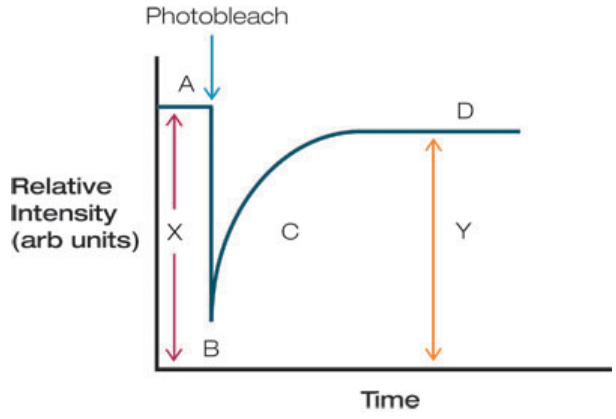
FRAPPA

Andor's FRAP solutions

Fluorescence Recovery After Photo bleach (FRAP) and Photo Activation (PA) provides a tool for precisely controlled and targeted application of the selected laser beam to initiate photo-effects, which includes: (FRAP) fluorescence recovery; (FLIP) rate of loss with repeated bleaching; (PA) activation and ablation (with Q-switched UV laser). Imaging the effects reveals qualitative and quantitative information about the cellular structures of interest and their environment. For example, photo-activated fluorescent proteins are becoming widely used to provide a spotlight on small numbers of activated molecules, UV uncaging is used to deliver agonists to specific cell compartments and photoactivation is used in drug discovery to study detailed effects of photo-sensitive topical compounds.



FRAPPA techniques allow measurement of molecular recruitment rates, trafficking and turnover in single cells and sub-cellular organelles and applications include membrane and protein binding, mytosis and cytoskeleton function. In these studies GFP is commonly used in a fusion protein complex to label a protein of interest, with the bleach or activation phase marking those molecules for observation. Quantification of motion or recovery can then provide a useful measure of molecular mobility.

FRAP is used increasingly in analytical devices to determine the identity of unknown substances (based on diffusion analysis), understanding cellular transduction, and identifying ligand binding sites.

The figure above shows a typical profile of fluorescence intensity collected during a FRAP experiment. Baseline fluorescence intensity is collected (A) before the photo-bleaching occurs (arrow) so that the amount of fluorescence is substantially reduced (B). This takes a finite time, as indicated by the period of low fluorescence following. After bleaching is stopped, fluorescence in the area increases as unbleached molecules diffuse into this area (C). A steady state is reached (D) when the recovery is complete. The percent recovery: (Y/ X) x 100 = % recovery. In practice, the percent recovery almost never reaches 100%. The lateral mobility is determined by the slope of the curve (C). A steeper curve indicates a faster recovery, reflecting higher molecular mobility or diffusion.

Reference: Sprague, B., R. Pego, et al. Analysis of Binding Reactions by Fluorescence Recovery after Photobleaching. Biophys. J. 2004 Jun;86(6):3473-3495