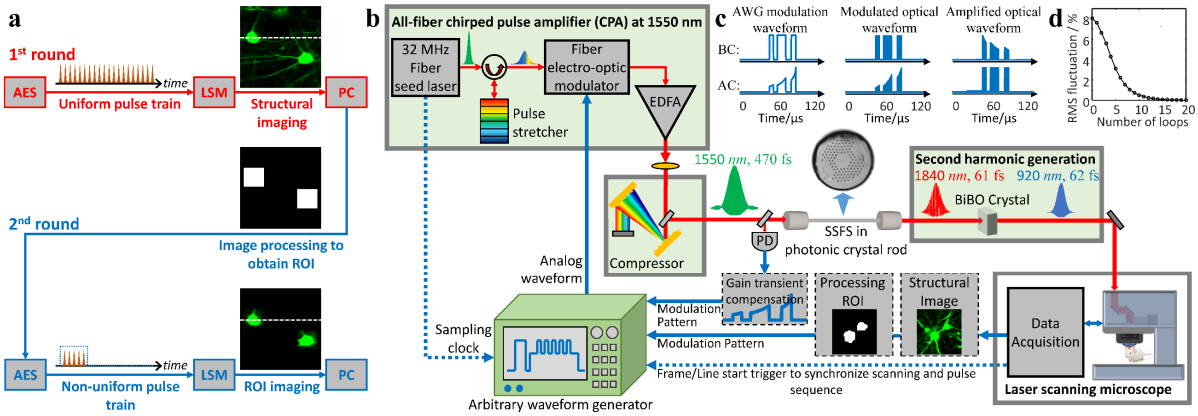

Fig. 1 (a) Principle of a microscope with the adaptive excitation source, AES: adaptive excitation source, LSM: laser scanning microscope, PC: computer. There are two steps for the adaptive process: 1. A structural image is obtained first to find the ROI. 2. An adaptive pulse train is generated so that only the ROI is excited. (b) Detailed experimental setup, CPA: chirped pulse amplifier. Inset (c): Demonstration of compensation of the gain transient in EDFA. BC: before compensation, AC: after compensation. Inset (d): RMS fluctuation of the pulse intensity as a function of the number of iterations by the feedback loop.

We developed a smart laser source that will adapt to the sample under study (i.e., an adaptive excitation source) to overcome the limitations of RAMP. Fig. 1(a) shows the principle of a MPM with the adaptive excitation source for recording neuronal activity. A high-resolution structural image is first obtained by raster scanning of the sample, and the image is processed to find the ROIs. For recording the activity of the neurons, for example, the bright regions of the somas define the ROIs. The ROI information is converted to a binary digital sequence in the time domain (i.e., the modulation pattern) to control an arbitrary waveform generator (AWG, Fig. 1(b)), which drives a fiber-integrated electro-optic modulator (EOM) that encodes the pulse pattern. The adaptive pulse sequence is then amplified to high pulse energy, and sent to a laser scanning MPM. The synchronization of scanning and the pulse sequence ensures that the excitation beam will only illuminate the ROI. By allocating all the permissible laser power on the ROIs (i.e., the laser is completely "turned off" outside the ROIs), the signal generation, and therefore, the imaging speed is increased by the inverse of the volume fraction of the ROI, without increasing the average or peak power on the sample. A salient feature of our design is that the "unwanted" pulses are removed before the final power amplifier (i.e., the erbium-doped fiber amplifier, EDFA) stage. While modulation of laser power has been done routinely in the past, e.g., for beam blanking or enhancing dynamic range, the modulation was always

performed after the laser or amplifier output (i.e., outside the excitation source). By placing the modulator inside the excitation source, our design not only allows a high speed, low power (only need to handle < 100 mW), and low cost fiber-optic modulator to perform the intensity modulation, but also enables the entire output power of the fiber amplifier to be used, which improves the robustness and power-efficient (>10X), and significantly reduces the cost.

