Università di Genova | Dipartimento di Fisica

PhD Program in Physics

first Year 2023

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Cycle: XXXVIII
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Tentative Research Project title:

Molecular level precision localization with minimal illumination applied to chromatin architecture by MINFLUX Nanoscopy

Annual Report of Year 1

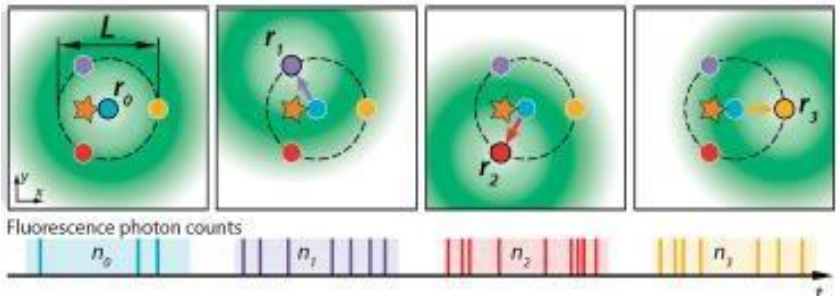
1. Research activity

The PhD project that I am working on in the group of Biophysics of the Department of Physics of the University of Genova is aimed at the exploitation of the novel concept of MINFLUX microscopy applied to eukaryotic living cells to disclose the mechanism of chromatin compaction and/or organization in response to various stages and conditions of the cell.

MINFLUX [1] is the contraction of MINimal FLUX of light, meaning that in this super-resolution optical microscopy technique the very high – actually molecular scale – localization precision is obtained with a very low amount of emitted photons, being collected from the fluorescent labels sticking to the features of interest. The paradigm of localization precision being only proportional to the inverse of the square root of the number of photons N is thus corrected, thanks to engineering of the excitation point spread function (PSF) – which is donut-shaped same as the depletion beam in stimulated emission depletion (STED) microscopy – and to advanced algorithms of scanning and analysis of the light intensity emitted at a given position in scanned region. We will use a commercial MINFLUX system (manufactured by Abberior GmbH, Goettingen, Germany) acquired with funds of the PNRR Project SeeLife embedded in the EuroBioImaging network [2]. Therefore, our work will not deal with implementation of the MINFLUX concept but will rather focus on its optimal use in a specific area of interest.

Obviously, MINFLUX makes non-invasive single-molecule localization microscopy (SMLM) possible. Thus, the biophysical targets can be not only small cellular compartments but even protein sub-units and DNA nucleotides. Availability of proper labelling probes and related techniques is critical for fundamental investigations aiming to elucidate details of DNA folding, replication, transcription and translation [3]. Given my interest in DNA and related topics, we will be arranging experiments addressing the response and organization of chromatin in live cells in the presence of different conditions, such as possible pathology or environmental agents affecting the overall functionality of the cell.

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



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 $\vec{r}_0\rightarrow$, $\vec{r}_1\rightarrow$, $\vec{r}_2\rightarrow$ and $\vec{r}_3\rightarrow$, in such a way that the fluorophore (star) position is identified as that one providing the minimum amount of emitted photons. Resolution σ does not depend any more on light wavelength but rather on pattern size parameter L.

During the first year I got acquainted with the basic concepts of super-resolution optical microscopy, starting with confocal technique and proceeding on to STED [4]. I have received a training in these techniques on a Stellaris8 microscope by Leica, available in the Biophysics group of the Department. So far the samples measured were just test samples for learning, such as Convallaria or pollen. I have also learnt about time-resolved techniques such as fluorescent life-time imaging microscopy (FLIM) and tau-STED. All the know-how obtained during this period will turn useful in the next phase, namely learning how to use the MINFLUX setup. The MINFLUX system has been delivered and installed in the laboratory in mid-October, and at present is still not accessible by the PhD students but only by more advanced personnel – both researchers and technicians – that is undergoing the training by the company.

2. Exams of former course

No	course	professor	CFU	status
i	Biosensing	Elena Angeli, Ornella Cavalleri	3	passed,12 october 2023
ii	Microscopic and spectroscopic techniques for the analysis of surfaces and interfaces	Renato Buzio, Andrea Gerbi (CNR-SPIN); Letizia Savio	3	passed,16 novemer 2023

3. Conferences, workshops, schools

No	Conferences, workshops, schools	place	date
i	International School of Biophysics A. Borsellino	Erice Sicily	16-22 October 2023
ii	Nikon school: Practical workshop on advanced microscopy	IIT Genova	27th November to1 st December 2023
iii	End of year 1 student poster	DiFi Genova	15 th December 2023

planned to be taken during the next year

- Microscopic and Spectroscopic Techniques for the Analysis of Surfaces and Interfaces (Prof. R. Buzio, A. Gerbi, L. Savio, DiFi)
- Biosensing (O. Cavalleri and E. Angeli, DiFi)
- Optical microscopy at the nanoscale (A. Diaspro, DiFi)
- Fluorescence Super Resolution Microscopy (G. Vicidomini, IIT)
- Advanced Fluorescence Microscopy" (P. Bianchini, IIT)

Until the end of this year, I plan to prepare a presentation on chromatin remodelling in neuroblastoma as a selected topic among those presented in the school that I have attended in Erice (see below) and give this presentation in front of the PhD Board, which will work as one course exam passed.

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. Kloecker *et al.*, Chem Soc Rev 2020, 49, 8749, DOI 10.1039/d0cs00600a
4. Diaspro A. and Bianchini P., La Rivista Del Nuovo Cimento 2020, 43, 385,