Università di Genova | Dipartimento di Fisica

PhD Annual Report

First Year 2023

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Cycle: XXXVIII
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Tentative Research Project title:

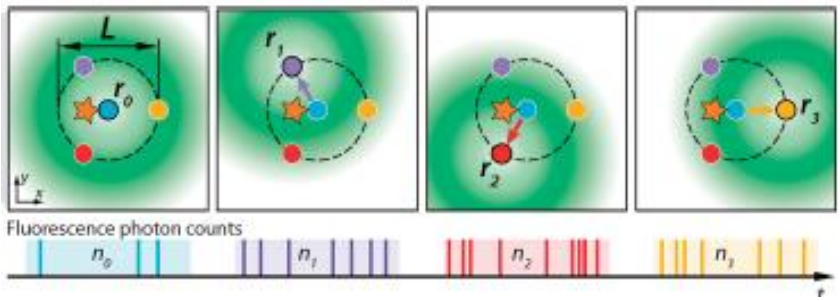
- **Observation and intracellular tracking of mesoporous silica nanoparticles within cells by MINFLUX Nanoscopy**

1. Research activity

During the first year, I acquainted myself with the fundamental concepts of super-resolution optical microscopy. This process began with an introduction to the confocal technique and advanced to the exploration of STED microscopy. On standard test samples, I have received a training in these techniques on a Stellaris8 microscope by Leica, accessible in the laboratories of the group. I have also extended my knowledge to time-resolved methods like fluorescent lifetime imaging microscopy (FLIM) and tau-STED. All the gained expertise is a prerequisite for the next step, which will be learning how to properly use the MINFLUX system.

MINFLUX, abbreviated from "MINimal FLUX of light," refers to a super-resolution optical microscopy method that achieves exceptionally precise localization at the molecular scale by using a minimal number of emitted photons. This precision is attained by collecting photons emitted from fluorescent labels that adhere to the specific features of interest. The established concept of localization precision σ that is typically linked to the inverse of the square root of the number of photons (N) is successfully overcome by employing clever engineering techniques point spread function (PSF) – which is donut-shaped (see figure) same as the depletion beam in stimulated emission depletion (STED) microscopy – and to advanced algorithms of scanning and analysis of the low light intensity spatially emitted at any given position in the planar projection plane of the sample surface.

$$\sigma \geq \frac{L}{4\sqrt{N}}$$



A vortex-phase mask is utilized to modify the PSF of an excitation laser beam (green), creating a doughnut-shaped intensity spot at the focal plane of the objective lens. The spot is redirected systematically at different positions, denoted by blue, violet, red, and yellow dots as $\vec{r_0}$, $\vec{r_1}$, $\vec{r_2}$ and $\vec{r_3}$, in such a way that the fluorophore (star) position is identified as that one providing the minimum amount of emitted photons. [1] Resolution σ does not depend any more on light wavelength but rather on pattern size parameter L.

We intend to utilize a MINFLUX system obtained through the PNRR Project SeeLife funding within the EuroBioImaging network. The system is a commercial product manufactured by Abberior GmbH, located in Goettingen, Germany [2]. Therefore, our work will not deal with implementation of the concept but will rather focus on its optimal use in a specific area of interest.

MINFLUX clearly opens the way to minimally invasive single-molecule localization microscopy (SMLM). As such, the target in biophysics can be single small cellular compartments and sub-units, down to single proteins and DNA nucleotides. Given my expertise in NPs, we aim to carry out experiments addressing the effect in eukaryotic live cells of the presence of NPs – engineered with proper coating to undergo endocytosis by the cells - for drug delivery or other interaction mechanism allowing biological – e.g., anti-cancer – action or affecting the overall functionality of the cell [3]. Direct observation of nanoparticles at a microscopic level within cells is crucial for gaining insights into their nanotoxicity and advancing their potential biomedical uses. The mesoporous silica nanoparticles (MSNs) are one type of NPs commonly used in controlled drug delivery, biosensing and biolabeling [4]. The unique properties of these particles such as tunable pore size and high pore volume/surface area can provide high loading capacity of fluorescence molecules while protecting the dye molecules from the harsh environmental factors, so they exhibit high photostability and concentrated optical signals with high spatiotemporal resolution. There are several approaches for preparing MSNs, we will use grafting of organic fluorescent dyes onto their surface. The

size, shape, electric surface potential and chemical modifications on the surfaces will influence cellular interaction and uptake [5].

As a contingency plan in case of issues with MSNs functionalization or performance, we will steer to different type of NPs also interesting in the life-science context, such as silver NPs, to gain better understanding of their biocides action, or calcium phosphate NPs, which are biocompatible and bioactive.

In the frame of the project, we will follow these purposes:

- Checking internalization to well-known cell lines, e.g. HeLa cells
- Test naturally fluorescent chemotherapy drugs (e.g. Doxorubicin, Paclitaxel) for loading and delivery inside cells
- Checking internalization to the living cells
- Investigating loaded MSN drug carriers, working inside cells in real time, aiming to identify patterns in cell response
- Understanding nanotoxicology of identified drug carrier system
- Optimizing the possible therapeutic efficiency

2. Courses and Exams:

• Biosensing, E. Angeli, O. Cavalleri	Unige	Pass
• Optical Microscopy at the nanoscale, A. Diaspro	Unige	Ongoing
• International PhD school	Erice	Exam to be taken

Following courses in the next year:

• Fluorescence Super-Resolution Microscopy, G. Vicidomini	IIT
• Advanced Optical Fluorescence Microscopy Methods, P. Bianchini	IIT
• Microscopic and Spectroscopic Techniques, R. Buzio, A. Gerbi, L. Savio	Unige

3. Conferences & schools

- International School of Biophysics A. Borsellino in Erice – Ettore Maiorana Foundation and Centre for Scientific Culture, October 16-22
- 8th NIC@IIT advanced microscopy- Practical workshop Italian Institute of Technology

4. References

1. Balzarotti et al., Science 2017, 355, 606, DOI 10.1126/science. aak9913
2. https://www.eurobioimaging.eu/nodes/advanced-light-microscopy-italian-node
3. Shang et al., J Nanobiotech 2014, 12, 1, DOI 10.1186/1477-3155-12-5.
4. X. Chen, Y. Wang , X. Zhang and C. Liu , *Biomater. Sci.*, 2021, **9**, 5484 —5496
5. Anal. Chem. 2019, 91, 9, 5747–5752 Publication Date:April 2, 2019
6. Diaspro A. and Bianchini P., La Rivista Del Nuovo Cimento 2020, 43, 385, DOI 10.1007/s40766-020-00008-1.