Grand unison in optical microscopy: an alliance between two-photon excitation microscopy and super resolution methods.

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KEYWORDS: Two-photon excitation microscopy, Fluorescence, Thre-dimensional imaging, Super resolution, Individual Molecule Localization, Nanoscopy.

Advances in fluorescence microscopy had a significative impact in cellular and molecular biology in the last 30 years. The advent of two-photon excitation (2PE) microscopy pushed to design new instruments and experiments. This also allowe to experience new imaging modalities from single molecule to organ 3D studies [1]. More recently, “super resolution” and "optical nanoscopy” approaches have been implemented in far field optical microscopes. Today, they are available for everyone to use without extreme complexity.

In order to improve the performances of 2PE we focused different super resolution approaches and combined architectures. IML-SPIM (Individual molecule localization selective plane illumination microscopy) [2] has been combined with 2PE towards imaging of 3D thick specimens[3]. 2PE-STED has been adapted to 2PE-SW-STED utilizing a single wavelength (SW) both for two-photon excitation and depletion[4]. Further advances are related to the more general RESOLFT concept extending its utilization to lithography [5].

Multimodality can be improved by coupling with scanning probe methods [6] while three-dimensional imaging of cellular aggregates and thick specimens is the main goal of our developments. So far, a variety of architectures will be critically outlined in regard to different applications demanding for investigations at the nanoscale.