Hexagonal lattice-SIM microscopy with three beams generated by a spatial light modulator

Hai Gong[#], Wenjun Guo[#], Mark A.A. Neil[*]

Photonics Group, Department of Physics, Imperial College London, United Kingdom
[#]Authors contributed equally to this work.
[*]E-mail: mark.neil@imperial.ac.uk

Key words: super-resolution, structured illumination microscopy, spatial light modulator, azimuthal polarisation, image transformation, image reconstruction, light sheet microscopy

Super-resolution structured illumination microscopy (SR-SIM) stands as an attractive approach for providing not only optical resolutions beyond the classical diffraction limit but also an imaging speed potentially suitable for high-throughput assays, while in the meantime demanding less excitation power as compared with other super-resolution microscopy techniques and therefore inducing less photodamage [1]. In recent years, various efforts have been made to improve the performance of SR-SIM-based techniques, among which is the use of a 2-dimentional (2D) grid illumination pattern (as opposed to the conventional 1D sinusoidal line pattern) that eliminates the need to rotate the illumination pattern for an isotropic improvement in the lateral image resolution [2, 3].

Here we present a SR-SIM implementation, hexSIM, which employed 3 beams diffracted from a ferroelectric liquid crystal on silicon (FLCoS)-based spatial light modulator (SLM) to generate a hexagonal-patterned illumination at the sample plane. The 3 diffracted beams were set to be azimuthally polarised relative to the optical axis by a "pizza" polariser (comprising 3 segmented linear polarisers, placed at a plane conjugated to the pupil plane together with a metallic mask to block other unwanted diffracted light from the SLM. The 3 beams were delivered to 3 radially symmetrical positions close to the edge of the pupil plane of the objective and then focused to the sample plane to form the hexagonal pattern. With such a setup, only 7 images were required for reconstructing a super-resolved SIM image, which can be realised simply by shifting the generating pattern on the SLM along a single direction. We also demonstrated that the 7-image hexSIM dataset can be readily transformed into the convention 9-image 2-beam SIM dataset via a simple matrix multiplication, and therefore the reconstruction process can be performed using software packages that are compatible with 2-beam SIM, e.g. fairSIM [4]. Our preliminary results confirmed that the theoretically maximum 1.87-fold lateral resolution improvement [5] was achievable for both 100-nm fluorescent bead samples and fixed BPAE cell samples.

We envisage that this 2D hexSIM modality is highly suitable for combination with light sheet techniques for optical sectioning with a higher signal-to-noise ratio, and that the combination could be integrated into a microscope-on-chip system for high-throughput applications [6].

[1] L. Schermelleh et al., Nat. Cell Biol. 21, 72–84 (2019).
[2] M. Schropp, R. UHL, J Microsc. 256, 23–36 (2014).
[3] M. Schropp, C. Seebacher, R. Uhl, Photonics. 4, 33 (2017).
[4] M. Müller, V. Mönkemöller, S. Hennig, W. Hübner, T. Huser, Nat Commun. 7, 10980 (2016).
[5] E. A. Ingerman, R. A. London, R. Heintzmann, M. G. L. Gustafsson, J Microsc. 273, 3–25 (2019).
[6] P. Paiè, R. M. Vázquez, R. Osellame, F. Bragheri, A. Bassi, Cytometry A. 93, 987–996 (2018).