# PhD Program in Physics and Nanoscience

**Student: Chantal Usai**
**Cycle: XXXVI**
**Tutor(s): Alberto Diaspro, Paolo Bianchini**

## ANNUAL REPORT

## 1. Research Activity

The PhD project I'm working on at the Nanoscopy & NIC@IIT group of the Istituto Italiano di Tecnologia (IIT) concerns the exploitation of advanced optical microscopy techniques to investigate mammalian chromatin architecture. I am specifically interested in the modification in the chromatin organization correlated with oncological processes and genetic diseases. My project aims to develop a biophysical tool to quantify DNA architecture modifications (e.g. 3D compaction) to compare healthy and diseased cells. Furthermore, the effect of mechanical stimuli on the cell nucleus will be studied. To address these questions, I exploit super-resolution microscopy techniques and expansion microscopy in the cell nucleus, trying to adapt them to such a thick and crowded system. Finally, I would like to investigate how modifications in DNA architecture affect the overall functionality of the cell.

During the 1st year of my PhD, I learned the basis of confocal and super-resolution microscopy[1]. I also studied and tried different expansion microscopy protocols. This technique is a novel super-resolution microscopy method that enables nano-scale biophysical studies by increasing the distance among molecules. The specimen is embedded in a swellable polymer network that forms a dense cross-linked matrix around the biomolecules, with a mesh size of ~ 2 nm. After a protein digestion or denaturation step, the polymer is expanded in water, resulting in a ~ 4.5-fold linear expansion factor[2]. To understand whether such a method is suitable for investigating the crowded nuclear environment, I then studied the changes in the DNA conformation induced by the application of different expansion microscopy protocols to fixed mammalian cells. I also performed a combination of confocal and super-resolution microscopy techniques to evaluate and compare the performances of different fluorophores for DNA staining.

During my 2nd year, I want to acquire three-dimensional (3D) STED images on the same cell before and after expansion to study DNA conformation and compaction, and the interplay between chromatin and nuclear Lamin. The next step will be to perform 3D microscopy acquisition of DNA of healthy and diseased cells to identify and quantify the differences.

## 2. Courses

- Optical Microscopy at the Nanoscale (Alberto Diaspro, DiFi, 20h)
- Biofisica (Ornella Cavalleri, Alessandra Pesce, DiFi, 48h)
- Metodi ottici e spettroscopici per lo studio dei materiali (Maurizio Canepa, DiFi, 48h)
- Fluorescence Super-Resolution Microscopy: Basis, Applications and Perspectives (Giuseppe Vicidomini, DIBRIS, 3 Credits)
- Advanced Optical Fluorescence Microscopy Methods (Paolo Bianchini, DIBRIS, 3 Credits)

## 3. Exams

- Optical Microscopy at the Nano-scale
- Biofisica
- Fluorescence Super-Resolution Microscopy: Basis, Applications and Perspectives
- Advanced Optical Fluorescence Microscopy Methods

## 4. Conferences

- Biophysical Society 65$^{th}$ Annual Meeting, held virtually, February 22-26, 2021
- Società Italiana di Biofisica Pura e Applicata XXV Congresso Nazionale, held virtually, June 26$^{th}$ – July 1$^{st}$ 2021
- 13$^{th}$ European Biophysics Conference, Vienna, Austria, July 24-28, 2021

## 5. Posters

- C. Usai, M. Oneto, I. Cainero, F. Baldini, A. Pierzynska-Mach, P. Bianchini, A. Diaspro, "*Evaluating the effect of different Expansion Microscopy protocols on mammalian DNA-Chromatin architecture*", 13$^{th}$ European Biophysics Conference, Vienna, Austria, July 24-28, 2021

## 6. Publications

- F. Baldini, I. Cainero, C. Usai, P. Bianchini, A. Diaspro, "Optical Microscopy to shed light on the chromatin landscapes", Biophysical Reports, in preparation

## References

1. Diaspro A., and P. Bianchini. 2020. Optical nanoscopy. *La Rivista Del Nuovo Cimento*. 43:385–455.
2. Bianchini, P., L. Pesce, and A. Diaspro. 2020. Expansion microscopy at the nanoscale: The nuclear pore complex as a fiducial landmark. *Methods Cell Biol*. 161:275–295.