ENHANCING THE RESOLUTION OF LOW EXPOSURE 4D WIDEFIELD MICROSCOPY BY USING 4D REGULARIZATION

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4D widefield microscopy provides a way of studying the dynamics of live cell processes with highest time resolution. The main issue in 4D imaging is either severe phototoxicity/photobleaching or unacceptably signal-to-noise ratios (SNR) if the illumination intensity is set at safe levels to ensure unperturbed cellular processes. This issue is more severe when recording multiple wavelengths to study the dynamics of multiple sub-cellular components. The consequence of low SNR imaging is a severe loss of resolution to the extent that the sub-cellular components appears to be blob-like structures—when currently available deconvolution methods are used—making the accurate study of live cell dynamics infeasible. Here we present a new deconvolution method that stands out from existing methods by the following factors: (i) it uses a regularization functional constructed using an entropy based formalism specifically tailored to exploit the spatial characteristics of the fluorescence images; (ii) it uses the regularization functional defined in 4D allowing to spatio-temporal correlations to be exploited. Due to these factors, our method gives significantly improved resolution when compared to existing methods. We name our method as “entropy regularized 4D deconvolution”, abbreviated as ER-Decon-4D. The following panel demonstrates that ER-Decon-4D can restore sufficient details from extremely noisy images, and compares the restored resolution of ER-Decon-4D to that of the one of the top methods in literature, known as Deconvolution-Lab.

The panel shows images obtained from yeast cells undergoing meiotic recombination where the Zip1 filaments (the central filament of the synaptonemal complex) were labeled with GFP. The top row displays a selected xy section of 3D raw images representing snapshots at various times within a 3D movie of meiotic recombination. The middle row shows the sections of 3D deconvolved outputs obtained from Deconvolution-Lab. The bottom row shows the sections of 3D deconvolved outputs obtained from the proposed method, ER-Decon-4D.

The images clearly demonstrate that ER-Decon-4D retrieves significantly improved resolution from images with very low SNR, and hence it will make accurate study of live cell processes possible without perturbing normal cell functioning. Details of the methods and results will be discussed in the presentation.