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Authors
Hellriegel, Christian
Caiolfa, Valeria R
Sidenius, Nicolai
et al.

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Receptor-Ligand Interactions in the Plasma Membrane of Live Cells Resolved in Space and Time by N&B Analysis

Christian Hellriegel[1], Valeria R. Caiolfa[2,1], Nicolai Sidenius[3], Enrico Gratton[4], Moreno Zamai[2,1].
[1]CNIC, Madrid, Spain, [2]San Raffaele Scientific Institute, Milano, Italy, [3]IFOM- Italian Cancer Research Foundation FIRC, Milano, Italy, [4]Laboratory for Fluorescence Dynamics, University of California Irvine, Irvine, CA, USA.

In this presentation we show how we push the Number and Brightness analysis (N&B) to the limits of applicability. We demonstrate that by N&B we can observe how a GFP labeled membrane receptor (namely uPAR) dimerizes upon ligand binding in live cells. We show how we obtain real time, spatially and temporally resolved images of the molecular reorganization of uPAR in the cell membrane. These results are backed by extensive simulations, and by well-defined live cell calibration experiments (using monomeric and dimeric GFP-uPAR constructs).

N&B quantifies the amplitudes of fluorescence intensity fluctuations as individual fluorescent species diffuse in and out of a pixel in a series of images. The basic idea is that the amplitude fluctuations of a diffusing molecule labeled with two dyes (e.g. a dimer, or a bound ligand-receptor pair) will be twice as large as the amplitudes of a molecule with only one dye (i.e. a monomer, or the unbound ligands and receptors), simply because the doubly labeled object is twice as bright as the individual one.

N&B is related to fluctuation spectroscopy such as fluorescence correlation spectroscopy FCS and photon counting histogram, PCH. These methods can resolve molecule-molecule interactions, but are usually restricted to the acquisition at one specific pixel. N&B was described recently for 2-photon scanning microscopy. There, N&B was typically used to distinguish between mobile molecules and large aggregates in cells, using time-sequences of about 50-100 frames (typically 512x512 pixels at 4s/frame).

However, when attempting to distinguish between monomers and dimers, as the smallest possible increment of molecule-molecule interactions, the experimenter is confronted with low signal-to-noise ratios and long-term perturbations (cell movement, vesicle trafficking). In this work we describe how we have resolved these issues.