Confocal Raman spectroscopy study of intracellular localization of graphene oxide nanoplatform under development for targeted delivery to cancer cells

Marianna Sohová[1,2], **Michal Bodík**[1,2], Peter Šiffalovič[1], Matej Jergel[1], Tibor Hianik[2], Nikola Bugárová[3], Zdeno Špitálsky[3], Mária Omastová[3], Martina Labudová[4], Silvia Pastoreková[4], Eva Majková[1]

[1]Institute of Physics, Slovak Academy of Science, Dúbravská cesta 9, 84511 Bratislava, Slovakia
[2]Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynská dolina F1, 84248 Bratislava, Slovakia
[3]Polymer Institute, Slovak Academy of Science, Dúbravská cesta 9, 84541 Bratislava, Slovakia
[4]Institute of Virology, Slovak Academy of Science, Dúbravská cesta 9, 84505 Bratislava, Slovakia
Marianna.Sohova@fmph.uniba.sk, Michal.Bodik@gmail.com

Abstract

The imaging of living cells using confocal Raman spectroscopy (CRM) is nowadays gaining on its importance. The immersion CRM in PBS buffer, the natural environment of living cells, allows real-time observation of living cells without any fixation and fluorescent dyes.

Diagnostics of oncological diseases stays at the forefront of the current medical research. A large number of oncological markers is available for the early diagnostics. The CA IX (Carbonic Anhydrase IX) is a cell surface, hypoxia-inducible enzyme that is expressed in aggressive tumors, hence, it can be used as a tumor biomarker [1]. Herein we propose a nanoscale graphene oxide (GO) platform functionalized with the antibody specific for the CA IX marker. The CA IX antibody is linked via amine functional groups to GO flake. In this work we study the interaction of pristine GO nanoflakes with two types of cell lines, namely MDCK cells (canine kidney epithelial cells) and the cancer cell line c33 (cervix cancer cells). Both types of cells were transfected and overexpressing human CA IX protein. For CRM studies of the cells directly in PBS buffer we employed a water immersion objective (63×, NA = 1). As a proof of principle, we present the localization capabilities of current CRM for nanoscale GO flakes with the lateral dimensions smaller than 300 nm, which is below the diffraction limit of the objective used. The small lateral size of GO flakes guarantees a successful penetration through the cell membrane. The Fig. 1(a) shows the C33 cell in PBS buffer using the standard reflection imaging mode. The cell located in the red rectangle was imaged by scanning CRM and its false-color image is displayed in Fig. 1(b). We used PCA (Principal Component Analysis) to find a finite set of spectra in orthogonal basis for background filtering. The final image in Fig. 1(b) was reconstructed using K-means clustering analysis based on PCA filtered original image. The average spectra of clusters from different parts of the cell are in Fig. 1(c). The micrometer-sized agglomerates of GO flakes can be clearly resolved in the cell interior (magenta color). This opens new opportunities of the CA IX real-time tracking by means of GO-antibody conjugated nanoplatforms in living cells as well as analysis of targeted drugs delivery into the cancer cells.

References

[1] Švastová, E., et al., FEBS Letters, **557** (2004), 439-445.

Figures

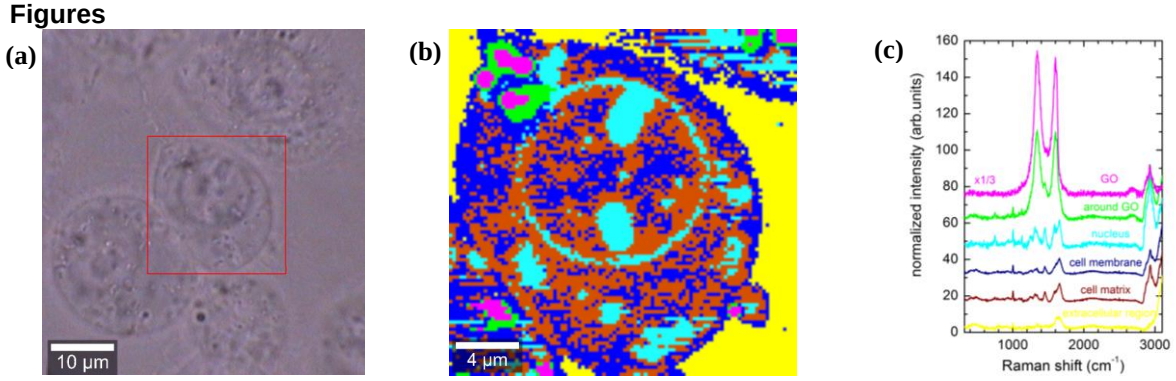

Fig. 1 The C33 + GO cell in bright field mode (a), false-color image of C33 + GO based on PCA/Cluster analysis (b), average spectra of clusters after PCA for different cell parts (c).

Acknowledgment

This work was supported by the Slovak Research and Development Agency under the contracts No. APVV-14-0120 and APVV-14-0267.