3D STRUCTURED ILLUMINATION MICROSCOPE WITH TUNABLE FREQUENCY AND REDUCED DATA ACQUISITION BASED ON 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 with engineered illumination. 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 two-dimensional SIM setup has been proposed by using a Wollaston prism illuminated from an incoherent slit [1,2]. In this contribution we present a new design for 3D SIM by extending the source irradiance distribution to several incoherent equidistant slits. The 3D illumination pattern generated with this 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. The optical layout of the setup optimizes the illumination pattern's visibility with respect to the achieved lateral field of view. Due to the particular form of the 3D illumination pattern generated with our proposed design, only 3 images per orientation of the illumination are required for the reconstruction as opposed to the 5 images required by traditional 3D SIM [3], resulting in a 40% reduction of data acquisition for the 3D restoration of the sample.

As computational methods are an integral part of the imaging scheme in SIM, we have been developing approaches to improve image restoration: an accurate method to compute phase-shift values in the structured illumination pattern, required for SIM-data processing [4]; and a 3D model-based (3D-MB) iterative approach for 3D restoration of 3D-SIM data [5, 6]. The 3D-MB method, shown to provide more accurate results than the non-iterative standard 3D deconvolution approach [3], is suitable for processing data from the proposed Wollaston-based 3D SIM system.

References

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