High-speed Two-photon Microscopy with Adaptive Sampling

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Abstract: We demonstrate a high-speed two-photon fluorescence microscope using line illumination with an adaptive sampling scheme. The illumination pattern is modulated by a digital micro-mirror device so only the regions of interest are illuminated and sampled.

1. Introduction

Two-photon laser scanning microscopy can image deep into the tissue with high signal-to-background ratio, tight axial confinement and low phototoxicity [1]. When used in conjunction with calcium indicators, two-photon microscopy provides a powerful tool to image the brain activity [2]. However, the conventional point-by-point scanning scheme in two-photon microscope has a tradeoff among sampling speed, spatial resolution and field of view (FOV), and is typically susceptible to oversampling. When imaging a large FOV sample, the temporal resolution could become very poor. This has limited its applications in calcium imaging, as it is important to record the fluorescence at high temporal resolution in order to capture the neuronal signals faithfully. Beam multiplexing techniques [3] increase the sampling rate but could be limited by the total allowed laser power that could be deposited to the brain [4]. Here, we propose an innovative adaptive sampling scheme which adopts a short line as the illumination/excitation pattern. The line is further modulated by a digital micro-mirror device (DMD) so only the regions of interest (e.g., neuronal cell body in population calcium imaging) are illuminated and sampled. Such a scheme could increase the imaging speed while keeping the overall laser power on the brain low. We demonstrated this adaptive sampling scheme in phantom samples, which shows great promise for high-speed calcium imaging.

2. Adaptive sampling scheme

The conventional point scanning scheme in two-photon microscopy (Fig. 1a) suffers from low frame rate. For population calcium imaging where the neuronal cell bodies are the regions of interest, we could use a short line as the illumination pattern (Fig. 1b) to improve the frame rate by sampling multiple points together and scanning fewer rows. However, this approach excites and collects the background during scanning, which could decrease the SNR and induce excessive phototoxicity in the sample through heat generated by the excitation light. To solve these problems, we propose an adaptive sampling method which modulates the pixel-wise intensity of the illumination line by a DMD (Fig. 1c). The DMD encodes a map of the neuronal cell bodies of the tissue. Excitation light impinges on the DMD, and the reflected light is modulated by the DMD and thus carries the tissue information. In this way, we could sample the neuronal cell bodies only and avoid exciting the unwanted background regions. Compared with other adaptive sampling scheme such as that based on raster scanning [5] or random access [6], our scheme samples a larger portion of the neurons at once and thus increases the imaging speed.

As a diffractive element, the DMD can be further used to achieve temporal focusing [7] while executing the adaptive sampling function. Temporal focusing ensures tight axial resolution when the illumination/sampling pattern is large.

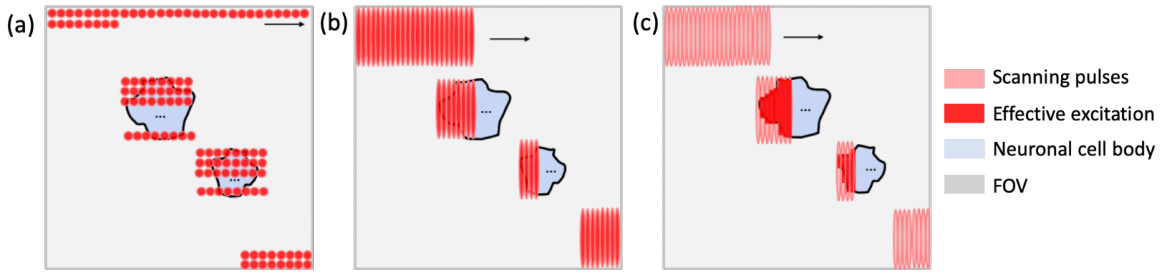

Fig. 1. Comparison of different sampling schemes in two-photon microscopy. (a) Point-by-point sampling. (b) Line sampling. (c) Adaptive sampling, with the prior knowledge of the sample, only the neuron cell bodies are illuminated and sampled.

