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**Photoactivatable Green
Fluorescent Protein (PA-GFP):**
a valuable tool for selective photolabelling of
proteins and cells

Abstract:

Fluorescent proteins are indispensable in the study of proteins in living cells. To study protein dynamics, however, it is necessary to selectively mark a sub-population of protein molecules. This is possible using Photoactivatable Green Fluorescent Protein (PA-GFP). PA-GFP is a mutant form of GFP, in which fluorescent intensity can be increased over a 100 fold after activation with ~400nm light. This technical note describes the evolution and characteristics of PA-GFP together with Nikon's hardware and software solutions that allow researchers to easily perform PA-GFP experiments.

Introduction:

Fluorescent proteins, fused to a protein of interest, allow the study of proteins and cellular processes in living cells, tissues and even complete organisms. Ever since the discovery and cloning of the first fluorescent protein (green fluorescent protein - GFP), researchers have attempted to improve the properties of these useful proteins. Directed evolution has removed unwanted oligomerisation, improved quantum yields and photostability, and has produced several sub-species with shifted spectra.

To study the dynamics of protein or cell movement it is necessary to selectively discriminate a sub-population of protein molecules or cells. One approach is to bleach a subpopulation of labelled molecules and to record the redistribution of unbleached molecules in the bleached area over time by a technique called Fluorescent Recovery After Photobleaching (FRAP). The selective activation of a subpopulation of proteins however, allows more flexible experimental design.

Technology

Wild-type GFP, normally exists as a mixed population of two forms, giving rise to a major and a minor absorbance peak at 397nm and 475nm respectively. With strong illumination at ~400nm, most of the molecules are converted to the 475nm form (Figure 1A). This capacity for photoconversion has led to

the development of a form of GFP, which has very low absorbance at 475nm and which can very efficiently be converted by ~400nm irradiation (Figure 1B). This form of GFP, better known as Photoactivatable GFP (PA-GFP), exhibits very low green emission (max 517nm) after 488nm excitation and can be activated ~100-fold by stimulation with 405nm light.

Selective activation of PA-GFP can be achieved both on a widefield fluorescent microscope as well a Laser Scanning Confocal Microscope (LSCM). In the case of a widefield microscope, selective activation of a portion of PA-GFP can be achieved by using Nikon's PA-GFP unit on a standard inverted microscope equipped for fluorescence (Figure 2A). This unit is placed on the back port of the microscope and uses a fibre-coupled laser (405 nm) to illuminate a diffraction limited spot on the specimen. After activation, time-lapse images are recorded by a digital camera using appropriate illumination through an epi-fluorescence attachment. Besides activation of PA-GFP this unit can be used for any type of laser-based point spot illumination.

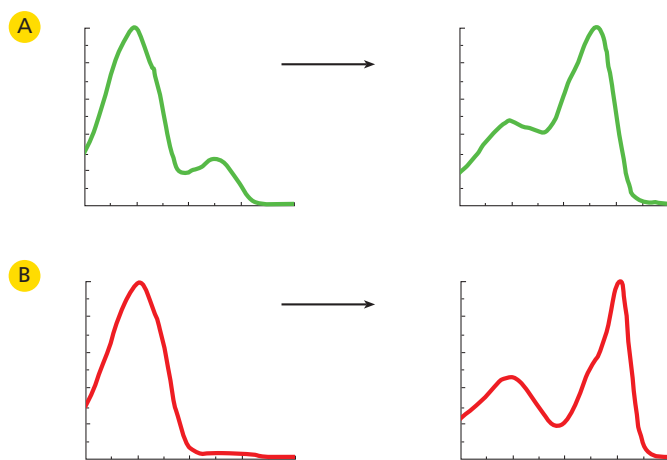


Figure 1. Absorbance spectra of WT-GFP (A) and PA-GFP (B) before and after activation with ~400nm light

