

BIOLOGICAL APPLICATIONS OF AN LCoS-BASED PROGRAMMABLE ARRAY MICROSCOPE (PAM)

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We report on the development and applications of a new generation, commercial, optically sectioning programmable array microscope (PAM) for rapid, light efficient 3D fluorescence imaging of living specimens. The stand-alone module, including light source(s) and detector(s), features an innovative catadioptric design and a ferroelectric liquid-crystal-on-silicon (LCoS) SLM instead of the DMD used in the first PAM design^{1,2}. The LCoS PAM (developed in collaboration with Cairn Research, Ltd.) can be attached to a camera port of any unmodified fluorescence microscope. The major advantages of the PAM are: (1) simple, inexpensive design with no moving parts; (2) increase in speed of optical sectioning due to an illumination duty cycle of each pixel of up to 50%; (3) optimal detection sensitivity, e.g. using electron multiplying CCD cameras; (4) continuously programmable, arbitrary, and adaptive optical sectioning modes between and/or within images using libraries of dot, line, or pseudo-random (Sylvester) sequence patterns; (5) maximally efficient and sensitive optical sectioning due to simultaneous detection and processing of both conjugate (in-focus) and non-conjugate (out-of-focus) light (a patented concept); (6) generation and detection of patterned polarized states; (7) minimal photobleaching; (8) compatibility with multiphoton illumination, strategies for superresolution, and other emerging techniques.

The prototype system currently operated at the MPI incorporates a 6-position high-intensity LED illuminator, a 120 W metal halide lamp (X-Cite 120, EXFO), laser sources, an Andor iXon emCCD camera, and is mounted on an Olympus IX71 inverted microscope. Further enhancements recently include: (i) point- and line-wise spectral resolution and (ii) lifetime imaging (FLIM) using phase-modulation². Using quantum dot coupled ligands we show real-time single molecule binding and subsequent trafficking of ligand-growth factor receptor complexes on live cells with greater speed and higher sensitivity in the PAM than conventional CLSM systems^{3,4}. The combined use of a 405 nm laser and LED inputs allows for photoactivation and rapid kinetic analysis of cellular processes with the photoswitchable visible fluorescent protein, Dronpa.

¹Verveer PJ et al. 1998. *J. Microsc.* 189:192; ²Hanley QS et al. 2005. *Cytometry* 67A:112; ³Lidke DS et al. 2005. *J. Cell Biol.* 170:619; ⁴G. Hagen, et al. In Single Molecule Dynamics, Ishii and Yanagida, eds., Wiley, (in press).