# REPEATED PHOTOPORATION WITH GRAPHENE QUANTUM DOTS ENABLES HOMOGENEOUS LABELING OF LIVE CELLS WITH EXTRINSIC MARKERS FOR FLUORESCENCE MICROSCOPY

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In the replacement of genetic probes, there is increasing interest in labeling live cells with high-quality extrinsic labels, which avoid over-expression artifacts and are available in a wide spectral range. This calls for a broadly applicable technology that can deliver such labels unambiguously to the cytosol of living cells. Here, we demonstrate that nanoparticle-sensitized photoporation can be used to this end as an emerging intracellular delivery technique.[1] We replace the traditionally used gold nanoparticles with graphene nanoparticles as photothermal sensitizers to permeabilize the cell membrane upon laser induced vapor bubble (VNB). We demonstrate that the enhanced thermal stability of graphene quantum dots (GQD) allows the formation of multiple VNBs upon irradiation with short laser pulses, allowing the delivery of a variety of extrinsic cell labels efficiently and homogeneously into live cells. Consequently, the same set of GQD can be used for repeated photoporation and, therefore, the gradual delivery of increasing amounts of labels into cells as showed in Figure 1 that HeLa cells were labeled with fluorescent nanobody. We demonstrate high-quality time-lapse imaging with confocal, total internal reflection fluorescence (TIRF), and Airyscan superresolution microscopy. As the entire procedure is readily compatible with fluorescence (super resolution) microscopy, photoporation with GQD has the potential to become the long-awaited generic platform for controlled intracellular delivery of fluorescent labels for live-cell imaging. [2]

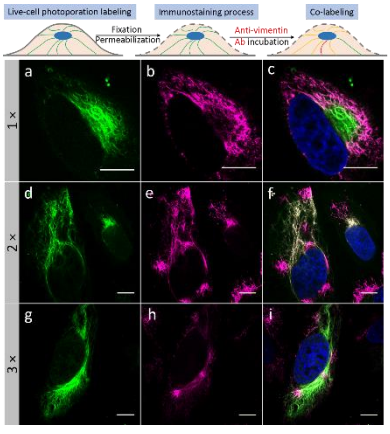


Figure 1. The top schematic illustration shows the labeling procedure. Confocal images of HeLa cells labeled with vimentin label (VL) nanobody by repeated photoporation. (**a**) HeLa cells were photoporated once (1×) with 40 µg/mL VL nanobody Atto 488 (green) and (**b**) co-stained with anti-vimentin primary Ab and goat anti-mouse secondary Ab (Alexa Fluor® 568) (magenta) after fixation and permeabilization. (**c**) The merged image of (**a**) and (**b**). The same procedure was repeated for cells labeled by **d-f** twice repeated (2×) and **g-i** third repeated (3×) photoporation steps. The scale bar is 10 µm.

[1] R., Xiong, et al. "Comparison of gold nanoparticle mediated photoporation: vapor nanobubbles out perform direct heating for delivering macromolecules in live cells." ACS nano **8.6**, 6288-6296 (2014).
[2] J., Liu, et al. "Repeated photoporation with graphene quantum dots enables homogeneous labeling of live cells with extrinsic markers for fluorescence microscopy." Light: Science & Applications **7.1**, 47 (2018).