2013

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Tracking protein dynamics with photo-convertible Dendra2 on Spinning Disk Confocal Systems

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Introduction

Eukaryotic cells regulate protein function by controlling their access to sub-cellular compartments. Knowledge of the dynamic associations of proteins with sub-cellular compartments is therefore a key determinant in understanding their function. In this context, we present two complementary techniques intended for characterising protein trafficking pathways in living cells. Firstly, we introduce the FRAP-PA unit (Andor) combined with the Nikon Eclipse Ti E Spinning Disk Confocal Microscope (SDCM) as a new & robust platform to exploit the photo-convertable properties of the fluorescent protein Dendra2 (Evrogen). A major advantage of the SDCM is the rapid acquisition speed, enabling high-temporal resolution of cellular processes. Furthermore, photo-conversion and imaging are less invasive on the SDCM as the cell exposure to illumination power is reduced, thereby minimising photo-bleaching and increasing cell viability. Using two fast trafficking proteins (UBC9, Fibrillarin) as proof of principle, we describe step by step procedures, with emphasis on image acquisition & processing parameters, to successfully characterise Dendra2-fused proteins trafficking pathways in live cells & in real-time. Subsequently, we present novel analytical software comprised of a simple user interface which allows the user to track the fluorescence of selected points over time, and we describe in depth the steps required to process the acquired data & analyse the resultant images. The image processing stage includes red/green image identification & separation, noise filtering, background extraction, contrast stretching & temporal smoothing. Image analysis includes the construction of mean & standard deviation images, classification of cell regions & photo-conversion point approximation.

Acquisition

Figure 1. Spectral properties of Dendra2.

Table 1: Characterisation of Dendra2. Spectral properties of the fluorescent protein Dendra2 (Evrogen). A major advantage of the SDCM is the rapid acquisition speed, enabling high-temporal resolution of cellular processes. Furthermore, photo-conversion and imaging are less invasive on the SDCM as the cell exposure to illumination power is reduced, thereby minimising photo-bleaching and increasing cell viability. Using two fast trafficking proteins (UBC9, Fibrillarin) as proof of principle, we describe step by step procedures, with emphasis on image acquisition & processing parameters, to successfully characterise Dendra2-fused proteins trafficking pathways in live cells & in real-time. Subsequently, we present novel analytical software comprised of a simple user interface which allows the user to track the fluorescence of selected points over time, and we describe in depth the steps required to process the acquired data & analyse the resultant images. The image processing stage includes red/green image identification & separation, noise filtering, background extraction, contrast stretching & temporal smoothing. Image analysis includes the construction of mean & standard deviation images, classification of cell regions & photo-conversion point approximation.

Applications

1. Tracking the inter-nuclear migration of nuclear proteins

2. Tracking protein nuclear-cytosolic & sub-nuclear trafficking

3. Delineating protein associations with sub-nuclear bodies

Analysis

Figure 2. Spectral properties of Dendra2. Dendra2c and Dendra2Fibrillarin vectors (Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia)

Data Analysis: The data set is exported as a Multi-TIFF file and analyses using an algorithm developed in MATLAB. The image sequence is first processed to remove noise, enhance contrast and generally improve image quality across the sequence. Dendra2 trafficking is characterised by analyzing the variation in red mean fluorescence intensity within cellular regions of interest (ROIs) over the time series. Selection and demarcation of the ROIs depend on the protein of interest trafficking characteristics and is a user input to the system. A mean image of the entire sequence is generated to aid in the user’s workflow. Points of interest have been selected, the algorithm automatically seeks out connected similar points in the nearby area whilst using a seeded region growing technique. The mean fluorescence of these regions is then tracked through the image sequence allowing graphs to be automatically produced of mean fluorescence over time. Finally a "migration map" is generated by classifying the signal variation (D) of individual pixels over the time series, providing an overview of Dendra2 trafficking during the time series with respect to the point of photo-conversion. The migration map is colored to represent different trafficking behaviors. Within Accumulation within a region: Dark Grey; Migration away from the region: light grey; Migration into a region: Black; Unclassified.

Applications

1. Tracking the inter-nuclear shuffling of nuclear proteins

2. Tracking protein nucleo-cytosolic & sub-nuclear trafficking

3. Delineating protein associations with sub-nuclear bodies

Acknowledgements:

The authors wish to acknowledge the access to and use of the UCD Conway Imaging Core Technologies, Conway Institute for Biomolecular and Biomedical Research, University College Dublin. In particular, we wish to acknowledge Prof. Dimitri Scholz director of Biologolecular Imaging, Conway Institute, UCD, for his assistance with image acquisition and photo-conversion on the Spinning Disk Confocal Microscope (funded by Science Foundation Ireland) and the authors wish to acknowledge Dr. Konstantia Papoutsaki for his generous gifts of the Dendra2 and Dendra2Fibrillarin vectors (Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia). This work is funded by the National Virus Reference Lab (NVRIL), University College Dublin.