BINARY PHASE PUPIL FILTER DESIGNS IN LINE-SCAN FOCAL MODULATION MICROSCOPE

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High imaging speed and good optical sectioning are always in demand in fluorescence imaging for biologists and medical researchers. Line-scan focal modulation microscopy (LSFMM) is a recently developed technique to achieve these two aims simultaneously[1]. The line-scan mechanism was the same as common line-scan confocal microscopes (LSCM), which introduce a cylindrical lens into the system to generate a diffraction-limited line in the focus for illumination at a time[2]. While scanning the line across the sample, a line camera behind the confocal slit detects the emitted signal from focal plane in parallel to form an image. LSFMM is upgraded from LSCM in that it utilizes modulation technique to provide better background rejection and optical sectioning. The mechanism behind focal modulation is a novel optical instrumentation method that generates periodic modulation of the excitation light intensity confined only to the focal volume[3]. To achieve this, a spatial phase modulator (SPM) with binary phase pupil is introduced into the illumination beam path and thus the beam consists of modulated and non-modulated zones with a relative phase difference varying from 0 to π periodically. After focused by an objective lens, interference between the modulated and non-modulated beams will introduce intensity modulation at the focal region[4]. We discovered that different binary phase pupil filter patterns can affect the modulated signal level as well as the signal-to-background ratio. Thus, it is necessary to theoretically study the effects of binary phase pupils in LSFMM and find out the optimal pupil patterns in order to improve the image quality. Exhaustive searching for the optimal solution and mathematical solution have been demonstrated. We present the results from a systematic investigation on binary phase pupils with different number of zones, duty cycles and irregular patterns. The scalar diffraction theory was implemented to evaluate light propagation and distribution in the system. Imaging performance indicators such as resolution, optical sectioning, and modulation depth have been fully discussed and then validated by experiments.

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