3D TUNABLE STRUCTURED ILLUMINATION MICROSCOPE USING A FRESNEL BIPRISM

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Recently, a three-dimensional (3D) structured illumination microscope (SIM) with an incoherent illumination has been implemented using a Fresnel biprism [1]. In this 3D-SIM system, the structured illumination (SI) pattern was generated by illuminating the biprism with the wavefront emerging from several equidistant spatially-incoherent slits [1]. This idea was first introduced by Saavedra *et al.* [2] with some initial proof-of-concept results. The main advantages of this system are: 1) its 3D structured pattern provides simultaneous optical sectioning (OS) and super resolution (SR) information overcoming the double-shot 2D-SIM approach we presented previously [3, 4]; 2) it is free from coherent noise; and 3) its modulation frequency is tunable up to the cutoff frequency of the imaging system, independently of both the objective lens and the wavelength used.

Fig. 1 shows simulated and experimental *xz*-section images of the 3D SI pattern generated by this SI setup using a different number of slits (*N*) in an open set-up implementation [1]. From this figure, it is evident that the higher the number of slits, the smaller the axial extent of the high-contrast regions of the illumination. Two key features of this pattern are that the fringes' contrast is kept invariant with *N* and it is not reduced through the illumination system. Also it is important to realize that the axial variation of the visibility of the fringes is periodic and its period depends on the slit separation and the lateral modulation frequency, which can be tuned by changing the axial position of the biprism with respect to the slits. Thus, by a proper tuning of this axial frequency one can achieve OS by any (or both) of the conventional mechanisms previously proposed in the SIM literature, namely, the axial confinement of the fringes (as in ApoTome©-based systems [5]) and the axial periodicity of the pattern (as in three-wave interference schemes [6]).

In this work, we derive a theoretical imaging model for our system and validate its behavior experimentally. Additionally, the performance of our 3D-SIM system is compared with the above-mentioned schemes [5, 6].

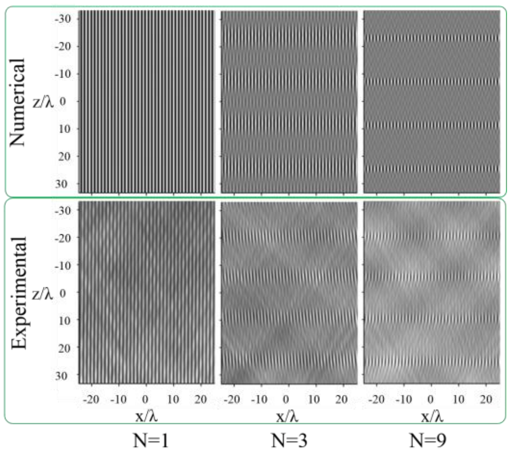

Fig. 1. Numerical and experimental *xz*-section images of the 3D SI pattern generated using a different number of slits *N*. The experimental images are recorded at the image plane by axially scanning a flat mirror along the sample volume. The cutoff frequency ($u_c = 2NA/\lambda$) is 1.9 μm^{-1} with the emission wavelength at λ = 515 nm and the imaging lens' numerical aperture (NA= 0.5). The lateral modulation frequency used in simulation and experiment is approximately $0.75u_c$.

References

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