IMAGE PROCESSING IN SUPER-RESOLUTION TWO-PHOTON POINT-SCANNING STRUCTURED ILLUMINATION MICROSCOPY

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Fluorescence microscopy with a lateral spatial resolution beyond the diffraction limit, i.e. super-resolution microscopy, is of great interest in cell biology. Unlike other super-resolution modalities, structured illumination microscopy (SIM) is fast and does not require a high illumination power. Furthermore, SIM can be made compatible with two-photon fluorescence by point scanning over the sample object with a focused pulsed laser while temporally modulating the excitation intensity, a crucial step for *in vivo* super-resolution microscopy [1].

SIM imaging of living cells is sensitive to motion artifacts [2]. We found lateral mechanical instabilities over 1 to 5 pixels (0.15 – 0.75 µm) in a Zeiss LSM 510 Axiovert 200M microscope with thermostated enclosure. We simulated the effect of sample stage drift on the SIM reconstruction with grainy, low signal-to-noise ratio fluorescence line patterns. The influence of the illumination modulation depth and the background signal was studied. We compared Tikhonov-Miller (TM, generalized Wiener) filtering [3] and total variation (TV) denoising [4] and show that the "best filter choice" for reconstruction depends on the imaged object spatial information structure.

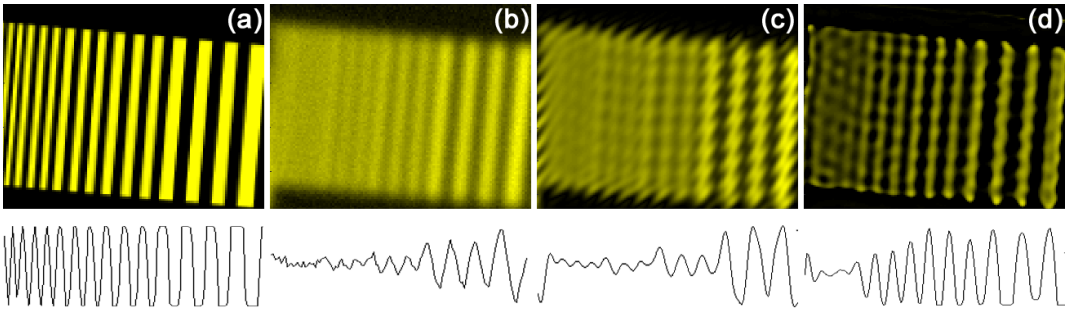

Figure 1: (a) Simulation of a line patterned object, (b) blurred image of the object with Gaussian white noise added to simulate the imaging process, (c) reconstructed image using SIM analysis with TM/generalized Wiener filtering and (d) TV denoising. All images are 320 x 250 pixels. The line plots show the corresponding profile in the images over a horizontal line. A stage drift of 3 pixels horizontally and 3 pixels vertically was assumed between every two scans. A SIM pattern periodicity of 6 pixels was taken, corresponding to the PSF $1/e^2$ intensity value that was chosen. The SIM reconstruction in this figure was done for 1 angle (45°) and 3 phase steps (0°, 120°, 240°).

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