EVALUATION OF THE RESOLVING POWER OF GROUND STATE DEPLETION SUPER RESOLUTION MICROSCOPY USING F-ACTIN ARRAYS IN CACO-2 CELLS

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Using the colorectal cancer cell line Caco-2 as a model, F-actin filaments were fluorescently labelled, imaged using super-resolution GSD and analysed. An easily reproducible and accurate methodology was developed to measure the widths of discernible actin fibers in digital images and comparing them to those in corresponding wide-field fluorescence images.

The Ground State Depletion-Super Resolution (GSD-SR) technique opens up new possibilities in light microscopy by providing a method by which the resolution restrictions imposed by the diffraction limit of light can be overcome. This advance in light optical resolution has narrowed the gap between the techniques of light and laser based microscopy and electron microscopy. In doing so, super-resolution light microscopy has increased the arsenal of the researcher who had no option but to sacrifice the specificity and localisation abilities of fluorescence microscopy for the high resolution of electron microscopy. This study employed a biological sample, the F-actin arrays of Caco-2 cells, in order to quantify the reported improvement in the lateral resolution obtained with GSD-SR over traditional wide-field (WF) fluorescence. An easily reproducible and accurate methodology was developed to measure the widths of discernible actin fibers in corresponding digital images obtained using the two different light microscopy techniques.

Figure 1. Schematic of image analysis workflow for evaluating the resolving power of the two techniques; Ground State Depletion-Super Resolution (GSD-SR) and Wide-Field (WF) fluorescence microscopy.

The GSD-SR microscopy technique has been shown qualitatively to be superior in resolving power to WF fluorescence and now based on the measured widths of F-actin this can now be quantified using image analysis program image J [1]. An approximately 5.5 fold improvement is of great benefit to the microscopists who which to measure, locate or co-localise structures of interest which were beneath the resolution limit of conventional light and laser based microscopy techniques. The method of data extrapolation used in this study will be later applied to correlative light and electron experiments (CLEM) to compare the resolving power of this new technique with that of transmission electron microscopy for further affirmation of GSD-SR’s resolving capabilities.

References