

Nanoscopy 2.0

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Three-dimensional (3D) fluorescence optical microscopy had a tremendous development in the last thirty years, related to the different converging disciplines [1]. Confocal, multi-photon and light-sheet approaches pushed the optical sectioning ability of getting 3D information to 3D imaging of thick specimens, including organs and tissues. The discovery and utilization of green fluorescent proteins opened new possibilities and incredible advances came out when unlimited resolution in space was demonstrated. So, terms like super resolution microscopy, super or ultra microscope, optical nanoscopy refer to the possibility of producing images at an unlimited spatial resolution, in principle. This is the scenario for fluorescence optical nanoscopy that can operate at the 10-50 nm scale surpassing the 200 nm of the optical microscope in x-y and at the 50 nm level along the z-axis. Nanoscopy 2.0 is related to the new developments using, for example, acoustic lenses for fast focusing, light-sheet architectures for thick samples, correlative approaches for multimodal information and the possible integration with scattering based methods like Mueller matrix signature. We will discuss targeted and stochastic readout methods expanded to multi-photon excitation (MPE)/absorption in a 3D framework. A variety of architectures will be outlined and further variations on the super resolution theme addressed.

A. Diaspro, "Circumventing the diffraction limit," *Il Nuovo Saggiatore*, vol. 30, no. 5, pp. 45–51, 2014.