Video-rate volumetric neuronal imaging using 3D targeted illumination

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Abstract: We describe a simple widefield based fast volumetric microscopy technique. Our technique enables neuronal imaging up to 500 × 500 × 240 μm 3D field of view at 100 Hz with high contrast and signal-to-noise ratio.

OCIS codes: (110.1080) Active or adaptive optics; (180.2520) Fluorescence microscopy; (180.6900) Three-dimensional microscopy.

1. Introduction

Fast volumetric microscopy is required to monitor large-scale neural ensembles with high spatio-temporal resolution. In general, point scanning techniques such as confocal or two-photon microscopy are too slow for real-time volumetric imaging applications, though efforts have been made to increase their temporal resolution [1, 2]. Widefield based techniques, on the other hand, can benefit from high sensitivity cameras with massive pixel counts that can capture wide 2D fields of view (FOVs) within a single snapshot. This is particularly advantageous for volumetric imaging since it obviates the need for 2D scanning, offering the potential for increased imaging speed and reduced system complexity. For example, light sheet microscopy with fast single-axis scanning can achieve video-rate volume imaging in the brain [3,4], though the requirement of side-on illumination often makes it impractical, prescribing in some cases oblique light sheet delivery [5].

A simple focal sweep add-on can also extend the capacity of widefield microscopy by enabling extended-depth-of-field (EDOF) imaging, but suffers from an inability to reject out-of-focus fluorescence background [6]. Specifically, objects in an EDOF image are both in focus and out of focus. The out-of-focus contributions constitute background haze that degrades both the image contrast and signal-to-noise ratio (SNR), which can introduce severe signal crosstalk and also limit the performance image postprocessing algorithms (e.g., deconvolution). This ultimately limits its practicability for volumetric imaging through long depth-of-field (DOF) extension.

2. Principle

In this work, we describe a simple widefield based microscopy technique that is capable of imaging up to 500 × 500 × 240 μm 3D FOV at 100 Hz acquisition rate with significantly improved contrast and SNR compared to conventional focal sweep techniques.

Our experimental setup is shown in Fig. 1. The system is analogous to a conventional epi-fluorescence microscope in that an objective (Olympus LUCPLFLN 20X/0.45) and tube lens f_1 image the sample onto an intermediate image plane, and an additional 4f system f_2 and f_3 relays the intermediate image onto a camera (Andor Zyla 4.2). However, there are two key additions to our system: a fast focal sweep mechanism and a targeted illumination system.

In our case, the focal sweep mechanism is an electrically tunable lens (ETL, Optotune VIS-EL-10-30-C), which has up to 100 Hz scan rate. The ETL is inserted in a pupil plane common to both the excitation and emission beams to ensure system telecentricity, so focus shift produced by it is given by $D = -n\frac{f_{obj}^2}{f_1^2}\frac{f_2^2}{f_{ETL}}$, where n is the refractive index of the sample, and f_{ETL} is the focal length of ETL. For example, in the case of mouse brain ($n = 1.35$) and with an ETL focal length that varies from from -666 mm to infinity to +286 mm, we can attain an EDOF range of 240 μm.

Our targeted illumination system is a digital micromirror device (DMD, Texas Instruments V-7000 VIS), which is capable of projecting high resolution (1024×768) excitation patterns at high switching speeds up to 22.7 kHz. The DMD is placed in a plane conjugate to the sample, and projects patterns onto the sample that are defined by the "on" pixels of the DMD. A LED provides collimated illumination to the DMD.

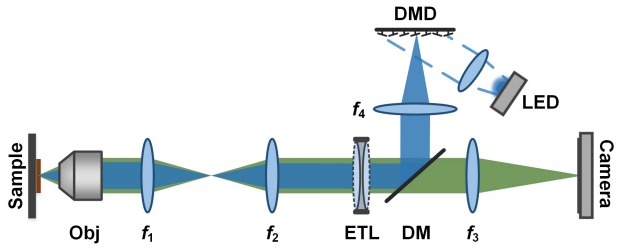

Fig. 1. Experimental setup. $f_1 = 150$ mm, $f_2 = 100$ mm, $f_3 = 150$ mm, $f_4 = 125$ mm. Obj: objective. DM: dichromatic mirror. Additional excitation and emission filters are not shown.

The basic idea is to generate an EDOF image using 3D spatially targeted illumination (TI) by rapidly switching DMD patterns during the ETL scan. Specifically, during the focal sweep, at each focal plane, instead of using uniform illumination (UI) we use patterned illumination that targets only in-focus sample structure. This essentially creates a sample-specific 3D illumination structure. All this is done within a single camera exposure time, enabling us to capture single-shot TI-EDOF images.

The 3D TI patterns can be predetermined using structured illumination microscopy (SIM) technique [7] that produces an optically sectioned image of the sample structure at every depth. Because SIM is only used in the calibration step, it will not affect the TI-EDOF imaging speed itself. For the application of neuronal imaging, depending on the sparsity of labeling, our 3D illumination patterns become commensurately sparse, thus reducing the light dose inflicted on the sample. And because the illumination patterns are tailored to the sample, this reduction in light dose leads to a reduction only of the out-of-focus background and not of the in-focus signal, thus leading to an increase in contrast and SNR. Additionally, we take advantage of an adapted deconvolution algorithm [6] to further enhance contrast and SNR, resulting in EDOF image qualities that approach those obtained by standard confocal microscopy, at 3D-volume acquisition rates orders of magnitude faster. The only requirement of our technique is that the sample be spatially fixed, though it can be temporally dynamic. This requirement can be realized in many applications.

In particular, we demonstrate that our system can increase contrast-to-noise ratio by 800% and image contrast by 4800% compared to conventional focal sweeping technique in fixed brain slices over an imaging volume of 500 $\times$ 500 $\times$ 150 µm. We also demonstrate in vivo calcium imaging for head-fixed mouse brains over 500 $\times$ 500 $\times$ 100 µm 3D FOV at video-rate.

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