

REPETITIVE AND TRANSIENT EVENT DETECTION IN FLUORESCENCE VIDEO-MICROSCOPY

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We worked on image sequences obtained from living cells stably expressing different xFP tagged proteins involved in exocytosis, recycling and fusion events. Since many years, an array of microscopy approaches has been used to get deeper insights in the dynamics of these cellular events. These include Fast 4D microscopy eventually associated with F techniques and total internal reflection fluorescence (TIRF). Among diverse molecular behaviors reported by previous video microscopies, membrane concentration of fluorescently tagged proteins often give rise to sudden spot appearance and/or disappearance. In order to quantify accurately, the numbers, the frequencies and the half lives of these transient membrane structures, a robust and automatic approach that could be adapted for either 3D or TIRF series of images, would be necessary. Yet, adapted quantitative and automatic analyses for such very fast occurring phenomenon are very limited and have never been compared for these diverse microscopy approaches. We present here two methods for the detection of these specific events occurring inside the living cell. These events correspond to temporal membrane concentration of fluorescently tagged proteins occurring in different optical planes and look like spotlights appearing or vanishing suddenly in videomicroscopy images sequences. Both approaches are based on the comparison of small image patches. The first one tries to locate in the next frame a small similar patch. The second one tries to estimate a small image patch in one frame by using a small set of patches taken in the next frame. If the restoration process fails then the algorithm concludes that the two frames were too different at this spatial location. This corresponds to a meaningful change in detection between the two frames. We validate these two methods by using simulated data as well as real image sequences. These methods allow statistical analysis of these types of events in different experimental conditions 1) on untreated living cells 2) on cells treated with drugs affecting the intracellular trafficking.

As considered in a more integrative view, none of the unique molecules within a cell works as an isolated entity. The gathering or the dispersion of many units of one type of them visualized here as spot appearing or vanishing, obviously correspond to a dynamics architecture involving the engagement of multiple partners. Knowledge on the biological and biophysical characteristics of some of these compounds at the level of the whole living cell, require methods allowing a rapid and objective comparison of mass data. Then, we have started to use these detection methods to compare the influence of diverse molecular regulators of one particular reporter protein in living cells by performing siRNA or mutant based approaches.