

Controlling photo-activation in 3D: optical tools for spatial confinement.

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Photo-activatable Green Fluorescent Protein (PAGFP) [1] exhibits peculiar photo physical properties making it an invaluable tool for protein/cell tracking in living cells/organisms [1-3]. PAGFP is normally excited at near UV wavelength (405 nm), with an emission peak centered at 520 nm. Absorption cross section at 488 nm is low in the not-activated form. However, when irradiated with high-energy fluxes at 405 nm, the protein shows a dramatic change in its absorption spectra making it efficiently excitable at 490 nm. Confocal microscopes can efficiently control excitation energy in the focal plane. However, irradiation extends to the entire illumination volume, reaching up to several microns and making impossible to limit the photo-activation process in the space.

In the present study, we characterized the activation volume with two different techniques to obtain efficient energy-spatial confinement. Total Internal Reflection Fluorescence Microscopy (TIRFM) allows for excitation of fluorescent molecules inside an evanescent electromagnetic field at interfaces such as cellular membranes. Optimization of the optical set up of an objective based TIRF system allowed to activate PAGFP fused to a variety of membrane localizing fusion proteins under total internal reflection. Characterization of the penetration depth showed that activation is efficiently confined in the third dimension. Two-photon fluorescence microscopy removes the restriction of localization at interfaces providing optical confinement at any focal plane. We therefore characterized two-photon excitation and activation properties of PAGFP [4], showing that two-photon imaging and activation is feasible in the 750-820 nm range producing a narrow confinement along the optical axis. Spatial extension of the activated volume shows energy dependence in good agreement with the theoretical expectations.

Reference:

1. Patterson GH, Lippincott-Schwarz J. A Photoactivatable GFP for Selective Photolabeling of Proteins and Cells. *Science* **297**, 1873-1877, (2004).
2. Patterson GH, Lippincott-Schwartz J. Selective photolabeling of proteins using photo activatable GFP. *Methods* **32**(4), 445-450, (2004).
3. Post JN, Lidke KA, Rieger B, Arndt-Jovin DJ. One- and two-photon photoactivation of a paGFP-fusion protein in live Drosophila embryos. *FEBS Lett.* **579**(2), 325-330, (2004).
4. Schneider M, Barozzi S, Testa I, Faretta M, Diaspro A. Two-photon activation and excitation properties of PA-GFP in the 720-920 nm region. *Submitted*, (2004).