High resolution restoration of 3D structures from extreme low dose widefield images

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The most important requirement in time-resolved widefield fluorescence microscopy (4D) is to keep the illumination dose small for maximizing the viability of the imaging specimen. In this context, the only factor that determines the minimum dose level required for obtaining an acceptable resolution is the efficiency of the deconvolution method. The minimum set by currently used deconvolution methods is still high enough to cause damage to the cells that are sensitive to light, especially when it is required to measure images for more than about 50 times points. The main reason is that the current methods use ad hoc energy functionals for noise suppression (regularization), and hence they have inadequate ability to discriminate the noise high frequencies against signal frequencies, thereby leading to the loss of signal frequencies when the noise is high. We present a novel deconvolution method that uses a regularization functional constructed using an entropy based formalism that is specifically tailored to exploit the spatial characteristics of the fluorescence images. The method has an exceptional power to discriminate the noise high frequencies against signal frequencies and hence leads to high resolution restoration of structures in the presence of large levels of noise. We name our method as the “Entropy Regularized Deconvolution (ER-Decon)”. We compare ER-Decon with two of the best among the currently available methods (i) Huygens; (ii) DeconvolutionLab.

For experimental validation, we imaged GFP labeled zip1 protein filaments of fixed yeast cells. We acquired two stacks with two different dose levels, where the one stack differs in dose level from the other by a factor of 400. Deconvolution results are given in Figure 1. It is clear from the images that the high dose results of all three methods have comparable resolution, whereas the low dose results differ significantly. In particular, ER-Decon’s output from the low-dose raw image reveals the filamentous structures despite the fact that the structure is nearly invisible in the raw images. On the other hand, the results of Huygens and DeconvolutionLab obtained from low-dose image only show blob-like structures. Figure 2 shows correlation between the images corresponding to low- and high-dose experiments as a function of resolution, where, for each value of resolution, the correlation is computed within a spherical shell in the Fourier space corresponding to the given resolution. It is evident from the plot that ER-Decon achieves considerably higher correlation even at the cut-off frequency (vertical line).