Journal of Visualized Experiments 2016 vol.2016 N111

Time-lapse video microscopy for assessment of EYFP-Parkin aggregation as a marker for cellular mitophagy

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Abstract

© 2016 Journal of Visualized Experiments.Time-lapse video microscopy can be defined as the real time imaging of living cells. This technique relies on the collection of images at different time points. Time intervals can be set through a computer interface that controls the microscope-integrated camera. This kind of microscopy requires both the ability to acquire very rapid events and the signal generated by the observed cellular structure during these events. After the images have been collected, a movie of the entire experiment is assembled to show the dynamic of the molecular events of interest. Time-lapse video microscopy has a broad range of applications in the biomedical research field and is a powerful and unique tool for following the dynamics of the cellular events in real time. Through this technique, we can assess cellular events such as migration, division, signal transduction, growth, and death. Moreover, using fluorescent molecular probes we are able to mark specific molecules, such as DNA, RNA or proteins and follow them through their molecular pathways and functions. Time-lapse video microscopy has multiple advantages, the major one being the ability to collect data at the single-cell level, that make it a unique technology for investigation in the field of cell biology. However, time-lapse video microscopy has limitations that can interfere with the acquisition of high quality images. Images can be compromised by both external factors; temperature fluctuations, vibrations, humidity and internal factors; pH, cell motility. Herein, we describe a protocol for the dynamic acquisition of a specific protein, Parkin, fused with the enhanced yellow fluorescent protein (EYFP) in order to track the selective removal of damaged mitochondria, using a time-lapse video microscopy approach.

http://dx.doi.org/10.3791/53657

Keywords

Cellular biology, FCCP, Fibroblast, Issue 111, Mitochondria, Mitophagy, Parkin, Time-lapse video microscopy