GENERATION 3 PROGRAMMABLE ARRAY MICROSCOPE (PAM) FOR HIGH SPEED, LARGE FORMAT OPTICAL SECTIONING

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We report on the current version of the optical sectioning programmable array microscope (PAM) implemented with a digital micro-mirror device (DMD) as a spatial light modulator utilized for both fluorescence excitation and emission detection. The PAM is based on structured illumination [1]. A sequence of HD (1920×1080) binary patterns of excitation light is projected into the focal plane of the microscope at the 18 kHz binary frame rate of the TI 1080p DMD. The resulting sequence of patterned emissions is captured in a single acquisition as two distinct images: *conjugate* (ca. “on-focus”) consisting of signals impinging on and deviated from the “on” elements of the DMD, and the *non-conjugate* (ca. “out-of-focus”) of those falling on and deviated from the “off” elements. The sectioned image is gained from a weighted subtraction of the conjugate and non-conjugate images.

This procedure allows for a high duty cycle (typically 30 to 50%) of on-elements in the excitation patterns and thus functions well with low light intensities, preventing saturation of the fluorophores. The corresponding acquisition speed is also very high, limited only by the bandwidth of the camera(s) (100 fps full frame with the current sCMOS camera) and the optical power of the light source (lasers, LEDs). In contrast to the static patterns typical of SIM systems, the programmable array allows optimization of the patterns (duty cycle and feature size), thus enabling a wide range of applications, ranging from patterned photobleaching, (FRAP, FLIP) and photoactivation, spatial superresolution (SIM, etc.), automated adaptive minimized light exposure (MLE) [2], and photolithography (see abstract N. Cook). This work is supported by BMBF VIP Grant 03V0441 (iPAM: "Intelligentes Programmierbares Array Mikroskop).
