

IMPROVEMENT OF RESOLUTION IN THREE-DIMENSIONAL FLUORESCENCE MICROSCOPY THROUGH THE WEB WITH “POWER-UP YOUR MICROSCOPE”

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The fluorescence microscope can image a specimen in its natural environment forming a 3D image of the whole structure allowing *in vivo* and *in vitro* observations.

Unfortunately the image formation process is affected by distortions mainly due to blurring and noise. These distortions hide fine details in the image hampering both the visual and the quantitative analysis. From a mathematical point of view the properties of an image formation system based on the optical microscope can be modeled by the knowledge of the so-called Point Spread Function (PSF), i.e., the image of a point-like source of subresolution dimension approximating impulse response of the system. Under some assumptions and in the absence of noise, the registered data are due to the convolution of the original object intensive properties with the PSF. “Power-Up Your Microscope”[1] implements different deconvolution algorithms [2] to compute the best approximation of the original object. This is possible by uploading own images and compiling a form with few acquisition microscope parameters. The software package determines automatically the theoretical PSF, which will be used for the image restoration process. Image deconvolution can be performed on images coming from wide field, confocal and multiphoton microscopy.

In order to compare the effective improvement in the quantitative image formation analysis, we compared the z-response of the optical system before and after restoration by using polyelectrolyte self-assembled ultra-thin (2-2.5 nm) layers transferred onto a coverslip or used for fabrication of round shaped shells [3]. Such a samples allows to test the performances of the different computational approaches implemented in the web-based package (<http://www.powermicroscope.com>).

- [1] Bonetto P., Boccacci P., Scarito M., Davolio M., Epifani M., Vicidomini G., Tacchetti C., Ramoino P., Usai C., Diaspro A., Three-dimensional microscopy migrates to the web with "PowerUp Your Microscope". *Microscopy Research and Technique*. 2004, vol. 64, pp. 196-203.
- [2] Bertero, M. and P. Boccacci, *Introduction to inverse problems in imaging*. 1998, Bristol, UK: IOP Publishing.
- [3] Diaspro, A., Krol, S., Cavalleri, O., Silvano, D., Gliozzi, A., Microscopical characterization of nanocapsules templated on ionic crystals and biological cells toward biomedical applications. *IEEE Trans. On Nanobioscience*, 2002, 1 (3), 110-115.