Slice-based out-of-focus reduction technique for 3D fluorescence microscopy

Awoke Negash[1], Thomas Mangeat[2,3], Simon Labouesse[1], Hugues Giovannini[1], Kamal Belkebir[1], Patrick C. Chaumet[1], Nicolas Sandeau[1], Renaud Poincloux[4], Anais Bouïssou[4], Anne Sentenac[1]

[1] *Aix Marseille Université, CNRS, Centrale Marseille, Institut Fresnel, UMR 7249, 13013 Marseille, France*

[2] *Universitié de Toulouse, UPS, LBCMCP, 31062 Toulouse, France*

[3] *CNRS, LBCMCP, 31062 Toulouse, France*

[4] *Institut de Pharmacologie et de Biologie Structurale, Université de Toulouse, CNRS, UPS,France*

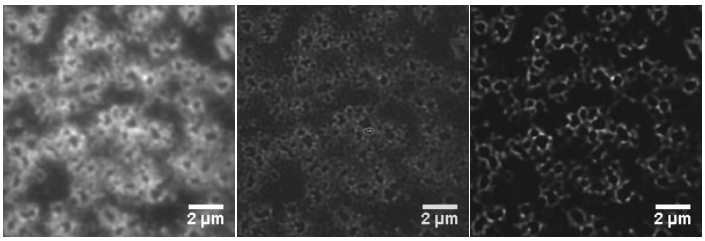
Key Words: 3D fluorescence microscopy, Positive widefield deconvolution, Resolution enhancement

In microscopy, the in-focus image of a 3D fluorescence sample suffers from out-of-focus blur due to the light coming from the specimen above and below the focal plane. This out-of-focus fluorescence degrades the contrast of the image and may hinder the visualization of in-focus sample features.

Recently, it has been suggested in the framework of Structured Illumination Microscopy that an appropriate three-dimensional deconvolution could be used to remove the out-of-focus fluorescence [1]. In this approach, the two-dimensional (2D) image is deconvolved using the three-dimensional (3D) point spread function of the microscope. In other words, one defines an investigation volume in which a three-dimensional sample is reconstructed so that its 3D convolution restricted to the focal plane best matches the recorded image. To improve the reconstruction, the deconvolution is performed under positivity constraint. Of course, the sample estimate outside the focal plane is not accurate, due to the lack of constraining data, but it is expected to carry out some of the out-of-focus fluorescence.

The algorithm presented in [1] was developed for blind-Structured Illumination Fluorescence Microscopy and it estimates alternatively the unknown illuminations and the sample. Here, the algorithm is significantly simplified, following [2], so that only the product of the illumination times the sample is reconstructed.

We first checked our algorithm on brightfield fluorescence microscopy data. It was shown that, in addition to the removal of out-of-focus fluorescence, the 3D deconvolution approach improves significantly the transverse resolution of the image. It yields significantly better results than the two-dimensional deconvolution that is usually performed on these images (with minimal additional computational costs), see Fig. 1.



(a) (b) (c)

Fig. 1: (a) Widefield image of podosomes. (b) Two-dimensional deconvolution. (c) Three-dimensional deconvolution.

Then we investigated its interest on fluorescence microscopy using speckle illumination. Once again, both the out-of-focus blur and the resolution were significantly improved compared to a two-dimensional reconstruction approach.

In conclusion, three-dimensional deconvolution should be preferred to two-dimensional deconvolution even when the data are restricted to a single plane image. It dims the out-of-focus fluorescence and significantly ameliorates the resolution. It is a simple data processing which can be used on most microscopy configurations.

Acknowledgement : Awoke Negash is supported by the Erasmus Mundus Doctorate Program Europhotonics (Grant No. 159224-1-2009-1-FR-ERA MUNDUS-EMJD)

References

1. A. Jost, E. Tolstik, P. Feldmann, K. Wicker, A. Sentenac, R. Heintzmann, "Optical Sectioning and High Resolution in Single-Slice Structured Illumination Microscopy by Thick Slice Blind-SIM Reconstruction," PLOS ONE **10(7)**, (2015).
2. A. Negash, S. Labouesse, N. Sandeau, M. Allain, H. Giovannini, J. Idier, R. Heintzmann, P. C. Chaumet, K. Belkebir, A. Sentenac, "Improving the axial and lateral resolution of three-dimensional fluorescence microscopy using random speckle illuminations," J. Opt. Soc. Am. A **33(6)**, 1089-1094, (2016).