ARBITRARY AND DYNAMIC PATTERNING IN A PROGRAMMABLE ARRAY MICROSCOPE

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The Programmable Arrray Microscope

The PAM is a confocal microscope that uses a spatial light modulator (SLM) in the primary image plane to construct a pattern of conjugate illumination and detection (figure 1). A confocal image is generated by integrating over a number of such patterns, including point scanning, line scanning, and pseudorandom (Sylvester) sequences (Verveer et al., 1998). A non-conjugate image consisting of light collected from pixels that were not used for illumination is also registered simultaneously. Both images serve to compute in real-time an optically sectioned image by compensating the background signal usually associated with multiple-pinhole systems, such as spinning disk confocal microscopes.

Arbitrary patterns of illumination

In addition to patterns for confocal image generation, the SLM can also generate arbitrary patterns for illumination or photoconversion. As opposed to CLSM systems, the number and size of the regions does not affect the imaging speed or switch time between photoconversion and imaging. We can thus illuminate or photoconvert arbitrarily shaped regions of interest. The ROI masks can be combined with the scanning patterns for confocal imaging.

Reduction of photobleaching

Much of the fluorescence in a confocal microscope is not used, as the emission from out-of-focus objects is rejected at the pinhole. These fluorophores therefore contribute no signal but are subjected to unnecessary photobleaching. By avoiding their illumination, we lose no useful signal and protect these molecules for further 3D imaging. Controlled Light Exposure Microscopy (CLEM, (Hoebe et al., 2007)) was introduced for dynamically adjusting the illumination to the appropriate level during acquisition in a point-scanning CLSM. We here adapted this principle to the PAM (Controlled Light Exposure PAM, CLEPAM) by dynamically masking out the SLM pattern where excitation is not needed (figure 3). Photobleaching is thereby minimized (figure 4).
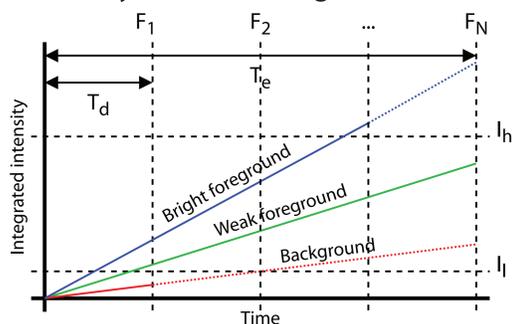
Figure 3: CLEPAM. After an initial image with integration time T_d (background) pixels with counts lower than I_l are switched off. After each subsequent image, illumination for (bright foreground) pixels with counts higher than I_h is similarly stopped. Only weak foreground pixels are illuminated for all N frames. After the complete exposure T_e, all values are extrapolated to N frames of illumination.
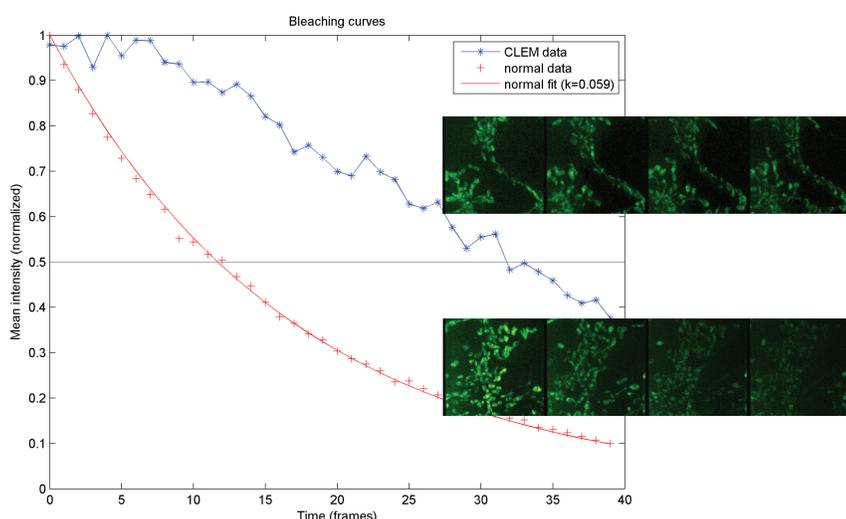
Figure 4: Bleaching curves comparing CLEPAM imaging versus widefield illumination. The exposure time for the weak foreground is the same in both situations. The CLEPAM sample takes 2.7 times as long to bleach half the original fluorescence. Sample: living HeLa cells stained with Mitotracker. Insets: maximum intensity projections over 10 sections of frames 0,10,20, and 30.
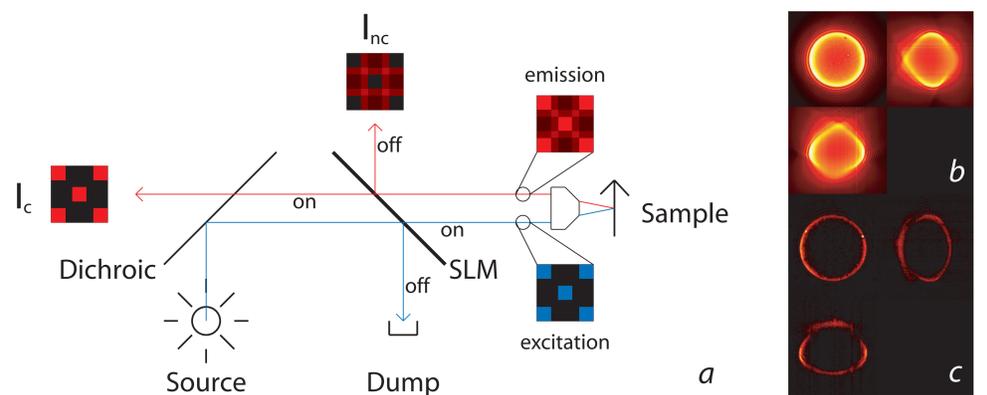
Figure 1: (a) Schematic depiction of the PAM. Excitation light is structured by application of an SLM. The emitted light is passed through the same SLM, resulting in the conjugate image (consisting of pixels conjugate to the illuminated spots) and the nonconjugate image (out of focus light from unilluminated pixels). (b)-(c) Comparison of widefield and PAM confocal images of a 6 µm shell coated bead using a 150x 1.45NA oil objective with a line scanning pattern.

