



PhD Program in Physics and Nanoscience

Student: Chantal Usai

Cycle: XXXVI

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ANNUAL REPORT

1. Research Activity

I am conducting my PhD research at the Nanoscopy & NIC@IIT group of the Istituto Italiano di Tecnologia (IIT). My work mainly focuses on investigating human chromatin architecture and its alteration correlated with genetic diseases at a single-cell level, exploiting the nanometer resolution of Super Resolution optical Microscopy (SRM) techniques¹. Particularly, I use an innovative technique, Expansion Microscopy (ExM)², that allows biophysical studies at the nanoscale by embedding biological samples into a water-absorbant hydrogel that can isotropically expand the specimen four-fold. Since the distance among all the molecules, fluorescent and not, increases, the ability to distinguish more details improves reaching a resolution over the diffraction limit. To further enhance the resolution capability, we combine ExM with other SRM techniques to investigate the morphological alterations at the nuclear periphery in a pathological cellular model of Hutchinson–Gilford Progeria Syndrome (HGPS).

HGPS is caused by a single nucleotide mutation in the LMNA gene, resulting in a truncated form of the protein Lamin A known as Δ LA50 or Progerin. This protein modification causes aberrant lamina network organization and functions, leading to nuclear and DNA structure abnormalities and causing the loss of peripheral heterochromatin and Nuclear Pore Complex clustering, making HGPS a relevant model to investigate the still not well-known intertwinement between chromatin organization and its function.

Our preliminary ExM analysis showed a significant variation in the peripheral association of chromatin to the mutated lamin, which seems to be detached from one another. In order to validate such results, we performed a colocalization analysis of super-resolution Stimulated Emission Depletion (STED) images on non-expanded cells. Moreover, by exploiting another SRM technique, Total Internal Reflection Fluorescence Stochastic Optical Reconstruction Microscopy (TIRF-STORM), we investigate the clustered re-organization of the Nuclear Pore Complex in our HGPS cellular model.

Our Microscopy analysis is integrated with Biomolecular essays that allow us to evaluate the DNA methylation levels (ELISA), the expression of specific enzymes acting on chromatin organization (PCR), and to investigate expression levels of specific epigenetic modifications selected as a marker for different chromatin regions (Western Blot).

In the last months of my PhD, I plan to perform STED-Correlative Light Electron Microscopy experiments to further investigate specific proteins' involvement in peripheral chromatin organization and their expression modification in HGPS with a nanometer-scale resolution.

2. Courses and Schools

- 7th Nikon School NIC@IIT, Practical Workshop on Advanced Microscopy, Istituto Italiano di Tecnologia, Genova, November 28th to December 2nd 2022
- Biocube Meeting, Sestriere, December 10-16 2022

3. Conferences

- Biophysical Society 67th Annual Meeting, San Diego, California, February 18-22, 2023

4. Posters

- C. Usai, I. Cainero, L. Cuneo, F. Baldini, P. Bianchini, A. Diaspro, "*Decrypting the Spatial Relationship between Peripheral Chromatin and Nuclear Lamina in Hutchinson-Gilford Progeria Syndrome Using Super Resolution Microscopy Techniques*", Biophysical Society 67th Annual Meeting, San Francisco, California, February 18-22, 2023
- C. Usai, I. Cainero, L. Cuneo, F. Baldini, P. Bianchini, A. Diaspro, "*Expansion Microscopy As a Tool to Explore the Spatial Relationship between Chromatin and Nuclear Lamin in Hutchinson-Gilford Progeria Syndrome*", FOM2023, Porto, Portugal, April 2-5, 2023

5. Oral presentations

- "*Validating Expansion Microscopy from macro- to nanoscale using NPC and other intrinsic reporters*", ExM User Group Meeting, held virtually, October 5th, 2022

6. Publications

- C. Usai, I. Cainero, F. Baldini, L. Cuneo, S. Civita, P. Bianchini, A. Diaspro, 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.
3. Vidak S., Folsner R., Molecular insights into the premature aging disease progeria, *Histochem Cell Biol* 145:401–417, 2016