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Scanless volumetric imaging by selective access multifocal multiphoton microscopy: supplementary material

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1. LINE-SCAN TEMPORAL FOCUSING MICROSCOPY COMPARED TO POINT-SCANNING TWO-PHOTON MICROSCOPY

The fidelity of the line-scan TF sub-system for structural imaging was evaluated by comparing with the conversional two-photon point-scanning approach. The results demonstrate advantages and disadvantages of line-scan TF. Imaging speed of line-scan TF is faster than point-scanning method by one to two orders of magnitude. At the same time, line-scan TF has nearly the same 3D resolution with point-scanning method, as shown in Fig. S1b and Fig. S1d, unlike normal line-scan without TF. As a parallel excitation method, line-scan TF requires a sCMOS camera for detection instead of a photomultiplier tube (PMT) as in the point-scanning case. The advantage of TF with sCMOS detection is the higher imaging speed. However, the disadvantage of imaging with a camera is that it is more influenced by tissue scattering of emitted photons that is not a concern for point-scanning approach that integrates all the emission signal using a PMT. Therefore, it is clear from Fig. S1 that there is a significant increase in background due to the presence of bright objects in the field such as the bright soma. This problem is potentially can be solved by structure illumination background rejection algorithm. Since we are interested in targeting along dendrites, the scattering from soma doesn't seriously affect our results. Thus, the line-scan TF provided "latest updated map" for saMMM targeting and recording.

2. LONG TIME RECORDING FOR PHOTODAMAGE TEST

We targeted one neuron with 125 spots, 169 spots and 200 spots sequentially (Fig. S2a). The cell was packed at 200 spots targeting. In each case, we continuously recorded calcium signals from all spots for 10 min (Fig. S2b). The time interval between changing targeted spots is within 1 min. In each case, the average power per spot was about 0.8 mW as a constant parameter. We increased the total power for more targeted spots. During these 30 min recording, the neuron kept firing, while the firing rate dropped gradually. However, the cell was outside of the incubator without CO_2 supply, which also influenced the health of the cell. Fig. S2c shows the neuron was photobleached after the 30 min recording.

3. GL PSF WITH SCATTERING AND ABERRATION MEASURED IN BRAIN SLICE

We measured the GL PSF with mouse brian slice as the scattering media. The mouse brain slice is 50 μm thick with YFP labeled neurons in L2/3. Compared to the measurement with fluorescence beads, the GL PSF inside brain slice is very similar except the symmetry of the two lobes is a little distorted. However, the DoF of GL PSF inside brain slice is the same as without scattering, which is the most important for us. Also, the lateral size of

