ASSEMBLING THE SPECTRUM OF 3D STRUCTURED ILLUMINATION MICROSCOPY IMAGES IN THE EVENT OF VISUAL ARTIFACTS

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Structured Illumination Microscopy (SIM) is a powerful technique that enables to double the theoretical resolution of a conventional fluorescent microscope. 3D SIM is a development of the 2D SIM method that increases not only the resolution but also optical sectioning[1]. To reconstruct 3D SIM image stacks, we need to carefully monitor the parameters of the system, and to reach the optimum double resolution, we need to deconvolve the signal from the microscope point response (PSF). Here we claim that not including the wide-field image in the final reconstructed 3D SIM spectrum helps increasing optical sectioning and reducing deconvolution artifacts with only limited impact on the signal to noise ratio.

DECONVOLVING THE SPECTRUM IN 3D SIM MICROSCOPY

Due to sample depth and photo-bleaching, 3D SIM produces only limited z stacks and deconvolution in this direction may induces some strong edges artifacts (Figure 1). An additional limit to deconvolution efficiency are spherical aberrations which can severely impact SIM acquisition[2]. We show that it is possible to limit deconvolution artifact when assembling the 3D SIM spectrum. We also detail a robust estimator to compute the contrast changes between the extracted components of 3D SIM. Our methods provide very stable final reconstructions from one sample to another with few computational efforts, leading to higher reliability on 3D SIM as a quantitative technique.

Figure 1
Left: Deconvolution artifact due to mismatch between the data and theoretical PSF.
Right: Reduction of the artifact by not including the wide-field image in the reconstructed spectrum.