FLEXIBLE 3D STRUCTURED ILLUMINATION WITH REDUCED DATA ACQUISITION BY MEANS OF A WOLLASTON PRISM

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Non-uniform illumination is a well-known strategy in microscopy that allows superesolution and optical sectioning after digital post processing of a set of images obtained on a conventional microscope. In particular, three-dimensional (3D) structured illumination microscopy (SIM) uses a set of illumination patterns that, in the simplest case, are one-dimensional periodic functions in transverse planes with an axial periodic envelope. Recently, an incoherent periodic 3D SIM setup has been proposed by using a Wollaston prism illuminated from several incoherent equidistant slits [1,2]. The 3D illumination pattern generated with this 3D SIM approach provides lateral and axial periodic variations, with frequencies that can be independently and continuously tuned, by simply axially shifting the position of the Wollaston prism and/or changing the slit separation. Also, the way in which the incoherent field is projected into the sample space provides an improved contrast of the illumination pattern respect to conventional SIM schemes. In this contribution we present a new design of the optical layout of the setup that optimizes the illumination pattern respect to its visibility, lateral field of view and brightness. Due to the particular form of the 3D illumination pattern generated with our proposal, only 3 images per orientation of the illumination are required for the reconstruction as opposed to the 5 images required by traditional SIM [3], resulting in a 40% reduction of data acquisition for the 3D restoration of the sample.

On the other hand, computational methods are an integral part of the imaging scheme in SIM. In a previous work [4], we developed a 3D model-based (3D-MB) iterative approach for 3D restoration of 3D-SIM data. This method was shown to provide more accurate results in noisy simulation than the non-iterative standard 3D deconvolution approach [3]. In this contribution, we present, based on the 3D-MB framework, a new method that enforces positivity of the solution and investigate its performance when applied to data from the Wollaston prism-based 3D-SIM configuration. In our new 3D-MB method the positivity constraint is included through the reconstruction of an auxiliary function using a conjugate-gradient method as it was previously suggested in speckle SIM [5]. Our model-based restoration utilizes the accurate phase-shift values of the illumination pattern provided by our recent approach based on an iterative comparison of the peak intensity of the spectral components that appear in the reconstructed images from estimated phase-shifts [6].

References

[1] S. Bedoya, A. Doblas, G. Saavedra, and C. Preza, "Tunable structured illumination system based on a Wollaston prism," in *Imaging and Applied Optics 2018* OSA *Technical Digest*, paper ITu2B.3 (2018).

[2] A. Doblas, S. Bedoya, and C. Preza, "Wollaston prism-based structured illumination microscope with tunable frequency," *Appl. Opt.* **58**, B1-B8 (2019).

[3] M. G. L. Gustafsson et al., "Three-dimensional resolution doubling in wide-field fluorescence microscopy by structured illumination," *Biophys. J.* **94**(12), 4957-4970 (2008).

[4] H. Shabani, S. Labouesse, A. Sentenac and C. Preza, "Three-dimensional deconvolution based on axial-scanning model for structured illumination microscopy," in *Proc. 2019 IEEE 16th International Symposium on Biomedical Imaging (ISBI 2019)*, pp. 552-555 (2019). doi: 10.1109/ISBI.2019.8759445

[5] A. Negash et al., "Improving the axial and lateral resolution of three-dimensional fluorescence microscopy using random speckle illuminations," *J. Opt. Soc. Am. A* **33**(6), 1089-1094 (2016).

[6] J. Sola-Pikabea et al., "Fast and robust phase-shift estimation in two-dimensional structured illumination microscopy," *PlosOne* **14**, e0221254 (2019).

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