HIGH-SPEED STRUCTURED ILLUMINATION MICROSCOPY IN THICK SAMPLES

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Structured illumination microscopy (SIM) [1] is amongst the fastest, least invasive of the super-resolution imaging techniques which have been developed in recent years. However the need to acquire multiple raw images for each super-resolution image increases both image acquisition time and overall specimen light exposure, limiting the effectiveness of SIM for imaging rapidly changing dynamic systems or samples prone to adverse phototoxic reactions and photobleaching. This is particularly acute for thick specimens imaged using 3D SIM which requires a focal stack of image planes.

We present details of a high-speed SIM system with emphasis on how the use of a spatial light modulator and fast electro-optic modulator allow rapid generation of illumination patterns leading to imaging frame rates in excess of 14 Hz [2]. We show how an optically sectioned super-resolution image can be reconstructed by attenuating individual SIM information passbands to remove out-of-focus light [3]. This method is applied to both 2D and 3D SIM datasets and we consider the relative merits of single-plane optically sectioned super-resolution SIM compared to traditional 2D and 3D SIM reconstructions in terms of imaging speed, spatial resolution and signal-to-noise-ratio.

[1] M. G. L. Gustafsson, "Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy," *J. Microsc-Oxford*, 198, 82-87 (2000).

[2] M. Shaw, L. Zajiczek & K. O'Holleran, "High speed structured illumination microscopy in optically thick samples." Manuscript under review (2015).

[3] K. O'Holleran & M. Shaw, "Optimized approaches for optical sectioning and resolution enhancement in 2D structured illumination microscopy", *Biomed. Opt. Express*, 5, 2580-2590 (2014).