3. Experiment results

We home-built a two-photon microscope with the adaptive sampling scheme (Fig. 2a). The system utilizes an 80 MHz repetition rate femtosecond laser at 920 nm excitation wavelength. The laser beam is shaped by a cylindrical lens set and a slit to form a line. The beam is then scanned by a resonant scanner and galvanometer system and focused on the

DMD, which encodes a pattern of the neuronal cell body of the tissue. The DMD is placed at the focal plane of the scan lens to ensure the best overlap of the spatial and temporal focusing planes. A tube lens and an objective lens (16X) then focus and illuminate the patterned beam on the sample plane. The fluorescent emission is collected by the PMT and digitized by a high-speed data acquisition card. We characterized the axial resolution of the illumination pattern to be ~16 μm full-width-at-half-maximum (FWHM) by taking a z-stack of a 5 μm fluorescent bead sample (Fig. 2b). We verified the lateral excitation shape to be ~2 μm × 10 μm FWHM by imaging the exciting pattern on a uniform fluorescent slab through a camera in a transmission way (Fig. 2c).

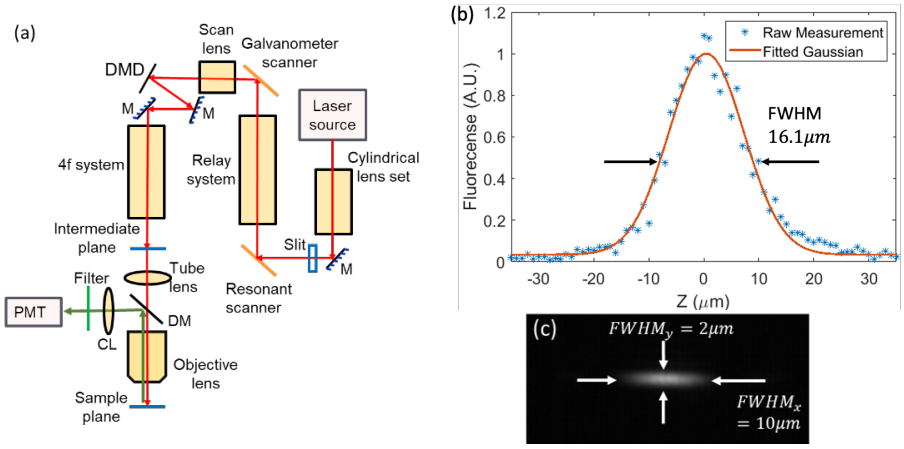

Fig. 2. (a) Schematic of the two-photon microscope setup with adaptive sampling scheme. M: mirror; DM: dichroic mirror; CL: collimating lens. (b) Measured axial point spread function (PSF) for line excitation. The FWHM is 16.1 μm. (c) Measured PSF in the lateral direction (xy) for line excitation, which shows 2 μm and 10 μm FWHM in y and x directions respectively.

We used the 5 μm fluorescent bead sample to verify the concept of adaptive sampling. Here, small clusters or single beads were considered as unwanted backgrounds, whereas some large bead clusters were considered as regions of interest. The mask was generated after system calibration and loaded onto the DMD. We compared the results without (Fig. 3a) and with (Fig. 3b) adaptive sampling, and confirmed that the backgrounds were not excited in the adaptive sampling scheme.

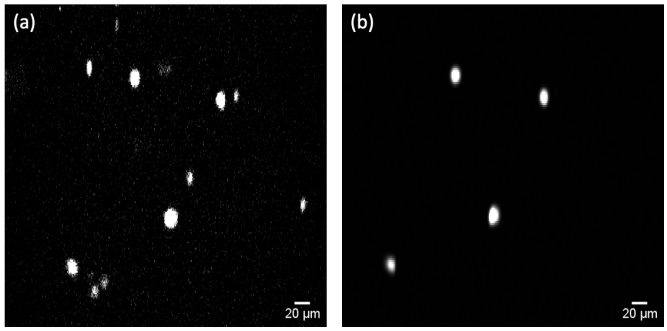

Fig. 3. (a) Acquired image of the two-photon microscope without the adaptive sampling scheme, by turning on all the mirrors of the DMD. (b) Acquired image of the two-photon microscope with the adaptive sampling scheme. The image of (a) and (b) were oversampled along the excitation line direction so as to visualize the feature details.

4. Conclusion

We proposed and demonstrated the concept of adaptive sampling in two-photon microscopy. By using line illumination and only exciting the regions of interest rather than the entire field of view, we could achieve a high imaging speed and reduce the overall laser power and thus phototoxicity on the sample. It holds great promise for high-speed calcium imaging.

Acknowledgement

We acknowledge support from NSF CAREER (1847141) and Burroughs Wellcome Fund (Career Award at the Scientific Interface: 1015761).

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