FLEXIBLE PRODUCTION OF LIGHT FRINGES IN STRUCTURED ILLUMINATION MICROSCOPY

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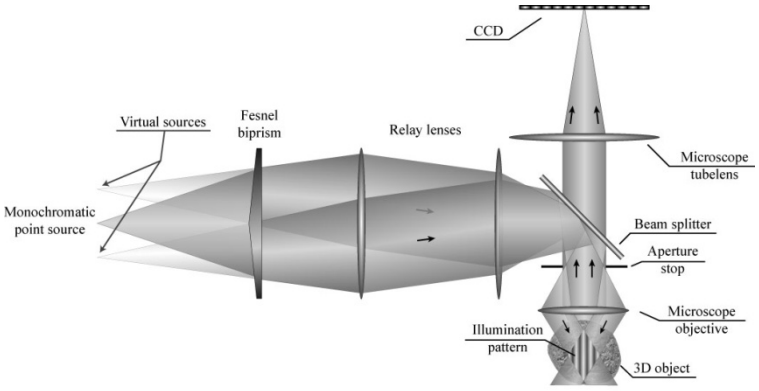
Confocal scanning microscopes possess a unique optical sectioning capacity due to their ability for rejecting the light proceeding from out-of-focus sections of the 3D samples. However, conventional widefield microscopes still have some advantages over confocal ones. The advantages result from the fact that practical biological samples are often too weakly fluorescent to yield a usable signal if pinhole diameter is too small. On the other hand, for studies of dynamic objects the imaging speed is of major importance. The required acquisition time cannot be achieved if a pixel by pixel scan has to be performed. It is therefore highly desirable to perform 3D imaging with parallel pixel acquisition

The first proposal for surpassing the diffraction limit of resolution by combining extended patterned illumination with widefield imaging was due to Lukosz and Marechand, who proposed the use of a matched detection mask for decoding the coherent image [1].

On the basis of Lukosz scheme, but extended to the general case of 3D fluorescence object, structured illumination microscopy was proposed [2]. In this new scheme the imaging is spatially incoherent, the patterned illumination is obtained either by the interference of two collimated beams [3], or by imaging 1 1D grating onto the sample [4], and the decoding is produced by computational reconstruction.

In this research we proposed the implementation of a very simple method for the flexible production of the 1D patterned illumination. Specifically, we propose the insertion of a Fresnel biprism after the monochromatic point source. As shown in the Figure, the biprism produces a pair of twin, fully coherent, virtual point sources. After imaging the virtual sources onto the objective aperture stop, the expected 1D periodic pattern is produced into the 3D sample.



The main advantage of using the Fresnel biprism is that by simply varying the distance between the biprism and the point source one can tune the period of the fringes. Additionally, a transverse displacement of the biprism, produce the required proportional transverse displacement of the fringes. In the presentation we will show the corresponding images to illustrate the utility of this new procedure.

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