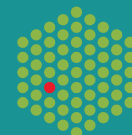




# Deconvolution allows reliable imaging of living cells at low fluorescence level

EMBL



## Introduction

Fluorescence signal of living cells is often limited by many factors:

- low excitation power to reduce fluorophore photobleaching and phototoxicity to the sample;
- short exposure times to increase temporal resolution;
- low protein expression to keep in physiological conditions.

Thus the spinning disk confocal microscope with its low degree of photobleaching, fast frame rate and high sensitivity is an instrument of choice, especially if an electron-multiplied CCD (EMCCD) camera is used instead of a normal CCD camera. However, the images of specimens with a weak fluorescence signal have low signal to noise ratio (SNR) and not suitable for quantitative analysis.

We applied deconvolution to images of living cells with low fluorescence intensity acquired with a spinning disc confocal microscope.

## Results

Images were obtained using PerkinElmer UltraView ERS spinning disc confocal microscope with C9100-50 EMCCD camera mounted on Zeiss Axiovert 200M microscope with 100x/1.3 NA objective.

Deconvolution of the images of spinning disc confocal microscope improves image quality and resolution. The quality of the deconvolved images drops with the increase of slice distance (Fig. 1).

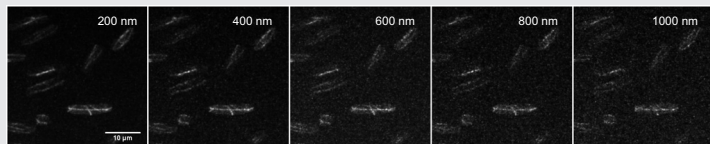


Fig. 1. Maximum intensity projections of deconvolved images of yeast cell expressing  $\alpha 2$ tubulin-GFP for different slice distance (200 nm, 400 nm, 600 nm, 800 nm, 1000 nm).

Deconvolution significantly improves the contrast of the images even for the specimen with low fluorescence intensity (Fig. 2).

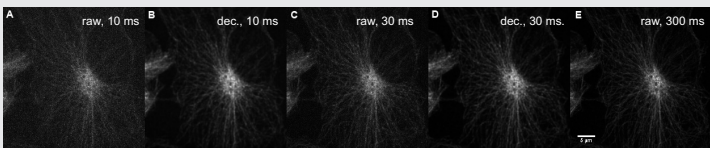


Fig. 2. Maximum intensity projection of raw (a, c, and e) and deconvolved (b, d) images of actin filaments in HeLa cells for different exposure times.

Maximum (Fig. 3) and average (Fig. 4) intensity projection of raw (a) and deconvolved with 15 iterations (b) z-stacks of HeLa cell expressing EB3-GFP and yeast cell expressing  $\alpha 2$ tubulin-GFP along with the intensity cross section over the images.

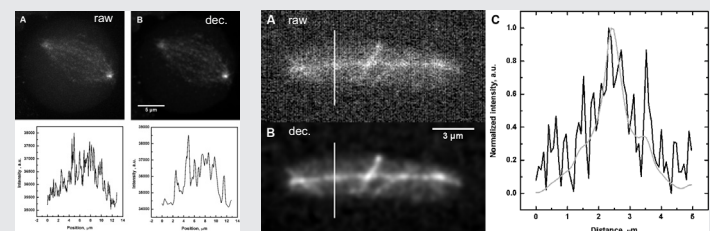


Fig. 3. HeLa cell expressing  $\alpha 2$ tubulin-GFP.

Fig. 4. Yeast cell expressing EB3-GFP.

The deconvolved images have a clearly better quality. For example, the microtubules are brighter and cytosol signal is remarkably visible.

## Deconvolution

Deconvolution was performed with classical maximum likelihood estimation algorithm with theoretical point spread function using Huygens Remote Manager (HRM) (developed by SVI, FMI, MRI, EPFL).

Main features of the algorithm:

- Relatively fast - about 1 minute for 100 Mb data set;
- Works for all microscopes: widefield, spinning disc, confocal;
- Very robust at high noise level;
- Requires only SNR estimation.

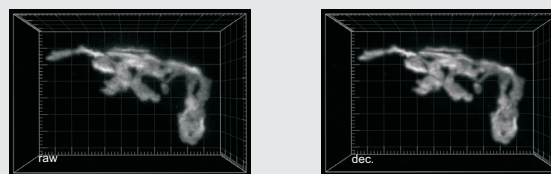


Fig. 5. Comparison of 3D rendering of raw (left) and deconvolved (right) Golgi in HeLa cell expressing GalNac-GFP.

## Discussion

CCD camera has a better performance at higher light level. On the other hand, EMCCD is better for imaging at low light level (less than several tens of photons per pixel, Fig. 6).

Image quality at low light level can be improved by several methods (Fig. 7): mean filter (b), Gaussian filter (c), or deconvolution. Both mean and Gaussian filters do improve image contrast, but the deconvolution significantly outperforms both methods.

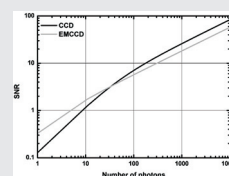


Fig. 6. Comparison of signal to noise ratio for CCD and EMCCD cameras.

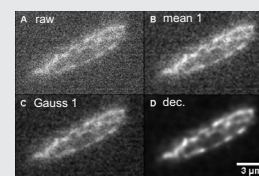


Fig. 7. Comparison of different methods for noise reduction of image of yeast cell.

## Conclusions

- Deconvolution is more efficient for the improvement of contrast, than traditional denoising methods such as averaging or Gaussian filtering.
- Deconvolution enables significant improvement of the live cell image quality at very low levels of fluorescence signal.
- Deconvolution makes it possible to reduce illumination, which leads to higher temporal resolution, lower photobleaching and phototoxicity.
- Deconvolution is a conservative method and can be potentially used for quantitative imaging.

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