The new excitation source starts with an all-fiber chirped pulse amplification system (CPA), Fig. 1(b). Because the pulse pattern is no longer periodic in time, the main challenge for the adaptive source is to overcome the gain transient in the EDFA, similar to what occurs for burst-mode amplification in telecom [2], as illustrated in Fig. 1(c), before compensation (BC). To equalize the intensity for the output pulses, we setup an automatic feedback loop to pre-compensate the gain transient. The intensity of the output pulses is measured by a photo-diode (PD). Based on the measured optical waveform, the AWG modulation pattern is adjusted to pre-shape the input pulse train to the EDFA. This process is repeated until the pulse-to-pulse intensity fluctuation is sufficiently reduced. As shown in Fig. 1(d), the root-mean-squared (RMS) fluctuation of the pulse intensity decreases with the number of iterations. After about 25 loops, which takes a total of approximately 60 seconds to complete, the RMS fluctuation decreased from 8% to 0.004%, as shown in Fig. 1(c), after compensation (AC). The intensity-equalized pulse train is then launched into a photonic crystal rod for soliton self-frequency shift (SSFS) to 1840 nm [3]. The frequency-doubled soliton at 920 nm is sent to the MPM for recording neural activity.

We performed in *vivo* two-photon imaging of brain activity of GCaMP6-labeled neurons in an awake mouse at 700 µm beneath the dura with a large FOV of 734x734 µm and at 30 frames/s. A uniform, periodic pulse train (with a 4 MHz repetition rate) is first sent to the microscope for structural imaging, as shown in Fig. 2(a). To obtain a high quality structural image, we averaged over 100 seconds because of the low signal-to-background ratio at such a depth for 2-photon imaging. An adaptive pulse train is then generated according to the information of the structural image. The average power at the brain surface is ~ 21 mW for recording the spontaneous activity of the neurons, as shown in Fig. 2(b). The adaptive excitation source has a 32 MHz repetition rate at the ROI, sufficient for recording neural activity using resonant galvo-scanners at 30 frames/s and 512x512 pixels/frame. We designed the ROI to be somewhat larger than the neuron in order to compensate for the motion of the awake mouse. The fluorescence time traces of 10 neurons are shown in Fig. 2(c). Since the ROI occupies ~ 5% of the FOV, approximately 400 mW average power would be required to obtain the same traces if we had used a conventional laser at 32 MHz repetition rate. Such a high power is beyond the thermal damage threshold (typically < 200 mW) of the mouse brain. The combination of the imaging FOV, frame rate, and depth cannot be achieved using a conventional laser system.

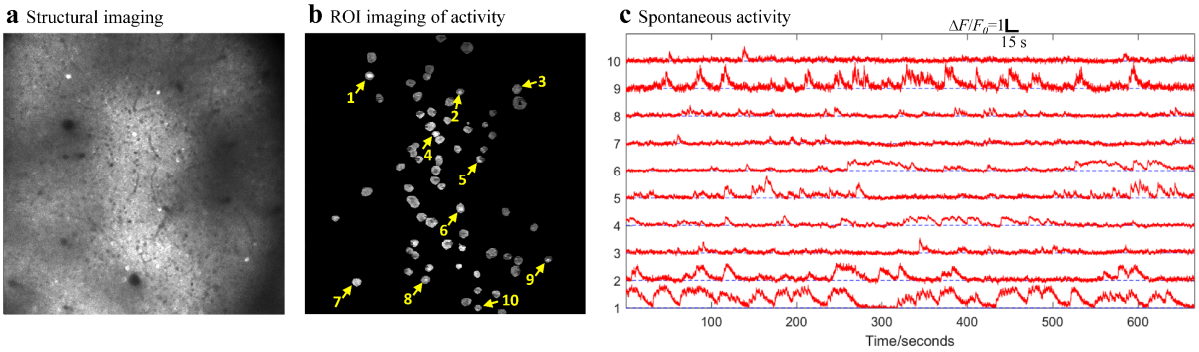

Fig. 2 Recording the spontaneous activity of neuronal population in an awake mouse brain. (a) Structural imaging of neurons located at 700 µm beneath the dura with a FOV of 734x734 µm, 512x512 pixels/frame, 30 frames/s, averaged over 100 seconds. (b) Activity recording sites (ROIs) with the same field-of-view and frame rate but no average. (c) Spontaneous activity recorded from the labeled neurons indicated in b.

Although our main motivation is to improve the speed of activity recording, the concept of an agile, adaptive excitation source represents a new direction in designing an imaging system. For the first time, the sample under study becomes an integral part of the excitation source, and trains the laser source so that optimum laser exposure is used to obtain the required information (e.g., neuron activity, blood flow speed, etc.). Our design takes full advantage of the robust, cost effective, fiber optic telecom systems. It requires no modification of the microscope hardware, has the same large FOV of a conventional MPM, and works at the excitation wavelengths for both two-photon and three-photon microscopy. In fact, the adaptive excitation source can be integrated with any existing MPMs, which will enable easy translation of the technique to the wider imaging community.

References

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