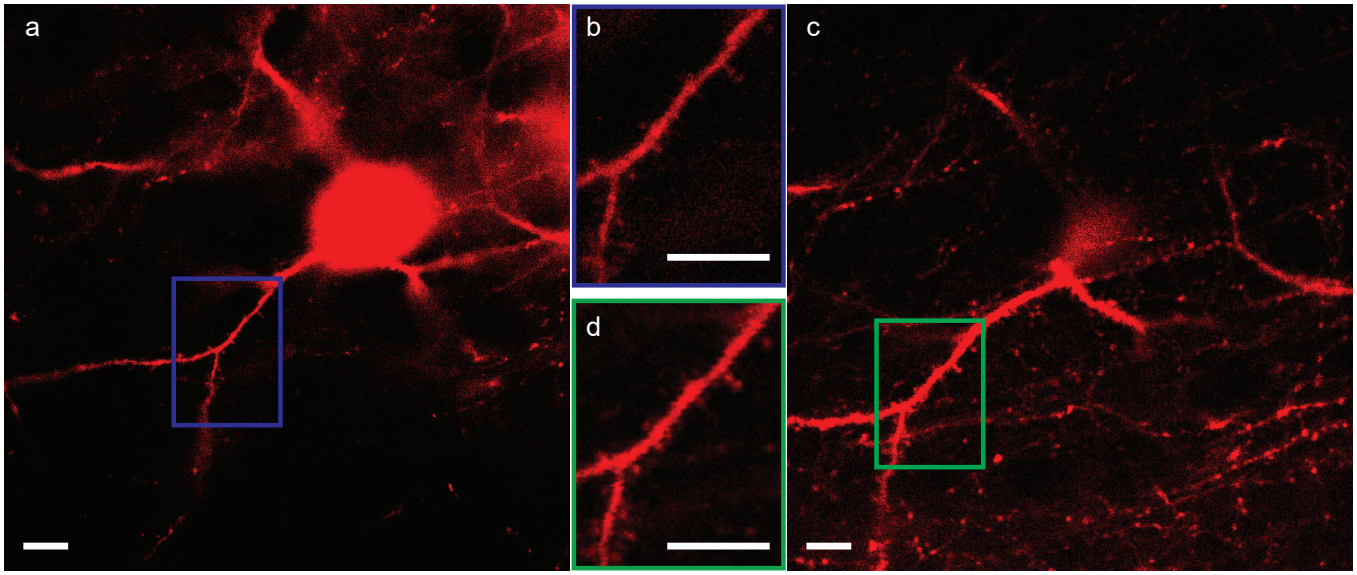

Fig. S1. The comparison of temporal focusing two-photon image and point-scanning two-photon image. (a) Temporal focusing two-photon image of a fixed neuron slice. (b) Zoom-in figure of the blue area in (a). (c) The same neuron imaged by point-scanning two-photon microscopy. (d) Zoom-in figure of the green area in (c). Scale bar (a-d), 10μm. All the spines on the dendrite in the selected region were shown clearly with nearly the same resolution and SNR.

the GL PSF keeps the same. Thus, GL PSF is robust enough to scattering for our application.

