

Photostimulation time-lapse imaging of live HeLa cells using confocal microscopy

The light induced dimerization (LID) system, an optogenetic tool, uses photo-responsive proteins showing homo- or hetero-dimerization upon light illumination, allowing us to manipulate cell signaling.

This application note introduces an example of time-lapse imaging of activation and deactivation of the red/far-red responsive proteins expressed in HeLa cells, using the AX/AX R confocal microscope and NIR imaging option.

Keywords: optogenetics, confocal microscope, NIR imaging option

Overview of experiment

A photoreceptor protein derived from higher plants, phytochrome B (PhyB), is characterized by binding to its binding factor, the phytochrome-interacting factor (PIF), by absorbing red light, and rapidly separating from the PIF by absorption of far-red light.

PhyB-mCherry-HRasCT, which is localized to the cell membrane, and its binding factor, PIF3-mEGFP, were expressed in HeLa cells. In addition, SynPCB, which synthesizes phytochrome chromophores was expressed in the HeLa cells, and the Blvr A gene was deleted.

It is assumed that, when irradiated with red light (640 nm), PhyB-mCherry-HRasCT should bind to PIF3-mEGFP and colocalization should be observable, and that irradiation with near-infrared light (730 nm) should separate the two and prevent their colocalization.

We observed binding and separation of PhyB and PIF3 using a confocal microscope that supports near-infrared (NIR) excitation.

Imaging conditions: scanning mode; galvano scanner, resolution; 1024 x 1024 pixels, zoom; 3x, objective; CFI Plan Apochromat Lambda D 40XC

Results

HeLa cells were photostimulated with red light (640 nm) and near-infrared light (730 nm), and images were acquired after each stimulation using 488 nm excitation light. As a result, the binding and separation of PhyB-mCherry-HRasCT and PIF3-mEGFP caused by photostimulation were observed.

Summary

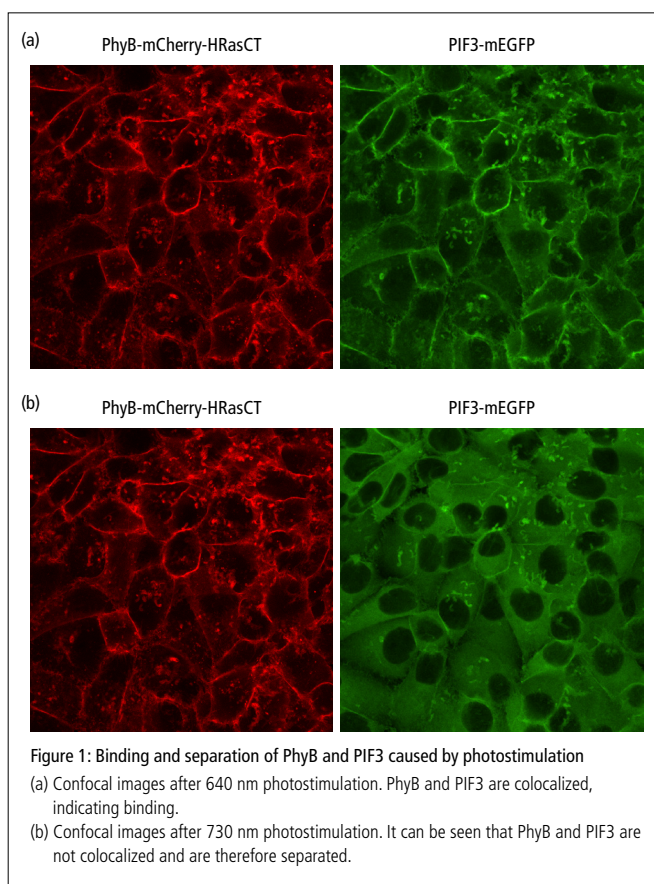
The optical response of phytochrome B could be visualized by mounting an option that enabled 730 nm or 785 nm near-infrared light excitation on a confocal microscope. This method can be applied not only to multicolor fluorescence imaging but also to optogenetics, and is expected to contribute to simultaneous acquisition of multiple biological reactions and structural information.

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Reference

Uda Y, Miura H, Goto Y, Yamamoto K, Mii Y, Kondo Y, Takada S, Aoki K
 Improvement of Phycocyanobilin Synthesis for Genetically Encoded Phytochrome-Based Optogenetics
ACS Chem. Biol. 2020, 15, 11, 2896–2906,
<https://doi.org/10.1021/acscchembio.0c00477>



Product Information

AX/AX R Confocal Microscope NIR Imaging Option

- High speed: Up to 720 fps (resonant at 2048 x 16 pixels)
- High resolution: Up to 8K (galvano) / 2K (resonant)
- High throughput: Ultra-wide FOV of 25 mm
- NIR imaging option that enables excitation at 730 nm or 785 nm, and two types of detector that can detect near-infrared light

