Fast optical sectioning obtained by structured illumination microscopy using a digital mirror device

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Abstract. High-throughput optical imaging is critical to obtain large-scale neural connectivity information of brain in neuroscience. Using a digital mirror device and a scientific complementary metal-oxide semiconductor camera, we report a significant speed improvement of structured illumination microscopy (SIM), which produces a maximum SIM net frame rate of 133 Hz. We perform three-dimensional (3-D) imaging of mouse slices at diffraction-limited resolution and demonstrate the fast 3-D imaging capability to a large sample with an imaging rate of 6.9 × 10^7 pixel/s of our system, an order of magnitude faster than previously reported. © The Authors. Published by SPIE under a Creative Commons Attribution 3.0 Unported License. Distribution or reproduction of this work in whole or in part requires full attribution of the original publication, including its DOI. [DOI: 10.1117/1.JBO.18.6.060503]

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Mapping neural connectivity of the whole brain is one of the key steps to understanding the brain in neuroscience. To map the neural networks of the entire brain, one is required to obtain sub-micron neural connection information on a centimeter scale. Recent progresses of micro-optical sectioning tomography (MOST) and fluorescence micro-optical sectioning tomography (fMOST) have achieved sub-micron voxel resolution imaging of the whole brain. But total data acquisition times still were 242 and 447 h, respectively, which was too slowly for neuroscience applications.

Structured illumination microscopy (SIM), which was first demonstrated by Neil et al., has optical sectioning ability comparable to confocal microscopy. In this technique, SIM projects a predefined high-frequency cosine illumination pattern on the specimen. Only in-focus information is modulated by this illumination fringe, owing to the rapid attenuation of high spatial frequency pattern with defocus. SIM acquires at least three images with different phase shifts to obtain an optical section.

The imaging speed of SIM depends on the process of shifting the cosine illumination pattern. Various methods of cosine fringe projection have been demonstrated in SIM systems, including mechanically moving grating, two beam interference illumination, micro-stripe array light-emitting diodes (LED), and liquid crystal spatial light modulators. Despite these efforts, an illumination pattern transition time of several tens of milliseconds still limits the net frame rate of SIM to a few hertz. A digital mirror device (DMD)-based SIM has been reported to achieve a cost-effective optical sectioning at an imaging speed of 1.6 × 10^7 pixel/s. However, because SIM needs three raw images to get a section image, the real net SIM sectioning speed had been reduced to approximately 5.3 × 10^6 pixel/s, similar to ones of MOST and fMOST. Relatively low imaging speed is a serious impediment to the high-throughput neuroanatomy application. In this study, by employing the fast modulation ability of DMD in binary mode and the fast imaging ability of scientific complementary metal-oxide semiconductor (CMOS) camera (sCMOS), we demonstrate a SIM image net rate of 6.9 × 10^7 pixel/s, an order of magnitude faster than existing high-throughput neural networks imaging methods.

We used a DMD in binary mode to make full use of the fast modulation ability of DMD. Patterns on the DMD plane can be defined as

$$S(x, y) = \begin{cases} 1, & \text{when mode}(x, 6^{-k}) < 3 \ast k, k = 1, 2, \ldots \text{others} \end{cases}$$

(1)

where x and y represent pixel coordinates on the DMD. Selecting different values of k can obtain various fringe periods.

By shifting the pixel values of the DMD to 2k and 4k, respectively, the phase shift steps of 2π/3 and 4π/3 on the specimen plane would then be executed, respectively. After acquiring three images, we can reconstruct the optical section image by

$$I_{s1} = [(I_0 - I_{2x/3})^2 + (I_{2x/3} - I_{4x/3})^2 + (I_0 - I_{4x/3})^2]^{1/2}.$$ 

(2)

Our experimental setup is shown in Fig. 1. A DMD (XD-ED01N, X-digit) was placed in a conjugate image plane of

![Fig. 1 Schematic of the optical setup. LLG, liquid light guide; CL, collimating lens; M, mirror; DMD, digital micro-mirror; TL, tube lens; EM, excit filter; DM, dichroic mirror; PZT, piezoelectric translational stage; OBJ, objective lens; and EM, emission filter.](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics on 06 Jan 2020)

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the excitation optical path. The DMD was a 1024 × 768 micro-
mirror array with a size of 13.68 × 13.68 μm. Excitation light
from a mercury lamp (X-cite Exacte, Lumen Dynamics) was
collimated and oriented toward the DMD. The specimen was
fixed on the XY stage (Aerotech), and axial scanning was
achieved by a piezoelectric translational stage (P-721, Physik
instrumente). Emission light from the sample was captured
by a 2048 × 2048 pixel sCMOS camera (ORCA-Flash4.0,
Hamamatsu), which was synchronized with the DMD pattern
transition.

