

LONG-TERM LIVE-CELL MICROSCOPY WITH NANOBODIES DELIVERED BY LASER-INDUCED PHOTOPORATION

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Fluorescence microscopy is the method of choice for studying intracellular dynamics. However, its success depends on the availability of specific and stable markers. A prominent example of markers that are rapidly gaining interest are nanobodies (Nbs, ~ 15 kDa), which can be functionalized with bright and photostable organic fluorophores. ^[1] Due to their relatively small size and high specificity, Nbs offer great potential for high-quality long-term subcellular imaging, but suffer from the fact that they cannot spontaneously cross the plasma membrane of living cells. We have recently discovered that laser-induced photoporation is well suited to deliver extrinsic labels into living cells without compromising their viability. ^[2] Being a laser-based technology, it is readily compatible with light microscopy and the typical cell recipients used for that. Spurred by these promising initial results, we demonstrate here successful long-term imaging of specific subcellular structures with labeled nanobodies in living cells. We illustrate this using Nbs that target GFP/YFP-protein constructs accessible in the cytoplasm, actin-bundling protein Fascin, and the histone H2A/H2B heterodimers. With an efficiency of more than 80% labeled cells and minimal toxicity (~ 2%), photoporation proved to be an excellent intracellular delivery method for Nbs. Time-lapse microscopy revealed that cell division rate and migration remained unaffected, confirming excellent cell viability and functionality. We conclude that laser-induced photoporation labeled Nbs can be easily delivered into living cells, laying the foundation for further development of a broad range of Nbs with intracellular targets as a toolbox for long-term live-cell microscopy.

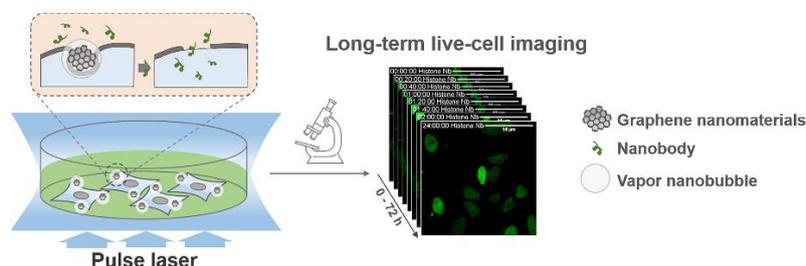


Figure 1. Schematic illustration shows fluorescent dye labeled nanobodies delivered into living cells by laser-induced photoporation for specific subcellular labeling. Long-term live-cell fluorescence microscopy imaging is performed up to 72 h.

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