Structured Illumination Microscopy: Improved Spatial Resolution using Regularized Inverse Filtering

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Structured illumination microscopy can provide enhanced resolution over both widefield and confocal microscopy. Practical applications often use a transmission grating in which case the OTF's missing cone becomes partially filled and the lateral region of support widened in the direction of the grating, more or less, depending on the modulation frequency. In these cases, the resolution enhancement is moderate compared to the theoretical limit and as shown in Gustafsson's work [1]. Common deconvolution techniques are known to be an inverse ill-posed problem, especially for widefield microscopy where the OTF approaches zero inside the missing cone. In structured illumination microscopy however, the problem can be modeled in such a way that singularities can be avoided where the spectral overlap occurs due to modulation. With some a-priori information about the grating, additional assumptions such as positivity are no longer required for high quality restorations. Therefore, the method presented consists of a simple modified regularized inverse filter. While still ill-posed, results with low spatial frequency gratings show substantially finer lateral and axial detail than achieved by a high end statistical restoration algorithm such as maximum likelihood restoration on widefield images.

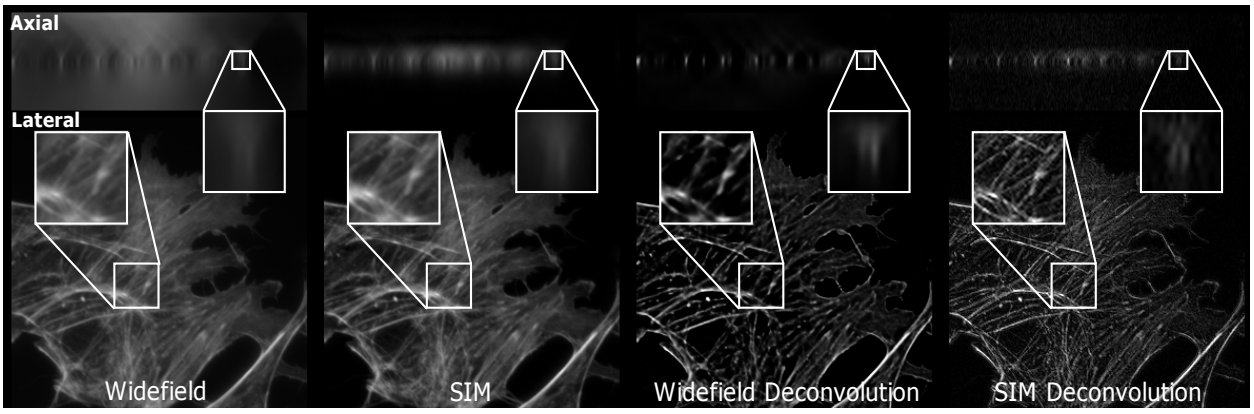

Figure 1: Actin micro tubule filaments, acquired with Zeiss Axio Imager™ Z1 (Objective: Plan Apo 20x/0.75, ApoTome™ slider with a grating frequency of 113 lines/mm in object space, fluorescence wavelength: 509nm). The z-stack image consisted of 40 slices with 9 phase shifts each. Images from left to right: Widefield; Sectioned; Widefield deconvolved (constrained iterative maximum likelihood method using conjugate gradients with 40 iterations) and SIM deconvolved.

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