doi:10.1017/S1431927614003663
Microsc. Microanal. 20 (Suppl 3), 2014

Live Cell Imaging With Spatial Light Modulator-based Optical Sectioning Structured Illumination Microscopy

Zdeněk Švindrych[1], Pavel Křížek[1], Evgeny Smirnov[1], Martin Ovesný[1], Josef Borkovec[1] and Guy M. Hagen

[1.] Institute of Cellular Biology and Pathology, First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

Structured illumination microscopy (SIM) is a method in fluorescence microscopy which works by acquiring a set of images using widefield detection. Each image in the set is made with a different position of an illumination mask, but with no mask in the detection path [1]. Subsequent image processing is used to produce an optically sectioned image (OS-SIM) [2–4], or an image with resolution beyond the diffraction limit (super-resolution SIM or SR-SIM) [5,6].

Previous OS-SIM microscopes relied on diffraction gratings to create suitable illumination patterns. This often limits imaging speed, as the grating must be precisely shifted, and the position must be stable during image acquisition to prevent artifacts. Also, different gratings are required to achieve optimum performance with different objectives and/or excitation wavelengths. An optimized illumination system and grating frequency (the optimum spatial frequency in the sample plane is λ/NA) can achieve significantly thinner optical sections than confocal laser scanning microscopes (CLSMs) with pinholes set to 1 Airy unit [3]. At the same time an increase in lateral resolution (by a factor of ~1.5×) can be achieved.

To increase the flexibility, the optical sectioning performance, and the imaging speed of OS-SIM, we used a reflective ferroelectric liquid crystal-on-silicon (FLCOS) microdisplay to create the illumination patterns (type 3DM, Forth Dimension Displays, Scotland). Use of the microdisplay allows us to use any desired pattern for structured illumination, including arrays of lines, dots, or random patterns, and thus, to find the most suitable scanning pattern for a given sample. This flexibility allows us to tune the desired optical section thickness. Similar LCOS microdisplays have been used previously in SIM [3], and in programmable array microscopy (PAM) [7]. Both incoherent (LED) and coherent (laser) illumination can be used in OS-SIM where the illumination pattern spatial frequency is not higher than λ/NA.

For processing the data we used a scaled subtraction method based on a calibrated camera as described previously [3]. Briefly, we illuminated a thin fluorescent layer with a calibration pattern. This allows us to determine a geometrical model describing the transformation between the microdisplay pixels and the camera sensor pixels and thus to create a digital illumination mask in the camera image. The geometrical model allows us to use arbitrary illumination patterns, to determine the exact pattern position in the camera image even with sparse samples, and to separate the in-focus and out-of-focus contribution using a simple linear procedure.

We used the OS-SIM approach with an LCOS-based illumination system for high resolution live-cell imaging of Lysosome-Associated Membrane Protein 1-GFP (LAMP1-GFP) in transfected U2-OS cells (Figure 1). We also imaged RNA polymerase 1-GFP (subunit RPA43; RPA43-GFP) in fixed HT-1080 cells (Figure 2). We used a line pattern with seven phase shifts and two orientations [9].

References:

[1] R. Heintzmann, in *Handb. Biol. Confoeal Mierosc,* edited by J. B. Pawley, 3rd ed. (Springer, New York, 2006), pp. 265-279. [2] R.
Heintzmann, Appl. Opt. **45** (2006), p. 5037.
[3] P. Křížek, I. Raška, and G. M. Hagen, Opt. Express **20** (2012), p. 24585.
[4] M. A. A. Neil, R. Juškaitis, and T. Wilson, Opt. Lett. **22** (1997), p. 1905.
[5] R. Heintzmann and C. Cremer, Proc. SPIE **3568** (1998), p. 185.
[6] M. G. L. Gustafsson, J. Microsc. **198** (2000), p. 82.
[7] G. M. Hagen et al., Microsc. Res. Tech. **72** (2009), p. 431.
[8] G. M. Lee, a Ishihara, and K. a Jacobson, Proc. Natl. Acad. Sci. U. S. A. **88** (1991), p. 6274.
[9] This work was supported by the Czech Science Foundation [P304/09/1047, P205/12/P392, P302/12/G157, 14-15272P]; by Charles University in Prague [Prvouk/1LF/1, UNCE 204022]; and by European Union Funds for Regional Development [OPPK CZ.2.16/3.1.00/24010].

