

COMBINED OPTICAL NANOSCOPY APPROACHES.

Alberto Diaspro, Paolo Bianchini, Francesca Cella, Zeno Lavagnino, Silvia Galiani, Emiliano Ronzitti, Marco Scotto d'Abbusco, Partha Pratim Mondal

Nanophysics, IIT – Italian Institute of Technology, 16163 Genova, Italy

E-mail : alberto.diaspro@iit.it

KEY WORDS: Fluorescence Microscopy, Bioimaging, super resolution, far-field optical nanoscopy.

Three-dimensional fluorescence optical far-field microscopy generated the design and realization of crucial experiments in cellular and molecular biophysics, although comparatively low spatial resolution when compared with electron microscopy or scanning probe near-field methods. Recently, an emerging family of fluorescence microscopy approaches exploiting the photo physical properties and the switching abilities of fluorescent markers allowed to achieve the surpassing of the diffraction barrier down to 10 nm resolution scale. Super-resolution microscopy and optical nanoscopy are the modern terms related to optical far-field methods [1]. Within this framework, focusing on the saturated depletion of the markers' fluorescent state by stimulated emission we have pointed our attention to different modalities for realizing STED (stimulated emission depletion) approach. In particular we are interested in the excitation modalities (including phase modeling, intensity control and scanning speed) and in evaluating the possible photo-bleaching/toxical effects as function of the light intensity levels needed. To this end we are working both on the "classical" solution using ps laser pulses both using white light laser generation and multi-photon based schemes and on the more recent CW approaches. In order to have a flexible set-up we are also approaching optical super-resolution using the FPALM (fluorescence photoactivatable localization microscopy) scheme coupled to two different ways for switching on the fluorescent proteins involved. The former being classical, in order to have a comparison with the STED approach in terms of possible photo-bleaching and photo-toxical effects, and the latter based on the utilization of the single plane illumination microscopy (SPIM) concept to extend far-field optical nanoscopy methods to large samples.

[1] A. Diaspro (ed.), Nanoscopy and Multidimensional Optical Fluorescence Microscopy, Chapman and Hall, New York, 2010).