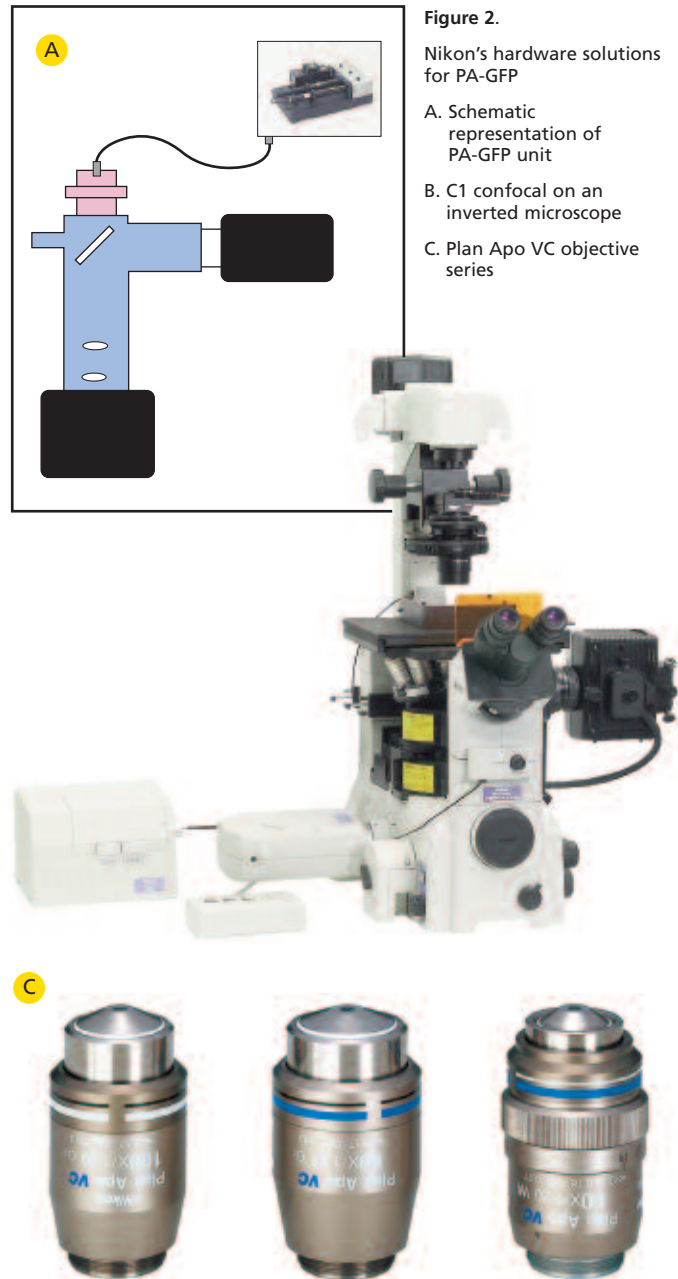


Figure 2. Nikon's hardware solutions for PA-GFP
 A. Schematic representation of PA-GFP unit
 B. C1 confocal on an inverted microscope
 C. Plan Apo VC objective series

Photoactivation on a LSCM (Figure 2B) can be achieved in two ways. Depending on the type of laser control, a region of interest (ROI) can be activated either by the zoom capacity of the scan mirrors or the local onset of laser power. With the zoom method, any size of a rectangle down to a single spot can be activated. The second method requires laser control through either an AOM (acousto-optic modulator) or AOTF (acousto-optic tunable filter) and allows for total flexibility in choosing the shape of the ROI.

To facilitate easy experimental set-up, Nikon provides dedicated software modules. These provide guidance through an experiment and deliver data, which can be further analysed by specialised third party software.

The use of PA-GFP requires activation with ~400nm light. Conventional optics are chromatically corrected for a maximum of four lines (436, 486, 588 and 656nm). Nikon has recently developed the Plan Apochromat VC (violet corrected) objective series (Figure 2C) in which chromatic correction is extended for the 405nm line. The use of these objectives result in activation with 405nm and acquisition with 488nm light occurring at the same axial level. This is illustrated in Figure 3. A confocal Z-stack was made from DAPI/FITC/TRITC triple stained cells with both a VC Plan Apo and a conventional non-VC Plan Apo objective. In the case of non-VC, the 405nm light used to excite DAPI is focused at a different Z-level compared to that of FITC and TRITC, which results in an axial shift in the emission of DAPI. By using the VC objective this axial shift is corrected (figure 3A).

