INTERACTION PATTERN ANALYSIS BY AUTOCORRELATION OF E-FRET IMAGES

Corentin Le Nézet, Gabriel Bidaux, Mélanie Henry, Alessandro Furlan, Mariano Gonzalez-Pisfil, Dorian Champelovier, Bernard Vandenbunder, Laurent Héliot

PhLAM, UMR 8523, CNRS and University of Lille 1
Bât. P5 - USTL-59655 Villeneuve d'Ascq cedex
E-mail : laurent.heliot@univ-lille1.fr

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FRET fluorescence microscopy is a well-established technique for detection of molecular interactions in living cells and tissues. Fluorescence lifetime measurement in time domain (TD-FLIM) using Time single photon counting hardware (TCSPC), is the most efficient method for FRET analysis in living cells thanks to its fluorochrome concentration-independence and its high spatial resolution. Current techniques allow to characterize the level of interaction between the molecules via statistical analysis of average lifetimes and FRET efficiencies (e-FRET). However, in most studies, the lifetime spatial distribution and the spatial organization (texture) of molecular interactions are analyzed via single images without a statistical approach.

We have developed a technique based on the study of e-FRET images by autocorrelation (named FICS) that takes advantage of information on spatial distribution of FRET efficiency. We have adapted the image correlation techniques previously developed by P. Wiseman et al. [1]. Analysis of lifetime images are produced by MAPI software with Phasor technique [2] and e-FRET patterns are processed by a FICS homemade software. The advantages of this approach are to allow an automatic analysis on a series of images, and to extract two parameters to characterize the e-FRET distribution in cells (average area of e-FRET patterns and density patterns). We applied this technique to the study of the transcription pause release by the Positive Transcription Elongation Factor (PTEFb) complex.

[1] P.W. Wiseman and N. O. Petersen, Biophys J, **76**, 963-977 (1999)

[2] A. Leray, C. Spriet, D. Trinel, Y. Usson and L. Héliot, *J Microsc*, **248,** 66-76 (2012)

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