Single Molecule Localization Microscopy has opened the possibility to study the cell nuclear architecture at radically enhanced optical resolution.\textsuperscript{1–3} Owing to the necessity to acquire several thousands of individual images, the resolution of SMLM reconstructions depends largely on correction of drift occurring during the acquisition process. Previously, drift correction was developed based on fiducial markers, on estimation from low-resolution wide field data, and on cross-correlation between reconstructions of subsets.\textsuperscript{4,5} These methods have been shown to operate reasonably well for 2D images, albeit the fact that the accuracy which can be achieved may be limited, as is its applicability to 3D SMLM datasets. Furthermore, with the exception of wide-field based correction, the application of present drift correction algorithms depends on a high signal density. Using suitable composition of the imaging media, Single Molecule Localization Microscopy (SMLM) technique based on photoconversion of classic DNA dyes (such as DAPI and Hoechst),\textsuperscript{2} 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.

We present a drift correction algorithm based on nearest neighbor analysis, and verify its applicability, robustness, and accuracy using both experimental and simulated datasets with lists of 2D and 3D positions. Validation of the resolution enhancement is shown on standard microtubule datasets and on nucleic DNA inside intact cell nuclei. Results obtained from drift correction based on nearest neighbor analysis are compared to those obtained using other methods. Resolution enhancement is verified indirectly by Fourier Ring Correlation analysis, and also directly based on profiles extracted from SMLM reconstructions of diffraction limited structures (e.g. microtubule, isolated unspecific bound dye molecules). It could be shown that based on nearest neighbor analysis, every single position potentially contributes to the extracted drift, with a prospective extremely high drift correction accuracy even in sub-optimally stained samples.

References