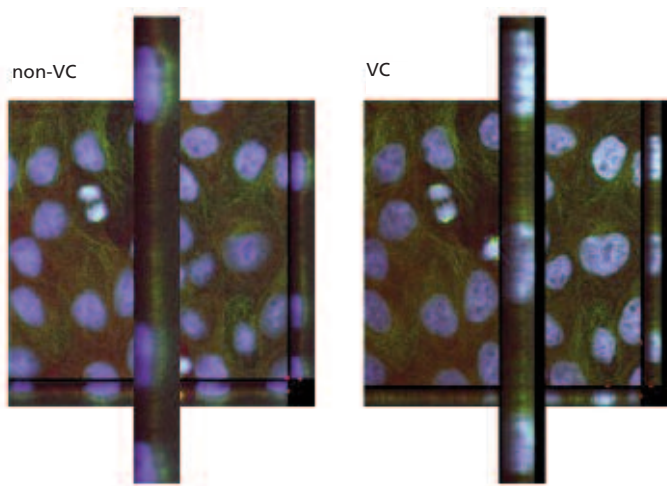


Figure 3a. Comparison of VC vs non-VC objective
Confocal Z-stack of triple stained cells (DAPI/FITC/TRITC)

Application:

The following experimental example demonstrates the use of PA-GFP in studying the dynamics of proteins within neurites/processes in neural cells. Microtubules are an important element of the cellular cytoskeletal structure and together with microtubule-associated proteins (MAP's) guide the formation and growth of, for example, axons in neural cells. To study the dynamics of tau, a MAP that is normally enriched in axons, a fusion construct between human tau and pa-gfp was stably transfected in neural cells. Photoactivation of a small spot, directed at a long segment of an individual process, was performed with a Nikon C1 confocal microscope equipped with a blue 405nm diode and an Argon ion laser using the zoom method. Nikon's dedicated PA-GFP software tool was used to

select the activation region and record intensity changes and spatial distribution of fluorescence over time. Figure 3B shows a result of such an experiment. Initial pre-activation PA-GFP fluorescent intensity is very low. After activation, a clear increase in fluorescent intensity at the site of activation can be observed and the redistribution of activated molecules can be followed over time.

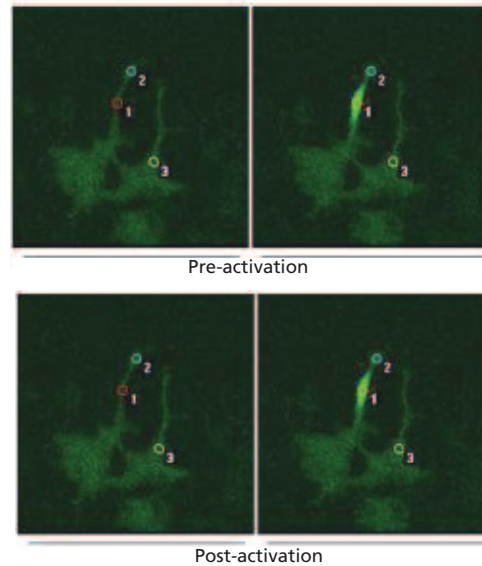
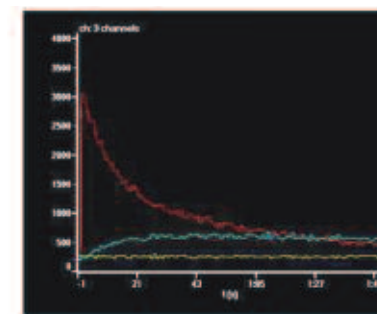


Figure 3b. pa-gfp-tau activation experiment pre- and post-activation images and graphical representation of intensities over time



Conclusion:

PA-GFP is a very useful tool for studying protein movement in living cells or tracking individual cells in a population of cells or in tissue. Recent developments have resulted in the discovery and creation of a whole range of similar photo-modulatable fluorescent proteins (Kaede, Dronpa Green, KFP1), which allow for even more flexibility and complexity in experimental design. Nikon provides useful hardware and software solutions to perform these types of studies and continuously develops new tools to keep abreast of these advances.

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Authors Background

Robert Stad is an application manager for Nikon Instruments Europe BV and is responsible for high end (biological) research applications. After finishing his PhD at the department of Molecular and Cell Biology and Centre for Biomedical Genetics of the Leiden University Medical Centre, he worked in the field of microarray and gene expression analysis for 5 years. He joined Nikon in 2005.

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Photo Activatable GFP (PA-GFP) is a valuable tool in the study of protein dynamics in living cells

Photoactivation can be performed on both widefield and Laser Scanning Confocal Microscopes

The use of 405nm light in combination with longer wavelengths requires violet-corrected optics

Nikon provides suitable hardware and software solutions to perform PA-GFP experiments

For more information on PA-GFP go to:

www.nikoninstruments.com/cpagfp



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