

Raster Image Correlation Spectroscopic Analysis of Intranuclear Molecular Dynamics

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KEY WORDS: Fluorescence correlation spectroscopy, image correlation spectroscopy, confocal microscopy, cell nucleus, diffusion coefficient.

In contrast to conventional epi-illumination microscopy in confocal laser scanning microscopy images are acquired sequentially either in point scanning or line scanning mode. This scanning approach therefore contains spatio-temporal information, which can be used to obtain insight into molecular dynamics, e.g. diffusion coefficients of fluorescently labeled probes. Raster Image Correlation Spectroscopy (RICS) [1] extends Fluorescence Correlation Spectroscopy (FCS) and thus makes the features of FCS accessible on commercial confocal LSMs, which are widely used in modern life science research. The basic principle of RICS is to calculate the temporal and spatial correlation between adjacent pixels, lines and frames and thereby provides access to three different time scales, on which dynamic processes can occur: microseconds, milliseconds, and seconds. Our aim is to supplement our commercial laser scanning microscope (Zeiss LSM 510 Meta) with the RICS method for measuring diffusion constants of fluorescent proteins and particles *in vitro* and also *in vivo*. Results will be correlated with experiments based on single-particle-tracking techniques developed in our research group [2-4]. We are planning to apply the RICS approach to the mobility of Balbiani Ring messenger-ribonucleoprotein particles (BR mRNPs) and the study of components of the nuclear pore complex in the nuclear envelope of eukaryotic cells.

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