

Development of a Novel DMD based Microscope to Combine Confocal, SIM and STORM to Study Biological Samples

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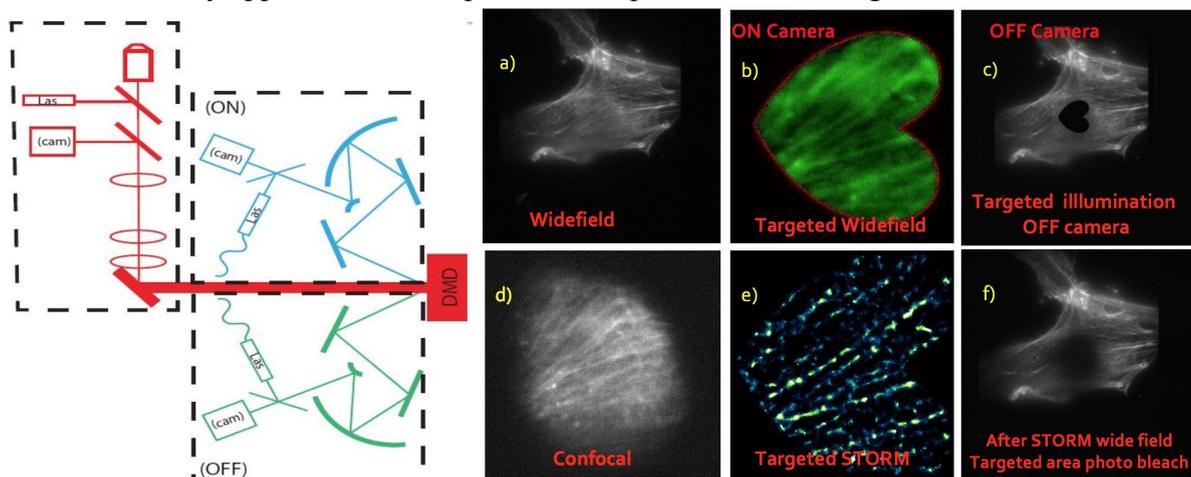
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Abstract: Super-resolution images of a biological sample are generally achieved by using high power laser illumination with long exposure times which unfortunately increases photo-toxicity of a sample, making super-resolution microscopy, in general, incompatible with live cell imaging. We have developed an illumination system based on a Digital Micro-mirror Device (DMD) which allows for fine control over the power and pattern of illumination.

The system is built to collect in-focus and out-of-focus light collected from the sample in two separate cameras as shown in the figure A, enabling us to perform post-processing on both in and out of focus information depending on the thickness and fluorescent labelling intensity of the sample. The major advantage of this system is to combine various imaging modalities such as confocal, Structured Illumination Microscopy (SIM), Stochastic Optical Reconstruction Microscopy (STORM). In real time, we can then change between these techniques in different regions of interest of a biological sample as shown in the figure B.

As an example we demonstrate targeted high-resolution STORM imaging for a small portion of a single cell without photobleaching the surrounding areas, establishing a new method in biological imaging. We have used this to show that we can reduce the power delivered to the sample to allow for long time imaging in one area while achieving sub-diffraction STORM imaging in another using higher power densities. We have also implemented dual colour imaging in this system. We show the application of this technique to NrCAM transfected or phalloidin stained NIH 3t3 mouse fibroblast cells and HeLa cells. Development of this method has many applications and opens wide capabilities for biological studies.



(Figure A) Optical setup of the DMD based system, B) Targeted imaging and combining widefield, confocal and STORM imaging techniques in one field of view)

1. Peedikakkal LV, Steventon V, Furley A, Cadby A. Development of Targeted STORM for Super Resolution Imaging of Biological Samples using Digital Micro-mirror Device. *Optics Communications*. 2017(404):18-22.