Università di Genova

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

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Research activity

Over the past year, my research has centered on the application of gold nanoparticles (AuNPs) in combination with 3D fluorescence optical microscopy to investigate behavioral differences between two subtypes of living cells, building upon preliminary experiments conducted during the first two years (see previous reports and Pakravanan et al., 2025). This knowledge will be useful in view of possible future use of the AuNPs as drug carriers (Huo et al., 2014; Kostka et al., 2024) or as radiosensitization agents for tumor radiotherapy (Petrovic et al., 2022; Schäfer et al., 2024). My work was structured in two main phases, each addressing complementary aspects of the study.

In the beginning of the third-year work, nanoparticle internalization and the corresponding morphological responses were examined in two variants of the SK-N-BE2 neuroblastoma cell line: Mock cells, displaying a tumor-like morphology, and S1.1 cells, which overexpress the non-coding RNA NDM29 and exhibit a more neuronal phenotype. Both cell subtypes expressed Green Fluorescent Protein (GFP). The cells were incubated with commercial spherical AuNPs of 150 nm size at several defined exposure intervals. Following each time point, the cells were also labeled. Then, the samples were fixed, imaging was carried out using a Stellaris8 confocal laser scanning microscope, and image processing and analysis followed. The AuNP uptake was quantified through 3D particle counting in Fiji, and the resulting internalization profiles were visualized and analyzed in SigmaPlot. Across the entire dataset, comprising more than 100 images per experimental run, this workflow enabled a robust and reproducible assessment of time-dependent nanoparticle internalization. Using 3D statistical analysis, changes in cell volume in the presence of AuNPs were also examined.

Previous results had shown that AuNPs as large as 150 nm in diameter are efficiently internalized into cells but are unable to penetrate the nuclear membrane. Building on these

findings, the study of this final year focused on evaluating how much smaller AuNPs score, especially in terms of nuclear uptake, and comparing these effects between healthy and cancerous cell lines. In contrast to our previous imaging approach, which relied on scattering-based detection of unlabeled AuNPs, the smaller commercial AuNPs of 20 nm size, fluorescently labeled with the fluorophore Alexa Fluor 568, enabled 3D visualization of their intracellular distribution despite their low scattering cross-section. In this part, the cells were also labeled with a fluorophore staining the nucleus, NucSpot650.

Before establishing the final imaging workflow, different DNA-labeling strategies were tested to identify combinations that avoided spectral overlap (using for nucleus a UV-excitable fluorophore, Hoechst, in combination with Two-Photon Excitation - 2PE) or circumvented it allowing for signal separation by means of Fluorescence Lifetime Imaging Microscopy (FLIM). This multimodal approach allowed a more accurate and reliable assessment of AuNP localization within the intracellular and nuclear environments. Data analysis is ongoing.

Preliminary to microscopy imaging and analysis, cell viability was assessed using the diMethylThiazol-diphenylTetrazolium bromide (MTT) assay to ensure that the AuNP concentrations used were biocompatible. The assay was performed for both cell variants across three AuNP concentrations 3.6×10^6, 3.6×10^7, and 3.6×10^8 particles/mL, and four defined incubation times 2, 4, 6, and 8 hours, which were tested each in triplicate. Results confirmed minimal cytotoxicity under all conditions, allowing reliable interpretation of the subsequent uptake data.

Courses

- Microscopic and spectroscopic techniques for the analysis of surfaces and interfaces (Prof. Renato Buzio, Prof. Andrea Gerbi; Prof. Letizia Savio)- Passed
- Optics for Microscopy and Spectroscopy (Dr. Alessandro Zunino, Dr. Eli Slenders)- Passed

Publications

- **Pakravanan, Kimiya**, et al. "Uptake of gold nanoparticles in HeLa cells observed by confocal microscopy shows dependency on particle size and shape." European Biophysics Journal (2025): 1-12.

- Salerno, M., Bazzurro, V., Angeli, E., Bianchini, P., Roushenas, M., **Pakravanan, K.** and Diaspro, A., 2025. MINFLUX nanoscopy: A "brilliant" technique promising major breakthrough. Microscopy Research and Technique, 88(5), pp.1264-1272.

Conferences

- 12th International Weber Symposium, June 15-20, 2025, Genova, Italy.
- IVSLA Nanoscopy Retreat, May 28-30, 2025, Genova, Italy
- International Day of Light, May 13, 2025, Genova, Italy
- 110th National Congress of Italian Society of Physics (SIF), 22nd - 26th September 2025, Palermo, Italy (Oral contribution).

References

- Pakravanan, K., Bazzurro, V., Salerno, M., & Diaspro, A. (2025). Uptake of gold nanoparticles in HeLa cells observed by confocal microscopy shows dependency on particle size and shape. *European Biophysics Journal*. https://doi.org/10.1007/s00249-025-01769-5

- Huo, S., Jin, S., Ma, X., Xue, X., Yang, K., Kumar, A., Wang, P. C., Zhang, J., Hu, Z., & Liang, X. J. (2014). Ultrasmall gold nanoparticles as carriers for nucleus-based gene therapy due to size-dependent nuclear entry. *ACS Nano*, *8*(6), 5852–5862. https://doi.org/10.1021/nn5008572

- Kostka, K., Sokolova, V., El-Taibany, A., Kruse, B., Porada, D., Wolff, N., Prymak, O., Seeds, M. C., Epple, M., & Atala, A. J. (2024). The Application of Ultrasmall Gold Nanoparticles (2 nm) Functionalized with Doxorubicin in Three-Dimensional Normal and Glioblastoma Organoid Models of the Blood–Brain Barrier. *Molecules*, *29*(11). https://doi.org/10.3390/molecules29112469

- Petrovic, L. Z., Oumano, M., Hanlon, J., Arnoldussen, M., Koruga, I., Yasmin-Karim, S., Ngwa, W., & Celli, J. (2022). Image-Based Quantification of Gold Nanoparticle Uptake and Localization in 3D Tumor Models to Inform Radiosensitization Schedule. *Pharmaceutics*, *14*(3). https://doi.org/10.3390/pharmaceutics14030667

- Schäfer, M., Hildenbrand, G., & Hausmann, M. (2024). Impact of Gold Nanoparticles and Ionizing Radiation on Whole Chromatin Organization as Detected by Single-Molecule Localization Microscopy. *International Journal of Molecular Sciences*, *25*(23). https://doi.org/10.3390/ijms252312843