

A DETECTION BASED FRAMEWORK FOR SYSTEMATIC ANALYSIS OF DUAL COLOR TIRF MICROSCOPY DATA

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Total Internal Reflection Fluorescence (TIRF) microscopy allows for the imaging of events occurring at a narrow depth (100nm-300nm) from a glass cover slip. Such ability is particularly useful in cell biology when events occurring at or near the membrane, involving communication between the cell and the external medium, are of interest. Those studies typically involve an important number of localised individual events which may be correlated across two channels. For this purpose an EM-CCD camera equipped with a image-splitter was installed on a TIRF microscope, allowing for in vivo simultaneous dual color TIRF acquisition. In this work is presented a framework for such study based on the automated detection of events at the membrane and their analysis.

The biological model under study involves trans-membrane protein endocytosis/recycling pathways. During their recycling, individual vesicles fuse with the plasma membrane. Due to the exponentially decreasing nature of the sensitivity of TIRF microscopy in z , it corresponds to a sudden appearance in the image. The proposed framework is based on a novel statistical patch-based change detection algorithm that allow for the exhaustive detection of appearances. When repeated across several acquisitions, there are enough individual events for a statistical or biophysical analysis of the resulting collection to be relevant.

Using that framework, we compare the recycling of langerin and Transferin receptor (TfR) and show that at least two biophysical mechanisms are responsible for the behaviour of langerin vesicles at the membrane, where TfR ones shows only diffusion. Dual color experiments involving langerin and Rab11, a protein known to be involved in vesicle recycling at the membrane, are also presented, opening the door for other pairwise experiments and allowing for the in vivo study of the spatio-temporal mechanisms of recycling at the membrane.

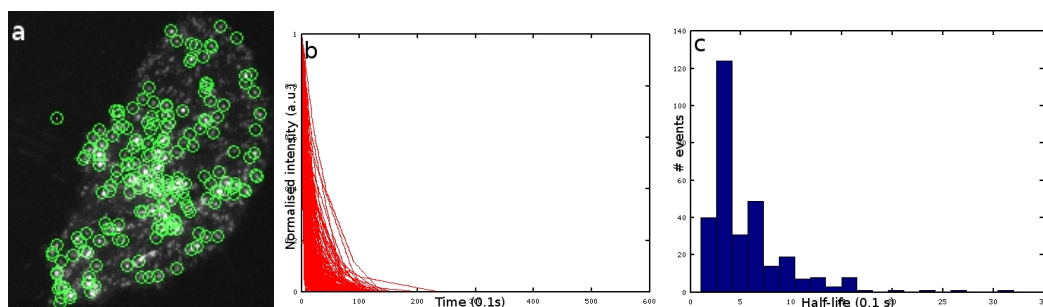


Figure 1: a) TIRFM, projection in maximum of recycling TfR, with events automatically detected in green; b) a normalised decrease in time is computed for all events; c) histogram of the half-lives of the events, computed using the normalised decrease.