Development of physical pinholing in confocal microscopy and targeted STORM of biological samples using Digital Micromirror Device

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ABSTRACT

Optical microscopy has been widely used in the study of biological samples due to its non-contact, minimal invasive nature enabling in vivo investigation. In this work, we use an implementation of a dual path Programmable Array Microscope using a Digital Micro-mirror Device (DMD) [1] to programmably control the source and the detection aperture in 2D in order to achieve the highest control over our excitation and collection pathways. The ability to carefully control the magnitude and spatial extent of the light delivered to a sample allows us to switch between a number of imaging modalities and gives us exquisite control over the amount of light delivered to the sample.

In our optical setup, a DMD is implemented in an inverse Schiefspiegler telescope setup to control the power and pattern of illumination and also to collect the in-focus and out-of-focus light in two separate cameras for super resolution microscopy. This allows us to, in real time, change between imaging techniques such as confocal microscopy, Structured Illumination Microscopy (SIM), Stochastic Optical Reconstruction Microscopy (STORM) and as such trade photo-toxicity for resolution. This work is the development of low power physically pin-holed confocal microscopy integrated with high power super resolution Stochastic Optical Reconstruction Microscopy (STORM) on targeted areas. Using this technique, the overall photobleaching of the sample is reduced enabling the selection of an area of interest in sample and rendering super resolution images. This technique is established and tested in NIH 3t3 mouse embryonic fibroblast cells.

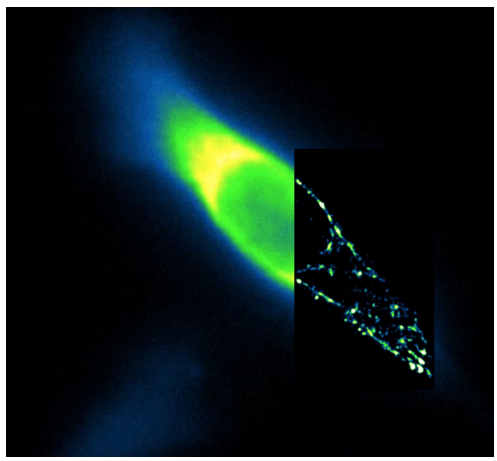

Figure: STORM image of the targeted area is overlaid on an epifluorescence widefield image.

1. R. Heintzmann et al., A dual path programmable array microscope (PAM): simultaneous acquisition of conjugate and non-conjugate images, Journal of Microscopy, Vol. 204, pp. 119-137, (2001).