STRUCTURED ILLUMINATION SCANNING MICROSCOPY: TACKLING OBJECT SPATIO-TEMPORAL INSTABILITIES

vandeVen, M.[1]; Slenders, E.[1]; Bovė[1,2] , H.; Ameloot, M.[1]

[1]Biomedical Research Institute, Hasselt University, Agoralaan Bldg C, 3590 Diepenbeek, Belgium

[2]KU Leuven, Centre for Surface Chemistry and Catalysis, Celestijnenlaan 200F, 3001 Leuven, Belgium

E-mail : martin.vandeven@uhasselt.be

KEY WORDS: Motion artifacts, Structured illumination, Non-Linear Optical microscopy, super-resolution imaging, simulations.

Structured illumination microscopy (SIM) allows the observation of enhanced 2D and 3D "super-resolved" detail in imaged cells and tissues: superimpose an intensity modulation pattern while point-scanning the fixed static object. A SIM reconstruction protocol for weakly fluorescent low Signal-To-Noise Ratio (SNR) image stacks has been proposed [1]. Photobleaching should be reduced as much as possible [2]. Motion artifacts during and in between scanned image frames should be detected and locally marked for living cells [3].
Stage mechanical instabilities due to the thermal history of a standard Zeiss 510 NLO Axiovert 200M microscope with thermostated enclosure were observed for up to several hours upon resetting from 37° C to 22° C room temperature. Long air-condition cycling periods may further aggravate the mechanical instability. Rather random lateral-plane object drift over several pixels (<~ 1 μm) was observed during the standard fastest 3 angle, 3 phase-step SIM approach. Artifacts were most noticeable when comparing object positions *between* successive frame scans. Mechanical instability *during* a frame scan may well go unnoticed hiding in the observed local cellular structure. Test targets with rather simple spatial information patterns are then preferred. Through in-silico simulations the nefarious effects of mechanical instabilities on SIM cell image reconstruction were evaluated. Parameter space variables included a varying amount of background signal, low modulation depth and poor SNR. Typical Tikhonov-Miller (TM, generalized Wiener) filtering [4] and total variation (TV) denoising [1] were compared-. "best filter choice" depends on the imaged object spatial information structure.

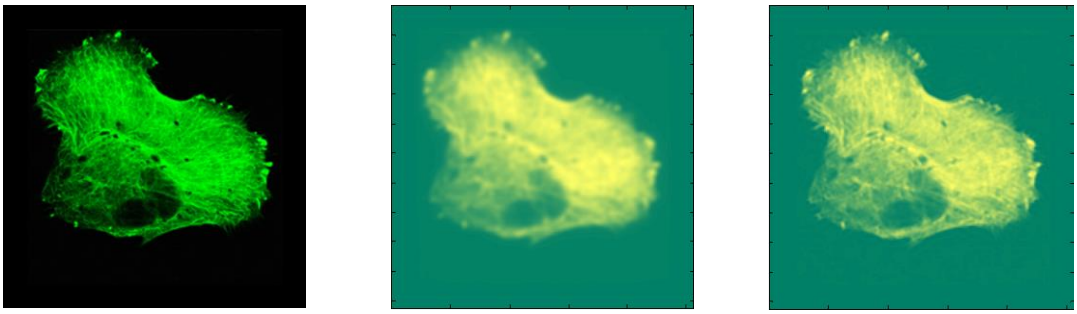

Figure 1: Tubulin network of fixed human lung fibroblast [5] (A); SIM simulation input: blurred shifted cell & noise added (B); Reconstructed filtered and denoised image (C). Size 1024 pixels (115 μm).

[1] Chu,K.; McMillan, P.J.; Smith, Z.J.; *et al.*, "Image reconstruction for structured-illumination microscopy with low signal level", Opt. Express, **22**, 8687-8702 (2014).

[2] Schaefer, L.H.; Schuster, D.; Scaffer, J. "*Structured illumination microscopy: artefact analysis and reduction utilizing a parameter optimization approach",* J. Microsc. 216, 165-174, 2004.

[3] Förster, R.; Wicker, K.; Jost, A.; Heintzmann, R. "Motion artefact detection in structured illumination microscopy for live cell imaging", Opt. Express, **24**, 22121-22134 (2016).

[4] Righolt, C.H.; Slotman, J.A.; Young, I.T.; *et al.*, "Image filtering in structured illumination microscopy using the Lukosz bound", *Opt. Express*, **21**, 24431-24451 (2013).

[5] Bovė, H.; Steuwe, C.; Fron, E.; et al. "*Biocompatible Label-Free Detection of Carbon Black Particles by Femtosecond Pulsed Laser Microscopy",* NanoLett., 16, 2899-3408, 2016.