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### Authors
Di Rienzo, Carmine
Gratton, Enrico
Beltram, Fabio
et al.



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**Fast Spatiotemporal Correlation Spectroscopy to Determine Protein Lateral Diffusion Laws in Live Cell Membranes**

Carmine Di Rienzo[1], Enrico Gratton[2], Fabio Beltram[1], **Francesco Cardarelli**[3].
[1]Istituto Italiano di Tecnologia, Scuola Normale Superiore & Istituto Nanoscienze, Consiglio Nazionale delle Ricerche, Pisa, Italy, [2]Laboratory for Fluorescence Dynamics, Biomedical Engineering Dept., University of California at Irvine, Irvine, CA, USA, [3]Istituto Italiano di Tecnologia, Pisa, Italy.

Here we present a straightforward image correlation analysis method to study the dynamics of fluorescently-labeled plasma-membrane proteins in live cells with high spatiotemporal resolution1. Notably, we don't extract and track each molecule, but we calculate population behavior using all molecules in a given region of the membrane. First, fast imaging of a given region on the membrane is achieved. Then, acquisitions at increasing time delays are correlated, for example each 2, 3, n repetitions. If particles diffuse, the width of the peak of the spatial autocorrelation function increases as the time delay between frames increases. Fitting of the series of autocorrelation functions enables to extract the actual protein 'diffusion law' from imaging, in the form of a mean square displacement vs time-delay plot (iMSD). The iMSD yields a quantitative view of the temporal evolution of the average molecular positions with nanometer accuracy, and no need for interpretative models. We demonstrate the potentiality of our approach by studying the regulation of protein lateral diffusion in live cell membranes. By using a GFP-tagged variant of the Transferrin Receptor (TfR) we are able to observe the regulation of protein diffusion imparted by the cytoskeleton meshwork on μm-sized membrane regions in the micro-to-milli-second time range. We show that our approach can successfully recover TfR diffusion parameters over many microns, and their variation in response to drug treatments or temperature shifts. Potential extension of this method to the 3D intracellular environment and differences with respect to other approaches will be discussed.

1. Di Rienzo, C., Gratton, E., Beltram, F. & Cardarelli, F. Proc Natl Acad Sci USA 110, 12307-12 (2013).

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