High-speed, large field-of-view and deep imaging with an adaptive excitation source

Li, Bo, Wu, Chunyan, Wang, Mengran, Charan, Kriti, Xu, Chris
High-speed, large field-of-view and deep imaging with an adaptive excitation source

Bo Li, Chunyan Wu, Mengran Wang, Kriti Charan and Chris Xu
School of Applied Engineering and Physics, Cornell University, Ithaca, NY USA

ABSTRACT

Conventional multiphoton microscopy uses periodically pulsed sources as excitation and the sample is illuminated uniformly by the laser. While necessary for structural imaging, monitoring dynamic biological functions such as neuronal activity in the brain typically only requires imaging of the region of interest (ROI), e.g., the neurons. The adaptive excitation source enables imaging of the region of interest only. It reduces the requirement for the output power of the excitation source (by at least an order of magnitude) and simultaneously reduces the excitation power to the sample for obtaining the necessary information (e.g., neuronal activity). We demonstrate three-photon imaging of brain activity in awake transgenic mice (jRGECO1a), with highest speed (30 frames/s), large field-of-view (620x620 µm/512x512 pixels) and deep penetration (750 µm beneath the dura).

Keywords: Jen Lab Young Investigator Award, Multiphoton microscopy, Brain research, Fiber laser

1. INTRODUCTION

Three-photon microscopy (3PM) increases the achievable imaging depth in mouse brain due to weaker scattering at longer excitation wavelengths and background suppression by higher-order nonlinear excitation [1-3]. However, high temporal resolution (≥ 30 Hz) and deep imaging within scattering brain tissue is still challenging. 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 image the region of interest (ROI) only [4-6]. 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. With an adaptive excitation source [7, 8], we demonstrate three-photon imaging of awake mouse brain with highest speed, large field-of-view (FOV) and deep penetration.

Fig. 1 Principle of a microscope with the adaptive excitation source.
Fig. 1 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, which drives a fiber-integrated electro-optic modulator that encodes the pulse pattern. The adaptive pulse sequence is amplified to high pulse energy, and then converted to longer wavelength centered at 1700 nm based on soliton self-frequency shift [9], 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) [10, 11]. 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.

Fig. 2 Comparison of neuronal activity recording with conventional raster scanning and adaptive excitation. (a1) Structural imaging of neurons located at 750 µm beneath the dura. (b1) Activity recording sites (ROIs) with the same field-of-view and frame rate. (a2) and (b2) are the spontaneous activity recorded from the labeled neurons indicated in (a1) and (b1).
We performed in vivo 3PM of brain activity of jRGECO1a-labeled [12] neurons in an awake transgenic mouse (~7 month) at 750 µm beneath the dura with a large FOV of 620x620 µm and at 30 frames/s (512x512 pixels/frame). A uniform, periodic pulse train (with a 2 MHz repetition rate) is first sent to the microscope for structural imaging, as shown in (a1). To obtain a high quality structural image, we averaged over 100 seconds because of the low signal with raster scanning at such a depth. An adaptive pulse train is then generated according to the information of the structural image, as shown in Fig. 2(b1). 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 average power at the brain surface for both structural and ROI imaging are ~ 35 mW. The fluorescence time traces of 10 neurons are shown in Fig. 2(a2) and (b2) for structural and ROI imaging, respectively. Although the same average power was used, the signal of ROI imaging is >30 times higher than that of structural imaging. Since the ROI occupies ~ 4.2% of the FOV and the fill fraction of the resonant scan is 71.3%, approximately 1.2 W 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 < 100 mW at 1700 nm) of the mouse brain. The combination of the imaging speed, FOV and depth cannot be achieved using a conventional laser system.

We also performed in vivo two-photon microscopy (2PM) imaging of the activity of GCaMP6s-labeled [13] neurons in an awake mouse (7 months old) at 680 µm beneath the dura with a FOV of 700 µm x 700 µm and at 30 frames/s (512x512 pixels/frame). A comparison of 2PM of neuronal activity with and without AES at the same location is shown in Fig. 3. The photon number per neuron per frame with the adaptive excitation is more than 7 times higher than that without the adaptive excitation even though the average power used for adaptive excitation is ~ 4.5 times lower. For ROI...
imaging with the AES, the average power at the surface of the brain was 18 mW. Since the ROIs occupy ~ 3% of the FOV and the fill fraction of the resonant scan is 71.3% in the temporal domain, approximately 800 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 average power is beyond the thermal damage threshold (typically ~ 200 mW at 920 nm) of the mouse brain [14]. The demonstrated imaging performance cannot be obtained by external modulation of a conventional femtosecond laser (e.g., the Ti:Sapphire laser) since an average power of ~ 3W would be required at 920 nm. Such a power level is beyond the reach of existing 2-photon excitation sources.

We demonstrated an AES for imaging neuronal activity with a large FOV, a large penetration depth and a high frame rate while using low average power. Although our main motivation is to improve the speed of activity recording, the concept of the AES represents a new direction in designing an imaging system where the excitation source itself is optimized according to the sample under study. The adaptive excitation technique requires no modification of the microscope hardware, has the same large FOV of a conventional MPM, and works at the excitation wavelengths for both 2PM and 3PM. In fact, the AES can be integrated with any existing MPMs, which will enable straightforward translation of the technique to the wider imaging community.

References