CONTROLLED DELIVERY OF FLUORESCENT LABELS INTO LIVE CELLS BY LASER-INDUCED PHOTOPORATION WITH GRAPHENE QUANTUM DOTS

Jing Liu^{1,2*}, Ranhua Xiong^{1,2}, Toon Brans^{1,2}, Sangram Keshari Samal^{1,2}, Rabah Boukherroub³, Kevin Braeckmans^{1,2,3}

¹ Laboratory of general biochemistry and physical pharmacy, Faculty of pharmacy, Ghent University, Belgium

² Centre for Nano- and Biophotonics, Ghent University, Ghent, Belgium

³ IEMN, Université de Lille1

*E-mail presenting author: jingli.liu@ugent.be

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It is imperative to observe subcellular structures as well as intracellular processes to gain insight in the role of biomolecules and biological pathways.^[1] A straightforward way to visualize intracellular processes in live cells is by fluorescence microscopy in combination with specific subcellular fluorescent labels. Delivering fluorescent probes, such as labelled antibodies or nanobodies, across the cell membrane into the cytoplasm of live cells is, however, a challenging task because of the cell-impermeable property. Laser-induced photoporation, in combination with membrane adsorbed plasmonic nanoparticles, is a broadly applicable method to deliver nanomaterials, such as fluorescent probes, into cells.^[2] Graphene quantum dots (GQD) are beneficial over the typically used gold nanoparticles (AuNP), since they are more resistant against the pulsed laser irradiation for vapor nanobubble (VNB) generation (Figure 1). Consequently, the same set of nanoparticles can be used for repeated photoporation and, therefore, the gradual delivery of increasing amounts of labels into cells. In this work, we got repeated VNB under dark field microscope and proved the multi-photoporation by gradually delivering FITC-dextran 10 kD (FD 10) into cells to get an increasing fluorescence intensity. At last, we delivered cell-impermeable phalloidin into live HeLa cells to label F-actin. After a second round of photoporation, it showed a much brighter and higher contrast labeling of actin.

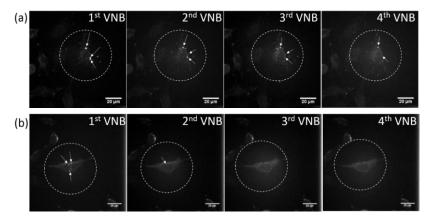


Figure 1: Multiple laser pulse irradiation to (a) GQD and (b) AuNP which were incubated with Hela cells for 30

^[1] S.-H. Shim, C. Xia, G. Zhong, H. P. Babcock, J. C. Vaughan, B. Huang, X. Wang, C. Xu, G.-Q. Bi, X. Zhuang, *Proceedings of the National Academy of Sciences* **2012**, *109*, 13978-13983.

^[2] R. Xiong, K. Raemdonck, K. Peynshaert, I. Lentacker, I. De Cock, J. Demeester, S. C. De Smedt, A. G. Skirtach, K. Braeckmans, *ACS nano* **2014**, *8*, 6288-6296.