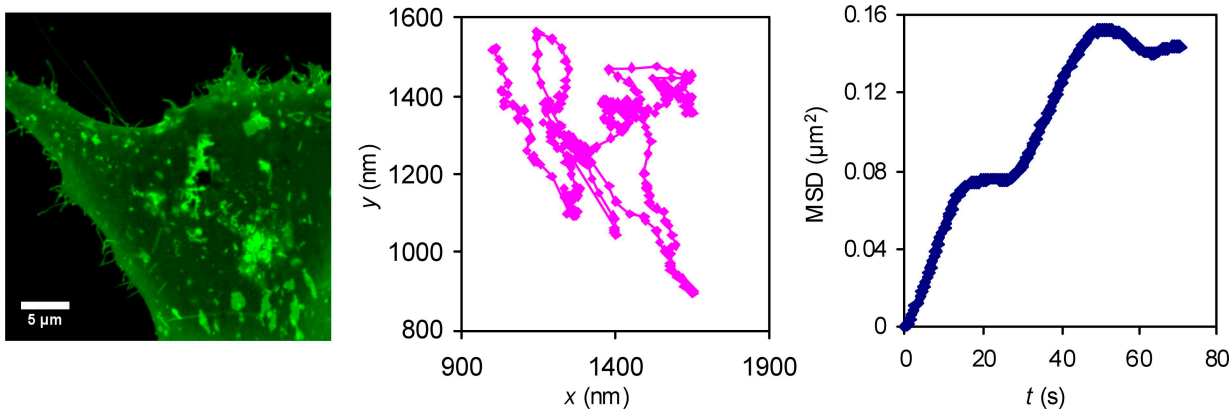

Figure 1. (left) LAMP1-GFP expressed in a U2-OS cell and imaged with OS-SIM. Single optical section. (center) trajectory of a single lysosome, 313 frames. (right) mean square displacement (MSD) according to [8], time between frames = 0.48 s, 470 nm LED illumination.

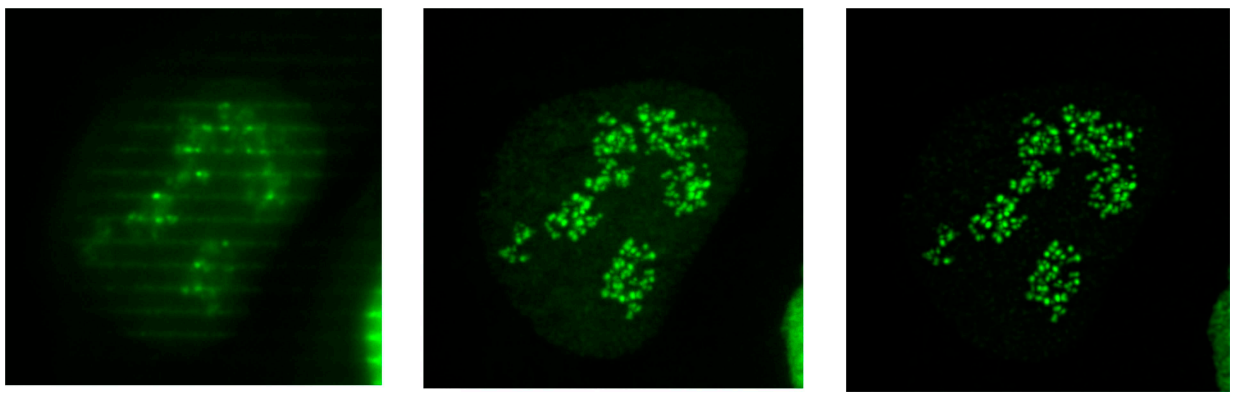

Figure 2. RPA43-GFP expressed in a HT-1080 cell and imaged with OS-SIM. (left) single SI frame. (center) maximum projection of processed dataset. (right) after nonlinear deconvolution. Acquisition time = 0.5 s per frame, 473 nm laser illumination.

https://doi.org/10.1017/S1431927614003663 Published online by Cambridge University Press