A ROBUST RECONSTRUCTION AND PATTERN CALIBRATION FRAMEWORK FOR STRUCTURED ILLUMINATION MICROSCOPY

Ning Chu, Roland Nussbaumer, Laurène Donati, Denis Fortun, Daniel Sage and Michael Unser
Biomedical Imaging Group, École Polytechnique Fédérale de Lausanne (EPFL)
EPFL/STI/IMT/LIB, Station 17, CH-1015 Lausanne, Switzerland
Email: laurene.donati@epfl.ch

KEY WORDS: *structured illumination microscopy (SIM), image reconstruction, sparsity, regularization, pattern calibration, fluorescent imaging.*

Structured illumination microscopy (SIM) is an effective and widely used method for producing high-resolution fluorescence micrographs. This imaging technique can reach up to twice the lateral resolution of conventional wide-field microscopy [1]. In SIM, the sample is imaged with varying configurations of an illumination pattern and a high-resolution image can be reconstructed from the collected data. The quality of the reconstructed SIM image is not only dependent upon the type of reconstruction method used, but also upon the correct setup of the illumination pattern. By taking advantage of recent advances in mathematical imaging and sparse signal recovery [2,3], we have designed a fast iterative algorithm that imposes sparsity constraints on the Hessian of the image. By doing so, we are able to outperform the current linear reconstruction methods (multichannel Wiener filter), while avoiding the staircase artifacts of total variation regularization.

SIM image reconstruction is also highly sensitive to imperfections of the illumination pattern and its performance depends crucially on the accurate calibration of the pattern parameters [4,5]. We are reporting experiments that document the influence of calibration errors on image quality and spatial resolution. To minimize these effects, we are also proposing a robust and rapid calibration procedure that estimates the phase shifts, angle rotations and pattern frequency merely from the observed wide-field fluorescent images.

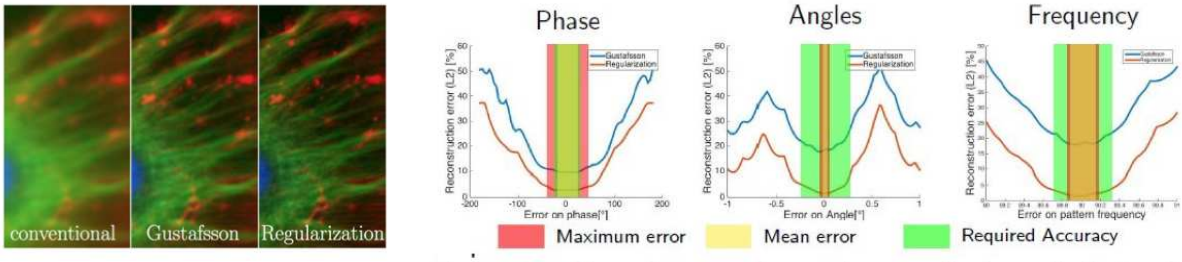

Figure: **(Left)** Illustration of the high-resolution reconstruction of biological data achieved by regularization algorithms (right frame). **(Right)** Performance of the proposed pattern calibration technique for various illumination pattern parameters.

[1] M.G.L. Gustafsson, Surpassing the Lateral Resolution Limit by a Factor of Two using Structured Illumination Microscopy, *Journal of Microscopy,* 198 (2000), pp. 82-87.
[2] E. Bostan, U.S. Kamilov, M. Nilchian, M. Unser, Sparse Stochastic Processes and Discretization of Linear Inverse Problems, *IEEE Trans. Image Processing*, 22 (2013), pp. 2699-2710.
[3] S. Lefkimmiatis, J.P. Ward, M. Unser, Hessian Schatten-Norm Regularization for Linear Inverse Problems, *IEEE Trans. Image Processing*, 22 (2013), no. 5, pp. 1873-1888.
[4] S.A. Shroff, J.R. Fienup, D. R.Williams, Phase-shift Estimation in Sinusoidally Illuminated Images for Lateral Superresolution. *Journal of the Optical Society,* 26 (2009), pp. 413- 424.
[5] K. Wicker, R. Heintzmann, Phase Optimisation for Structured Illumination Microscopy, *Optics Express,* 21 (2013), pp. 2032-2049.