ARBITRARY AND DYNAMIC PATTERNING IN A PROGRAMMABLE ARRAY MICROSCOPE

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KEY WORDS: Confocal microscopy, programmable array microscope, spatial light modulator, structured illumination, dynamic illumination, controlled light exposure microscopy.

1. PROGRAMMABLE ARRAY MICROSCOPY

Programmable array microscopy [1] falls within the category of structured illumination methods for fluorescence microscopy. Excitation is conducted through a programmable spatial light modulator, forming a pattern of illumination in the sample. The emitted light is passed through the same pattern, yielding an image with conjugate illumination and detection. In the case of the PAM developed at the MPI, the emitted light that is rejected by the pattern is collected in a second so-called non-conjugate image. By scanning the pattern during camera integration and combining these two images, an optical section can be generated even if the duty cycle of the pattern is high (up to 50% on pixels). By modifying such properties as the duty cycle and pattern style (pinhole array, slit array, pseudo-random) different tradeoffs between confocality, signal-to-noise ratio and background level can be achieved.

2. DYNAMIC PATTERNING

In addition to the use of scanning patterns for confocal microscopy, static patterns can be generated to achieve localized photoactivation, photobleaching or photoconversion [2, 3]. Static masks superposed on scanning patterns allow for arbitrary regions-of-interest. However, dynamic patterns achieved by automatically adjusting the scanning pattern or mask using information from a previous image (such as in a time series) open up even more possibilities. We present a statically patterned photoactivation experiment and dynamically patterned controlled light exposure (CLEM) application. In our photoactivation experiment, we have studied H2AVD-paGFP transfected Drosophila embryos. By activating a sparse subset, we can distinguish and track individual nuclei in what would normally be a large aggregate of fluorescent material. CLEM is designed to prevent photobleaching and phototoxicity by limiting the light dose in background and bright foreground pixels. It was originally proposed for laser scanning microscopes [4], but we show here that it is effective in the PAM as well, leading to a more than 2x decrease in the rate of photobleaching.

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