Enhance imaging depth in wide-field two-photon microscopy by extended detection and computational reconstruction

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Abstract: Line-scanning temporal focusing microscopy is competitive in high imaging speed but still suffers from tissue scattering. We propose the extended detection and computational reconstruction technique, to extract signals from scattering photons and enhance imaging depths.

OCIS codes: (110.0113) Imaging through turbid media; (110.7050) Turbid media; (180.0180) Microscopy; (180.2520) Fluorescence microscopy; (180.4315) Nonlinear microscopy.

1. Introduction

Multiphoton microscopy (MPM), as the golden standard in biomedical studies [1], has advantages in deep penetration, 3D sectioning capability, and low phototoxicity, *etc*. However, in conventional point-scanning MPM, the inertia of mechanical scanners limits the imaging speed, which hampers most studies of biological dynamics. Recently, temporal focusing microscopy (TFM) has been proposed to increase the imaging speed via wide-field imaging, while maintaining optical sectioning [2][3]. Specifically, in line-scanning TFM (LTFM), biological samples are excited by a sweeping line, which ensures a well balance between imaging speed and axial resolution.

However, as a wide-field imaging technique, TFM is susceptible to cross-talk induced by tissue scattering [4][5]. Even though employing the mechanism of multi-photon excitation could reduce the scattering of the excitation beam for deep penetration, the scattering of the emitted fluorescence sets the bottleneck in parallel reading-out. In LTFM, confocal slit detection has been adopted to filter out the crosstalk in the direction orthogonal to the excitation lines. Apparently, such a technique would fail to handle the crosstalk along the excitation lines.

Here we propose the extended detection-LTFM (ED-LTFM) that could extract signals from scattering photons and enhance imaging depths. Instead of employing the confocal slit, we record a 2D fluorescent image at each line-shape excitation position so that the signals, including the scattering signals, are fully recorded. Then we perform computational reconstruction to recover the signals. We demonstrate the enhanced performance of ED-LTFM in *in vivo* deep imaging of mouse brains.

2. Experimental methods and results

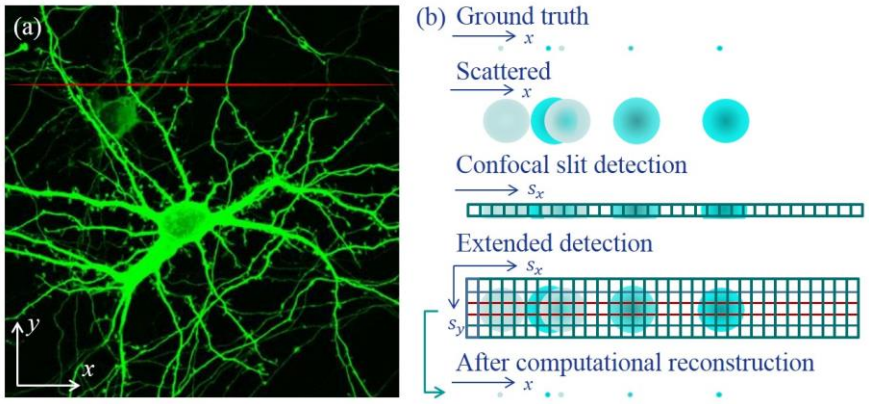

Fig. 1. Diagram of the experimental principle. (a) Illustration of a neuron (in green) illuminated with a line-shape excitation beam (in red, scanning along *y* axis). (b) The excited, detected, and reconstructed signals along the *x* direction. Scattered signals distributed on the detectors are shown with grids for pixels.

Figure 1 shows the diagram of the experimental principle. We numerically verify that the pattern induced by tissue scattering is circularly symmetric. To detect such scattered patterns, confocal slit was proposed to filter out the scattering signals along *y*. However, the crosstalk along *x*-axis remains, as shown in Fig. 1b, which will mix together to decrease the contrast and signal-to-noise ratio. Instead, we propose to fully record the scattering signals by extended detection, and perform computational reconstructions to recover original signals. In ED-LTFM, we acquire a 2D image for each excitation line. In the image reconstruction, we can also conduct Hessian regularized deconvolution to improve the image quality.

We evaluate the performance of the proposed method via numerical simulations, and demonstrate the advantages of the ED-LTFM in strong scattering and noisy conditions. We compare the retrieved images of the microtubules (data not shown) by wide-field detection (WD), confocal slit detection (CSD) and ED, and find that the results of ED are the best in the metric of structural similarity index.

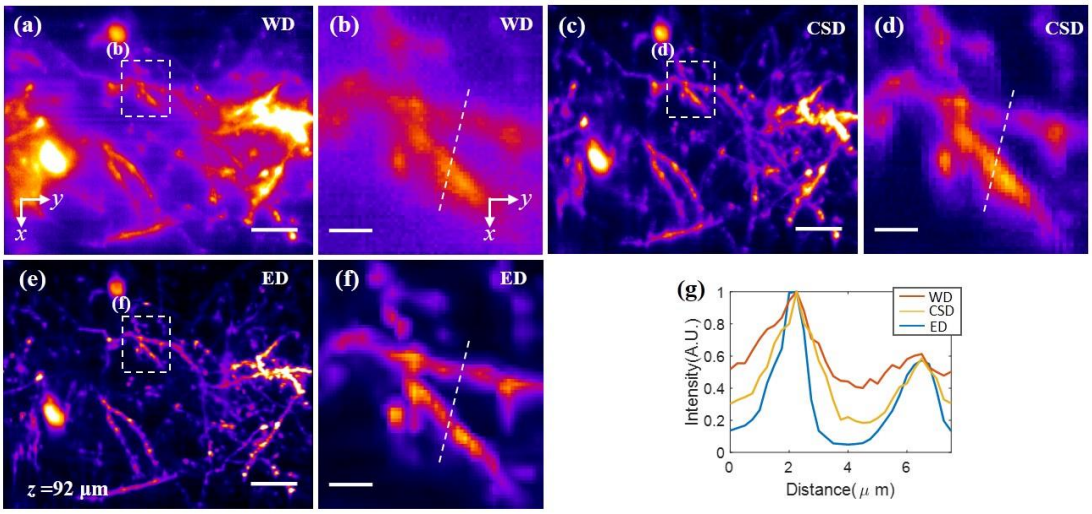

Fig. 2. Performance comparison of *in vivo* imaging among different imaging techniques. Maximum intensity projection of neurons (80–92 μm under the dura) recorded with (a) wide-field detection (WD), (c) confocal slit detection (CSD), and (e) extended detection (ED). Zoom-in views of the regions labeled in (a, c, e) are shown in (b, d, f), respectively. (g) Intensity profiles along the indicated lines in (b)(d)(f). Scale bars in (a, c, e) are 10 μm, in (d, e, f) are 3 μm.

We then demonstrate the effectiveness of ED-LTFM through *in vivo* deep imaging of neurons in Thy1-YFP mouse brains. In Fig. 2, we show the maximum intensity projection of neurons along the *z*-axis of a 13-μm-thick image stack. It can be seen that the dendrites are blur in WD due to the strong scattering, while CSD techniques help to eliminate the crosstalk induced by scattering along *y*-axis, and ED effectively eliminates the crosstalk along both *x*-axis and *y*-axis.

3. Conclusion

In summary, we have demonstrated the extended detection and computational reconstruction technique that could enhance the imaging depth in wide-field two-photon microscopy. We validated the performance improvement of ED-LTFM via *in vivo* imaging of mouse brains.

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