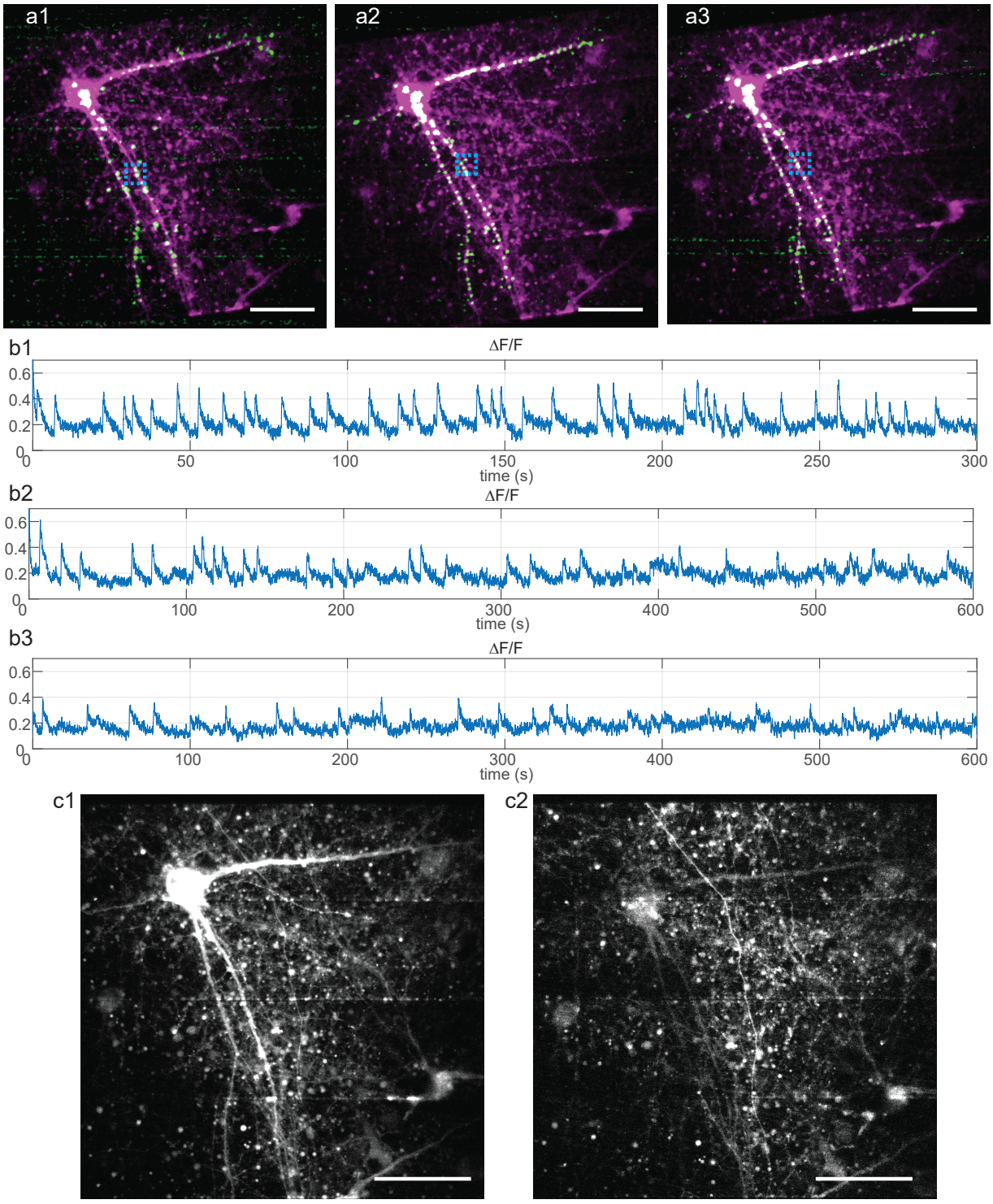

Fig. S2. Long time recording for photodamage test. (a) The neuron with 125 spots (a1), 169 spots (a2) and 200 spots (a3) targeting (green) overlapped with the line-scanning temporal focusing structure image (magenta). (b) Calcium signal from one targeted spot (dashed box in (a)) in each case for 10 min continuous recording. The time between b1, b2 and b3 is about 1 min to change the targeted spots and power. (c) Structure imaging of the neuron before (c1) and after (c2) the 30 min recording. Scale bar, 50 μm.

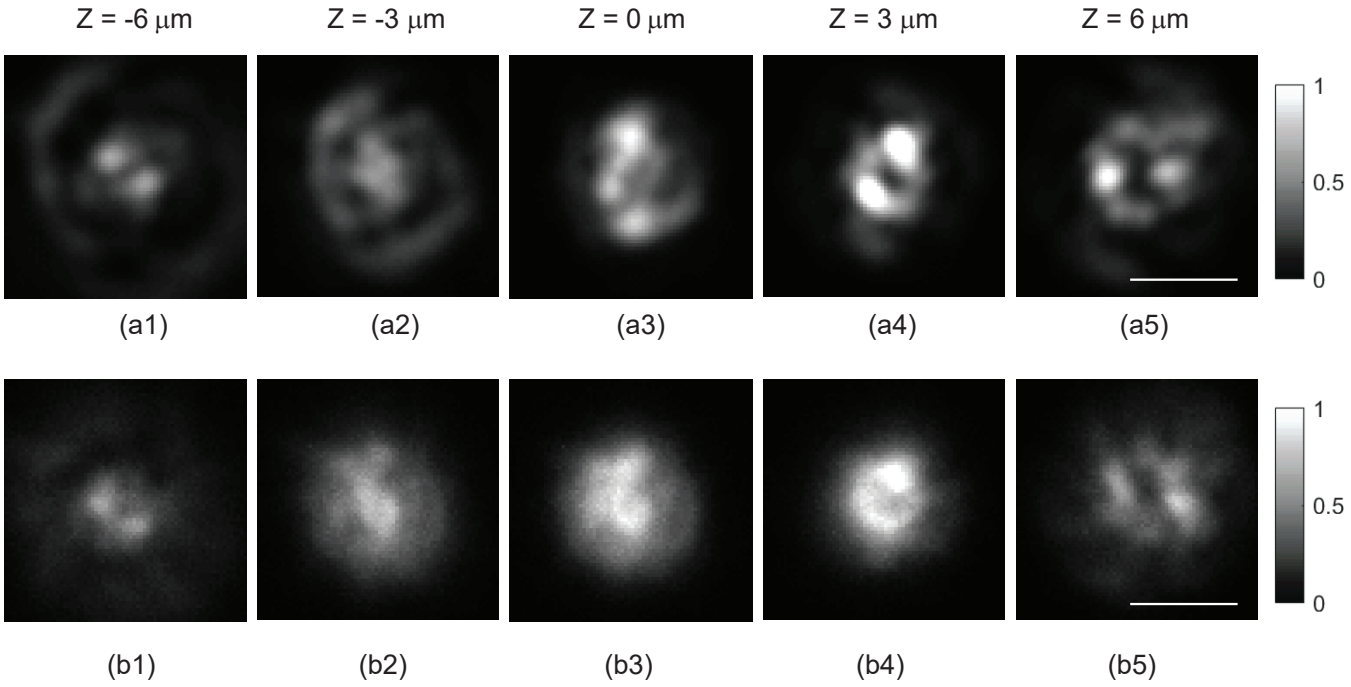

Fig. S3. The influence of scattering and scattering to GL PSF. a. GL PSF without scattering and aberration. a1-a5. GL PSF on different Z plane. b. GL PSF measured inside brain slice. b2-b6. GL PSF on different Z plane. Scale bar, 5 μm.