

Photoswitching and Vesicle Tracking Applications using the Ultra *VIEW* PhotoKinesis accessory

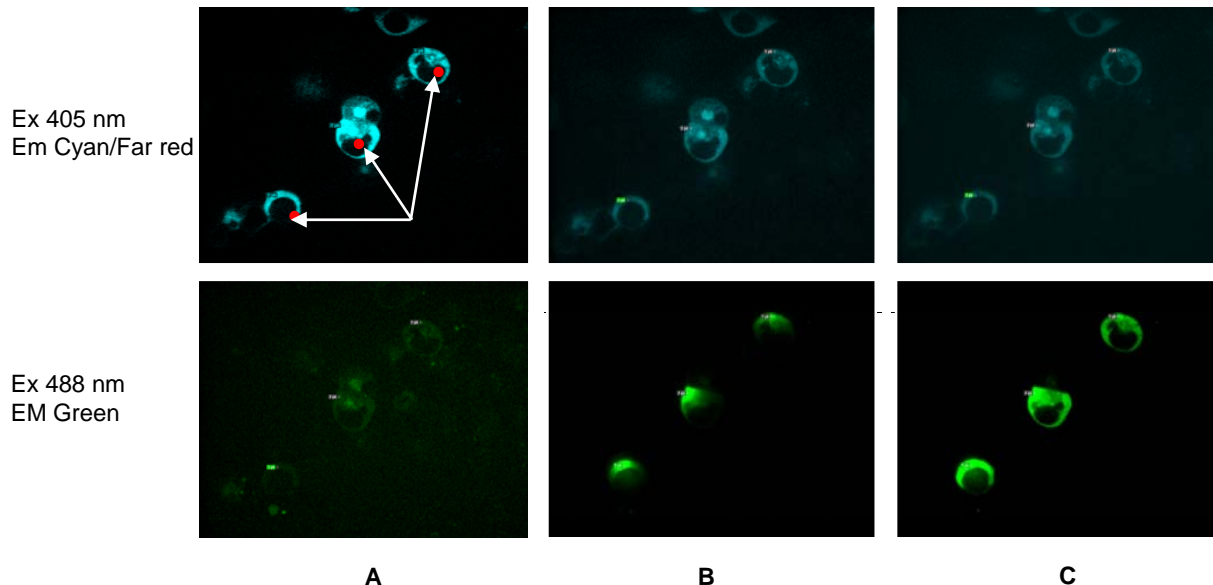


Figure shows photoswitching of HeLa cells transiently transfected with PSCFP2-SNX1. Using the **Ultra *VIEW* PhotoKinesis™** accessory, cells in the chosen region of interest (ROI) were successfully photoswitched within 100ms. Visualization of PSCFP2-SNX1 was performed using 405nm excitation and tracking at 488nm at 70% laser power. **A)** Shows proteins before photoswitching, **B)** shows proteins photoswitched and **C)** shows proteins 15 s after photoswitching.

The discovery of photoactivatable and photoswitchable fluorescent proteins which can be manipulated by laser energy has made a significant impact in the cellular research of endosomal transport and fast intracellular dynamics. PerkinElmer Application Specialists in collaboration with the research group of Professor Pete Cullen at the Department of Biochemistry, University of Bristol have focused on sortin nexin 1 (SNX1), a protein which has been implicated in the regulation of the intracellular trafficking of internalized cell surface receptors. In this study HeLa cells were transfected with PS-CFP2-SNX1. PS-CFP2 (photoswitching cyan fluorescent protein) is a photoactivatable, dual-color, monomeric protein capable of irreversible photoconversion from cyan to a green fluorescent form in response to 405 nm light irradiation.

To investigate the photoswitching capabilities of PS-CFP2 and thus the intracellular trafficking of SNX1, live cell imaging was performed with the **Ultra *VIEW* ERS** spinning disk confocal imaging system equipped with the **PhotoKinesis™** accessory. Cells (**figure**) were successfully photoswitched within 100 ms at 100% laser power and 120 bleach cycles. Visualization of PS-CFP2-SNX1 was performed using 405 nm excitation and tracking at 488nm at 70% laser power. To track PS-CFP2-SNX1 the "Track-it" feature of the PhotoKinesis accessory was used. Results showed that PS-CFP2-SNX1 was effectively transported along the endosome, TGN (trans Golgi network) pathway.

This experiment demonstrated the unique abilities of PSCFP2 to be targeted to acidic organelles and the ability of the **PhotoKinesis™** accessory to track the dynamics of rapidly moving SNX1. Additionally, it was shown that the PhotoKinesis accessory offers **Ultra *VIEW*** spinning disk users the ability to target concentrated light onto a user-defined region of interest (ROI) and photoactivate a region of any shape size and position whilst minimizing photobleaching associated with sequential image capture. These experiments also demonstrate the unique abilities of the 'Track-it' technology to isolate and track vesicles of interest providing more information on the trafficking in the endosomal to TGN pathway.