ROI tracking

One application of arbitrary regions of interest is to track a sample over time by only illuminating that part of the field in which there is a signal. After every frame, the illumination is calculated by segmentation and subsequent dilation (to account for movement of the sample). This creates the impression that the sample is "pushing" the illumination forward (figure 2). In this way, continuously moving structures such as filopodia or migrating cells can be tracked.
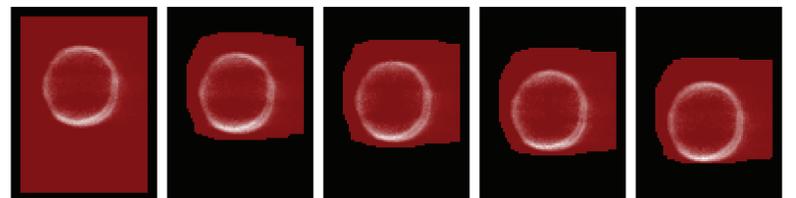
Figure 2: Tracking a moving sample (a 6 µm shell coated bead). The illumination (red) is continually adjusted to the sample, such that each subsequent acquisition is taken with the illumination calculated from the previous image.

Photoconversion in arbitrary regions of interest

By area selective illumination of a photoactivatable probe, one can choose which subset of the sample to image. In a study of H2AVD-paGFP transfected Drosophila embryos, we activated only a sparse subset of the specimen so as to distinguish and track individual nuclei or chromosome segments in what would normally be a large aggregate of fluorescent material (figure 5). In previous work, we applied the same method for fluorescence recovery after photobleaching (FRAP), obtaining diffusion times and immobile fractions for different parts of the sample at the same time (Hagen et al., 2009).
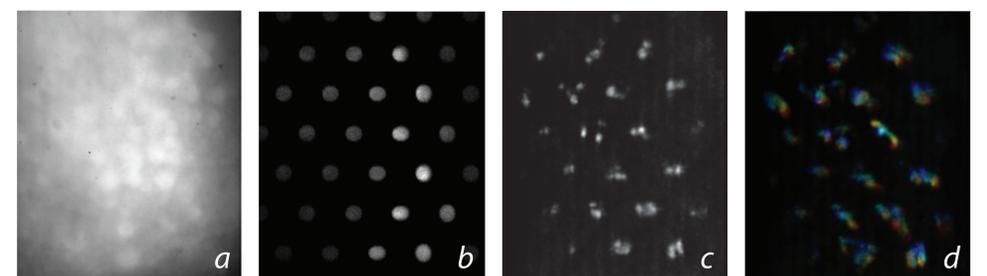
Figure 5: Photoactivation of H2AVD-paGFP in Drosophila embryos. (a) Widefield activation. (b) Activation pattern. (c) MIP of the first stack in the time series after activation. (d) Projected time series (jet colormap: blue = 0s, red = 490s).

References

Verveer et al. (1998), *Theory of confocal fluorescence imaging in the programmable array microscope (PAM)*. J. Microsc. 193:50-61.

Hoebe et al. (2007), *Controlled light-exposure microscopy reduces photobleaching and phototoxicity in fluorescence live-cell imaging*, Nat. Biotech., 25:249-253.

Hagen et al. (2009), *Fluorescence Recovery After Photobleaching and Photoconversion in Multiple Arbitrary Regions of Interest Using a Programmable Array Microscope*, Microscopy Res. Tech., accepted for publication.