To test the performance of our system, we used fluorescence
beads with a diameter of 170 nm (505/515, Molecular Probes)
to measure imaging resolution. By setting the pattern period
on the DMD plane to 164.16 μm (13.68 μm × 12), a three-
dimensional (3-D) stack of the fluorescence beads was acquired
with a z step of 250 nm using a 40× water-immersion objective
(0.8 NA, LUMPLFLN 40XW, Olympus). Figure 2 shows
that the measured lateral and axial FWHM values from a selected
bead were 0.39 and 2.15 μm, respectively.

Figure 3(a) and 3(b) shows the conventional wide field image
and corresponding SIM optical section image of fluorescence
microspheres (10-μm diameter), demonstrating the optical sectioning
ability of SIM. The image was captured by a 20× water-
immersion objective (1.0 NA, XLUMPLFLN 20XW, Olympus),
and excited light exposure power on the sample was measured to
be 2.9 mW. SIM suppressed most of the out-of-focus blurring
information. To demonstrate that it was capable of imaging the
dynamic process, we developed an optical flow phantom model
that consists of 1-mm diameter glass tubing and a micro-syringe
pump. By using a micro-syringe pump, we could control the
flow speed of fluorescent microspheres inside the glass tubing.
The illumination grating is oriented perpendicular to the flow.
Figure 3(c–3g) shows SIM images that were taken under different
frame rates for five different moving fluorescent microspheres.
The imaging pixel format of the sCMOS was set to
2048 × 192, and 2 × 2 binning by camera was used to enhance
the signal. These microspheres could be tracked and the mean
flowing rate was calculated to be 46.2 ± 2.9 μm/s. When the
net frame rate of SIM was 133 Hz, there were no movement
artifacts in the SIM section image. Once the frame rate
decreased to 50 Hz, movement artifacts began to appear in the
section image. The artifacts became increasingly severe as the
frame rate decreased further, until the imaged fluorescence
microsphere completely separated into three consecutive struc-
tured light modulated raw images. The reconstructed SIM
section image would look like a superposition of three micro-
spheres [Fig. 3(g)].

Finally, we demonstrate that the video-rate SIM is a fast im-
ageing method which can be applied to large tissue imaging.
the XY stage moves the sample under the objective to extend the

![Fig. 2] Normalized lateral (a) and axial (b) responses of the system.

Fig. 3 Images of fluorescent microspheres. (a) Conventional wide-field
image of motionless fluorescent microspheres; (b) corresponding opti-

cally sectioned image captured by structured illumination microscopy
(SIM); (c to g) structured illumination sectioned images of moving
microspheres taken at different SIM imaging frame rates: faster recording
rate (c) shows clear shape of the microsphere, while slower recording
rate (d to g) shows deformed shape. Scale bars: 40 μm (a, b) and
15 μm (c to g), respectively.

The lateral field of view of each mosaic image was 310 × 290 μm. The refresh rate of the DMD was set at 50 Hz and each
60-μm thick mosaic 3-D volume was acquired in approximately
3.5 s. It took approximately 1 h to acquire the whole coronal 3-D
data volume by 40x objective. The depth discrimination ability
of SIM makes block-face imaging available for the resin-
embedded whole brain samples. A whole mouse was approx-
\text{approximately} 1 \times 1 \times 1 \text{ cm}^3, and the imaging field of view was 620 \times 580 \text{ μm}^2 when using a 20x objective. 17 \times 18 mosaics were
needed to cover a coronal section, and each 60-μm thick 3-D
mosaic took 3.5 s to image. We could estimate that the imaging
time for a whole brain would be (3.5 s × 17 \times 18 + 60 s) ×
10,000/60 = 52.36 h (60 s for sectioning). It indicates that
whole brain data acquisition at sub-micron resolution would
require no more than three days.

The imaging speed of this video-rate SIM here is limited by the
exposure time and readout speed of the camera. The full
frame rate of sCMOS is limited to 50 Hz in external trigger
mode. Without any compromise in the field of view, 16.6 Hz is the fastest SIM net frame rate we can currently achieve. Further acceleration of the imaging process depends on the use of a brighter light source and a faster camera with a shorter readout time.

In conclusion, by taking full advantage of the high-speed binary modulation capability of the DMD and fast capture ability of sCMOS, we demonstrate a video-rate structured illumination microscope for fast optical sectioning. At present, the imaging throughput of video-rate SIM can reach $6.9 \times 10^7$ pixels/s ($2048 \times 2048$ at a 50 Hz imaging frame rate). Meanwhile, the system enables one to resolve the fine structures of neurons, such as spine in thick brain tissue. In combination with appropriate brain tissue preparation and mechanical sectioning methods, this video-rate SIM has the potential to map neuron connectivity information for entire brain in a relatively short time.

Acknowledgments

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