

The effects of antioxidants on photo-toxicity in fluorescence live-cell imaging

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Keywords: Live cell imaging; phototoxicity; Vitamine E, Trolox; mitosis.

Abstract: Live cell imaging has become an increasingly important tool in biomedical research. In combination with fluorescent markers such as Green Fluorescent Protein (GFP), it has allowed the visualization of subcellular distribution of molecules and study of the dynamics of cellular processes. However, cells observed under fluorescence microscopy may be subject to photodamage due to the production of light-induced reactive oxygen species (ROS). These ROS may disrupt the redox homeostasis of the cells and result in physiological damage of the cells. Therefore, various antioxidants have been used anecdotally in live cell imaging to protect cells from photodamage. The aim of this study was to investigate the protective effects of various antioxidants in live cell imaging. Using the photobleaching half-life of a fluorescent DNA dye (Hoechst 33342) as an indirect assay for phototoxicity, we have established that Trolox, a derivative of Vitamin E, demonstrated a significant reduction in the photobleaching rate of the dye in live HeLa cells. The reduction rate increases linearly with increasing concentration of Trolox within the tested concentration range (100-800 μ M). Additionally, we have demonstrated that there is no significant increase in cell death and mitotic index change in HeLa cells within the tested concentration range. We have also compared different treatment periods and demonstrated that there is no significant difference in the reduction of photobleaching rate between cells treated for 24hrs versus 1hr. Compared to other antioxidants which have been used in live cell imaging (Vitamin C, NADPH, epinephrine), Trolox exhibited the most effective protective effect in fluorescence microscopy. Taken together, our data suggests that a 300 μ M, 1hr Trolox treatment can be used to minimize phototoxicity in live cell fluorescence microscopy.