

NANOMETRY OF BIO-MOLECULAR COMPLEXES USING PSF RECONSTRUCTION IN SMI MICROSCOPY

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During the last decade, light microscopy and three-dimensional (3D-) image analysis have made considerable progress; this opens an avenue to analyze the genome nano-architecture of specific gene domains in 3D-conserved cell nuclei by fluorescence microscopy. As examples, chromatin folding and the formation of biomolecular complexes indicate a significant nuclear structure-function relationship, where condensation and clustering play an important role. In order to obtain reliable, quantitative structural information about these processes, monitoring of the extension of e.g. gene regions could provide an essential method employing far field microscopy based on point spread function (PSF) engineering techniques. Here, an improved size measurement method using Spatially Modulated Illumination Microscopy (SMI) [1] in combination with reference objects of known size and shape to reconstruct the microscope PSF is applied to determine the size of individual fluorescent objects far below the conventional optical resolution limit. Reconstruction of the PSF allows to overcome the assumptions about the shape of the SMI-PSF. These assumptions were necessary in former SMI nanosizing measurements [2]. For this, reference objects with known dye distribution have to be put additionally to the unknown objects on the object slide or on the cover slip. We present data from measurements on fluorescent microspheres with diameters between 40 and 200 nm using an excitation wavelength of 488 nm.

In quantitative molecular cytogenetics this “nanosizing” method allows a variety of applications. Experimental SMI measurements of fluorescent labeled objects inside of cryosections and 3D conserved cells were performed using 488 nm excitation. The nanosizing data shown comprise results of gene expression analysis, transcription factory analysis, protein cluster analysis, and gene compaction analysis. An important practical feature of the SMI-nanosizing method described is that such studies may be conducted using conventional microscope object slides and cover slips. The size values obtained are about two orders of magnitude smaller than the illumination or observation volume, respectively, in a confocal laser scanning microscope using a high numerical aperture objective lens [3].

References

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