

RASTER IMAGE CORRELATION SPECTROSCOPY (RICS): DIFFUSION IN GUVS AND CELL MEMBRANES USING ONE PHOTON EXCITATION AND ANALOG DETECTION.

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Raster Image Correlation Spectroscopy (RICS) allows for mapping the variations in local translational diffusion coefficient(s). The applicability of the technique has recently been extended to confocal laser scanning microscopes (CLSM) equipped with one-photon laser excitation and analog detection [1-4]. For a better understanding of the reproducibility and accuracy of RICS analysis of the top membranes of Giant Unilamellar Vesicles (GUVs) and cellular membranes [4], the influence and the constraints imposed by instrumentation characteristics and sample properties on the retrieved diffusion values have been simulated similar to Brown et al [1]. Influence of scan speed choice and detection noise was explored. Evidence is presented for a drop in mapped D values near the GUV perimeter and on the influence of a small total number of particles and small mapping brick size. Experiments show that the magnitude of correlated detection noise in our Zeiss LSM 510 META confocal system (build 2002) along the fast x-scan axis ($\psi = 0$) is considerably larger than reported for other confocal instruments [1-3]. Both the RICS package supplied by the Laboratory for Fluorescence Dynamics, University of California at Irvine as well as our Matlab software based on code supplied by Dr. D. Kolin (McGill University) gave similar simulation and translational diffusion mapping results. This work was supported by the Research Council of the UHasselt, tUL, the K.U.Leuven (GOA/2006/02) and by a Ph.D grant of the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen). Support by IAP P6/27 Functional Supramolecular Systems (BELSPO) is also gratefully acknowledged.

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