TUNABLE 3D STRUCTURED ILLUMINATION MICROSCOPE USING A FRESNEL BIPRISM: OTF DESIGN

Hasti Shabani[1], Ana Doblas[1], Genaro Saavedra[2,*], Chrysanthe Preza[1,+]

[1] Dept. of Electrical & Computer Eng., University of Memphis, Memphis, TN, USA.
[2]3D Imaging&Display Laboratory, Universitat de València, Burjassot, Spain.
e-mail: *genaro.saavedra@uv.es; +cpreza@memphis.edu

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Recently, a three-dimensional (3D) structured illumination microscope (SIM) with an incoherent illumination has been implemented using a Fresnel biprism illuminated from N incoherent equidistant slits [1]. Because the generated 3D structured illumination (SI) pattern presents simultaneous lateral and axial variations, with this 3D SIM approach there is a simultaneous improvement in the resolution limit and optical sectioning capability of the native imaging system.

Among the several advantages that this system presents over traditional 3D SIMs [2-4], the lateral (u_m) and axial (w_m) modulation frequencies of its 3D SI pattern can be controlled separately through the design of the geometrical configuration of the slits used to create the structured pattern. This separate tunability allows us to engineer the compact support of the synthetic optical transfer function (OTF) in our 3D SIM system rendering it suitable for specific biological imaging needs [5]. Fig. 1 shows the relationship between the 3D SI pattern and the associated synthetic OTF, which is the sum of the conventional widefield fluorescence microscope (WFM) OTF and two new components, each composed of the sum of N axially-shifted replicas of the conventional OTF separated by $2w_m$, and shifted laterally by $\pm u_m$. The axial locations of the conventional OTF replicas in the synthetic OTF are given by the Fourier transform of the 3D SI pattern (Fig. 1a). Fig. 1b shows that both the lateral and axial OTF compact support (white dashed line) have been extended compared to the compact support of the WFM OTF (black dashed line). Note that the lateral and axial resolution limit (pink arrows in Fig. 1b) have been increased by a factor of $1+(u_m/u_c)$ and $1+[(N\text{-}1)(w_m/w_c)]$ where u_c and w_c are the native lateral and axial cutoff frequencies, respectively, of the WFM system. Regarding the OS capability, the upper portion of the missing cone is filled by $N/2$ axially-shifted OTF replicas, with axial separation between them equal to $2w_m$ (Fig. 1b).

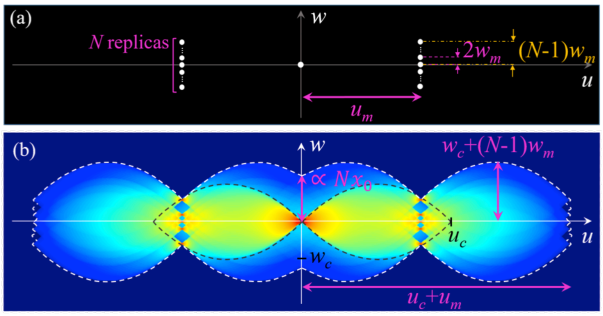

Fig. 1 Relation of the SI design and synthetic OTF of the tunable-SIM system: (a) *uw*-section of the 3D SI's Fourier transform; (b) *uw*-section of the synthetic OTF.

In this contribution, we evaluate the OTF's extent for different number of slits and separation to determine how they affect both the resolution improvement and optical sectioning capability of our system. Additionally, the performance of our 3D SIM system is compared with conventional SIM systems [2-4], including the impact of noise.

References

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