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IMC-PL-6095 Light Microscopy at the Nanoscale

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Novel developments in optical technology and photophysics made it possible to radically overcome the diffraction limit (ca. 200 nm laterally, 600 nm along the optical axis) of conventional far-field fluorescence microscopy. Presently, three principal “nanoscopy” families have been established: “Nanoscopy” based on focused laser beams, like 4Pi-, STED- (STimulated Emission Depletion)-, and RESOLFT- (Reversible Saturable OpticaL Fluorescence depletion Transitions) microscopy; nanoscopy based on Structured Illumination Excitation (SIE), like SMI (Structured Modulated Illumination) microscopy, SIM (Structured Illumination Microscopy) and PEM (Patterned Excitation Microscopy); and nanoscopy based on various modes of Localization Microscopy, like PALM (PhotoActivated Localization Microscopy) and FPALM (Fluorescence Photoactivable Localization Microscopy), GSDIM (Ground State Depletion Imaging Microscopy), SPDM Spectral Precision Distance/Spatial Position Determination Microscopy), STORM (STochastic Optical Reconstruction Microscopy) and dSTORM (direct STORM). These and related far-field light microscopy methods have opened an avenue to image nanostructures down to single molecule resolution; they made possible to measure the size of molecule aggregates of few tens of nm diameter and to analyze the spatial distribution of individual molecules with a light optical resolution down to the few nanometer range, corresponding to ca. 1/100 of the exciting wavelength. Application examples obtained by focused, structured, and localization techniques cover a variety of biostructures, such as membrane complexes, neuronal synapses, cellular protein distribution, nuclear nanostructures, as well as the “nanoimaging” of individual viruses and lithographically generated nanostructures. Each of the nanoscopy methods described has its peculiar advantages; as a whole, they provide a tool set of light microscopy approaches to the nanoscale and open a wide range of perspectives in Biology, Medicine and the material sciences. Further improvements are expected to make possible a three-dimensional lightoptical resolution down to the 1 nm scale. The combination with Electron- and X-ray microscopy techniques is anticipated to provide further nanostructural insights.

C. Cremer, Optics far Beyond the Diffraction Limit: From Focused Nanoscopy to Spectrally Assigned Localization Microscopy (2012). In: Springer Handbook of Lasers and Optics, 2nd edition (F. Träger, Edit.), pp. 1351 – 1389.
C. Cremer, B.R. Masters (2013) Resolution enhancement techniques in microscopy. Eur. Phys. J. H 38: 281–344.