

PHASOR ANALYSIS OF LOCAL RASTER IMAGE CORRELATION SPECTROSCOPY (RICS) PROVIDES QUANTITATIVE MAPS OF DIFFUSION IN LIVING CELLS

Lorenzo Scipioni^{*†}, Melody Di Bona^{†§}, Simone Pelicci^{†§}, Giuseppe Vicidomini[§], Alberto Diaspro^{†§@}, Luca Lanza[†]

[†]Nanoscopy, [§] Molecular Spectroscopy and Microscopy and [@]Nikon Imaging Center, Istituto Italiano di Tecnologia, via Morego 30, 16163 Genoa, Italy

^{*}Department of Informatics, Bioengineering, Robotics and Systems Engineering, University of Genoa, Via All'Opera Pia, 13, 16145 Genoa, Italy

[§]Department of Physics, University of Genoa, via Dodecaneso 33, 16146, Genoa, Italy

KEY WORDS: ICS, RICS, Confocal Laser Scanning Microscope (CLSM), PLICS, Phasors

Image Correlation Spectroscopy (ICS) can be considered the imaging analog of Fluorescence Correlation Spectroscopy [1]. A wide variety of ICS-based methods (e.g. STICS, RICS, ICCS, kICS) have been developed as quantitative tools to investigate properties related to the transport (diffusion and flow), oligomerization state and interactions of fluorescent molecules in cells. An advantage of ICS-based methods is that they can be applied to image series obtained from commercial confocal laser scanning microscopes (CLSMs). For instance, Raster ICS (RICS) can be carried out on almost any CLSM for assessing the concentration and diffusion constant of proteins diffusing in living cells [2].

Although powerful, most ICS-based techniques do not perform very well on highly heterogeneous samples. This limitation comes from the fact that a single spatial correlation function, averaged over the entire image, is used to characterize the properties of the system. In this respect, we recently introduced a method, based on the phasor analysis of local ICS (PLICS), that extends ICS to heterogeneous systems without the need of a priori assumptions [3]. Being the cell an intrinsically heterogeneous system, we expect PLICS to improve performances of ICS-based methods for cellular applications.

In particular, here we present application of the PLICS concept to RICS for assessing spatial heterogeneities in molecular diffusion properties. Performing a phasor analysis of multiple local image correlation functions we obtain a high resolution map of diffusion constant in a heterogeneous system, with performances dependent on the number of frames analyzed. The analysis can be implemented on any commercial CLSM, even on those working with non-linear scanning patterns. As an application, we show maps of the diffusion constant of GFP in the nuclei of HeLa cells.

[1] Wiseman, P. W. "Image correlation spectroscopy: principles and applications" *Cold Spring Harb Protoc* 2015:336-348.

[2] Brown et al. "Raster image correlation spectroscopy (RICS) for measuring fast protein dynamics and concentrations with a commercial laser scanning confocal microscope". *Journal of microscopy* 2008 229:78-91.

[3] Scipioni et al. "Phasor Analysis of Local ICS Detects Heterogeneity in Size and Number of Intracellular Vesicles" *Biophysical Journal* 111:619-629