Novel developments in optical technology and photophysics opened the possibility to study the cell nuclear architecture at radically enhanced optical resolution. Previously, studies aiming at a molecular resolution of DNA inside of fixed cell nuclei was hampered by the availability of suitable fluorescent labels, often resulting in a poor Nyquist sampling of the fluorescent structures, with signal densities of a few 100 fluorophores per $\mu m^2$. Our recently developed Single Molecule Localization Microscopy (SMLM) technique based on photoconversion of classic DNA dyes (such as DAPI and Hoechst), has largely increased the number of signals to be extracted, thus facilitating for the first time a true structural resolution and therefore the analysis of changes in chromatin structure upon various biological processes.

Validation of the resolution enhancement is shown on nucleic DNA both inside intact cell nuclei and from spreads. Characteristic superstructure folding patterns of chromatin are compared in conventional and superresolution microscopy images. Advanced algorithms for correction of mechanical instabilities and for visualization of the extracted list of localizations are used. Simulations were performed to validate structural resolution capabilities. Thus we are able to present a perspective of multi-color superresolution microscopy (Spectral Position Determination Microscopy/SPDM) for enhanced structural resolution using conventional fluorophores under linear excitation conditions, i.e. without the requirements to induce blinking.

References