Artifacts in structured illumination microscopy: Analysis and reduction, utilizing a parameter optimization approach

L. H. Schaefer[†], D. Schuster[‡] & J. Schaffer[*]
[†]**Advanced Imaging Methodology Consultation, Kitchener, Ontario, Canada, N2P 2A2,**
E-Mail: lschafer@golden.net,
[‡]**Department of Computer Science and Mathematics, University of Applied Sciences,**
D-93025 Regensburg, Germany,
[*]**Carl Zeiss Göttingen, D-37081 Göttingen, Germany**

Key words: Structured illumination microscopy, least-squares solution, merit function.

Practical applications of structured illumination microscopy can suffer from various artifacts, which result from imprecise instrumental hardware and bleaching properties of the sample. These artifacts become observable as residual stripe patterns originating from the illumination grating. We investigated some significant causes of these artifacts and developed a correction approach that can be applied to images after acquisition. Most of these artifacts can be attributed to fluctuations in illumination and detection intensities during acquisition. Analyzing the impact of associated changes in the frequency domain of the reconstructed image, we have developed a model-based method utilizing parameter optimization by minimizing a merit function. To apply these parameters and efficiently reconstruct the optically sectioned image a generic least squares method is used. This enables taking images at arbitrary lateral grating shifts. With more images taken, the signal to noise ratio will improve. Furthermore, for applications in fluorescence structured illumination microscopy we developed a spatially adaptive bleaching correction method (SABC). It takes into account, that bleaching rates of different structures in the sample may vary locally. The correction algorithm appears to be reliable on noisy image data and produces satisfactory results in daily routine laboratory work [1].
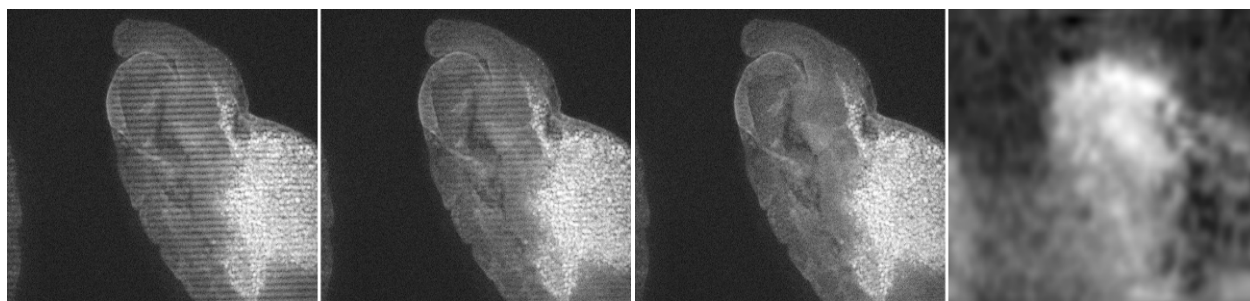
Figure 1: From left to right: Uncorrected; globally corrected; corrected with SABC and normalized array of correction coefficients for one phase of SABC. Image (Zeiss Axiovert™, Plan Apo 20x0.75 with Apotome™ slider using a 17.5 lines/mm grating): Developing drosophila embryo, about 9-10 hrs old, stained primary with ftz-Gal4 + UAS-actin GFP and secondary with α-armadillo + goat α-mouse FITC, courtesy of Dr. B. Reed, Toronto Hospital for Sick Children. Including the auto fluorescence in the yolk spheres, there are three distinct fluorophores present. With different rates of bleaching, a global correction appears less efficient than SABC.

[1] Schaefer, L.H., Schuster, D. and Schaffer, J. "Structured illumination microscopy: Artifact analysis and reduction utilizing a parameter optimization approach", *Journal of Microscopy,* (submitted, under review).