Università di Genova

Dottorato in Fisica e Nanoscienze - Ciclo XXXVIII - Second Year Report

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Provisional research project title:

Observation and tracking of functional nanoparticles within cells by optical Nanoscopy

Research activity

In the second year of my PhD, I have focused on the investigation of cellular uptake of nanoparticles by the already tested and user-friendly Stellaris8 confocal microscope available in the laboratory. Confocal laser scanning microscopy (CLSM) actually provides a robust and versatile approach for imaging nanoparticles NPs, facilitating detailed studies of their interactions with biological systems. As the model NPs we decided to work with gold ones (AuNPs), whose biocompatibility is well-known [1]. AuNPs have garnered significant attention as a promising option for biomedical applications such as drug and gene delivery and cancer therapy [2]. The effects of NPs on living systems are obviously linked to their internalization into cells. Therefore, there is a significant need for techniques that can both quantify the uptake of NPs and possibly reveal their localization and interactions with cells simultaneously. By integrating in our imaging protocol both fluorescence detection – for the cell structures - and reflection-mode detection – for the AuNPs - we could obtain high-resolution label-free 3D mapping of the distribution of AuNPs around and inside the cells, after different incubation times in culture (in vitro). The selected cell line is also a common model, namely HeLa.

In the study done so far, we conducted a quantitative analysis of the cellular uptake of commercial AuNPs already available in the laboratory, having distinct size and morphology, namely 80 nm gold nano-urchins and 150 nm gold nanospheres. HeLa cells expressing green fluorescent protein (GFP) were cultured on coverslips and maintained in appropriate growth medium. Then, cells were incubated with bare AuNPs at a constant concentration for varying

durations between 0.5 and 8 h. We repeated the experiments twice to increase the reliability and accuracy of our results. An example of the 3D images obtained is shown in Fig.1.

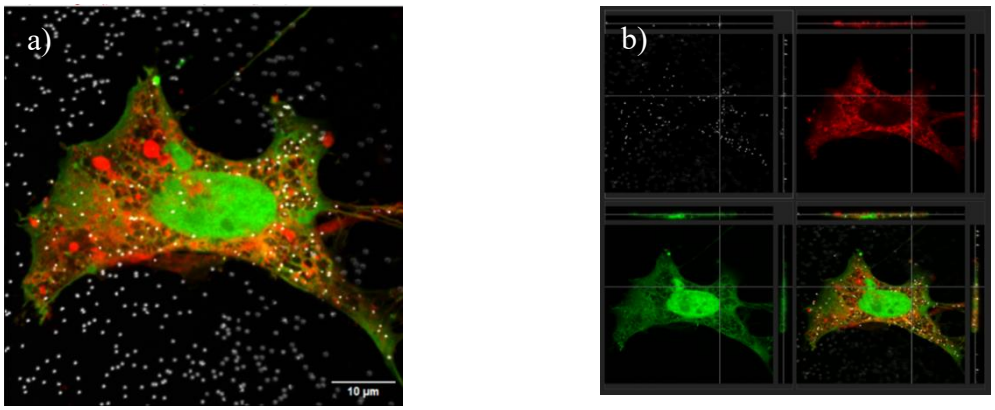

Fig.1. Typical HeLa cell exposed to 150 nm AuNPs for 6 h. a) 2D image with focus on the glass coverslip, and b) images of split channels at intermediate height in the 3D stack, showing CellMask Deep Red stained membrane (red), GFP stained whole cell (green) and reflectance from the AuNPs (Gray).

Nanoparticle internalization was quantified by counting particles within the cell volume using a 3D object counting method in Fiji software, with adjustments for thresholding, Gaussian filtering, and normalization based on the cell's volume. Data for nanoparticle internalization were analyzed and visualized in SigmaPlot, using a bar graph to represent the number of particles within the cell volume with error bars indicating standard deviation across replicates. Statistical analysis was performed using a one-way ANOVA in SigmaPlot to determine significant differences in nanoparticle internalization across incubation time, followed by post-hoc tests to identify specific group differences, with significance set at $p < 0.05$.

It should be mentioned that, for the first runs (a) of each case, the NPs endocytosis imaging has been complemented with parallel tests of cell viability (live-dead, up to 24 h) and vitality (MTT). In both tests, the results were close to 98% for viability. These tests assessed the insensitivity of the living cells to the administration of gold NPs, with respect to their vital condition and functions, and thus confirmed the expected biocompatibility of the AuNPs.

Courses

- Bioimaging: biology seen through the eyes of chemistry: Fabio De Moliner (Andrea Basso)

Publications

- Minflux nanoscopy: a “brilliant” technique promising major breakthrough, Salerno et al., submitted to Microscopy Research and Technique, under review.

Conferences

- Focus on Microscopy 2024, 24-27 March, Genova, Itay.
- XXVII Congresso Nazionale SIBPA 2024, June 16-20, Genova, Italy.

1. Biocompatibility and Cytotoxicity of Gold Nanoparticles: Recent Advances in Methodologies and Regulations, Kus-Liskiewicz et al., Int. J. Mol. Sci. 2021, 22, 10952, DOI: 10.3380/ijms222010952

2. Gold nanoparticles: Synthesis properties and applications, Hammami et al., Journal of King Saud University 2021, 33, 101560, DOI: 10.1016/j.jksus.2021.101560

3. Determining the Size and Shape Dependence of Gold Nanoparticle Uptake into Mammalian Cells, Chithrani et al., Nano Letters 2006, 6, 662, DOI: 10.1021/nl052396o

4. The Effect of shape on Cellular Uptake of Gold Nanoparticles in the forms of Stars, Rods, and Triangles, Xie et al., Scientific Reports 2017, 7, 3827, DOI: 10.1038/s41598-017-04229-z