Dipartimento di Fisica
Università degli studi di Genova

ISTITUTO ITALIANO DI TECNOLOGIA

PhD annual report
First Year

PhD student: Sajedeh Kerdegari
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Cycle: XXXVI
Year: 2020-2021
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Research and Activity:

Objective:
My research project is focused on the use of advanced microscopy approaches in the characterization of biomaterials. The idea is to merge the capability of different techniques, specifically Atomic Force Microscopy (AFM) and STimulated Emission Depletion (STED), to achieve an accurate prediction/characterization that no single technique is yet able to provide. The match between the AFM and optical nanoscopy offers optical specificity while the sample is scanned by the tip of a sensitive cantilever probe [1].

For this purpose, experience and studies on atomic force microscopy and fluorescence microscopy must be achieved separately in the beginning. Furthermore, to image biological samples, a basic knowledge of biology and extensive experience in the biology lab is required for sample preparation as well.

Case studies:
One of the main subjects of investigation will be the affection induced in the normal physiological properties of the cell nucleus by pathological states. The goal is to achieve new information on the Physico-chemical properties of nuclei from patients affected by Progeria, a disease that induces faster aging in the patients.
In the first years of my PhD I started working on a model system. In particular, I measure the mechanical properties of skin fibroblast nuclei. I employed the AFM, performing indentation experiments, and different experimental approaches. First, I indented nuclei in their natural environment (within the cell), localizing their position with phase-contrast optical microscopy. Second, I measure the stiffness of nuclei isolated from the cell. This work is in collaboration with Prof. Massimo Vassalli from the University of Glasgow, and Dr. Reinier Oropesa-Nunez from the University of Uppsala, and Dr. Andrea Mescola from the CNR-Nanoscience Institute-S3. The idea is to develop an experimental and theoretical model that provides the best result in terms of reliability and reproducibility in the definition of the mechanical properties of the cell nucleus, and, in a second step, to apply it to the case of Progeria nuclei.

Sample preparation:
During the past year, I have mainly worked on gaining the required skills by working on human fibroblast skin cell. For this study, the cells were cultured in the bio lab at the physics department. The whole process was a collaboration with the Post Doc biologist researcher Virginia Bazzurro in the biophysics group at UNIGE. The cells were cultured in flasks and kept in an incubator, controlled regularly, and split for 16 passages in culture. To prepare samples for the AFM measurement, an adequate number of cells were put into pre-poly-lysed Petri dishes to ensure the attachment of cells to the substrate and kept in an incubator until the measurements took place. Furthermore, nuclei of cells were also extracted, to perform the experiments and compare the mechanical

properties of isolated nuclei with nuclei in intact cells. In this phase of study, to prove the suitability of the extraction protocol, two different fluorescence dyes DAPI and MitoTracker were used to label both samples of whole cell and extracted nuclei. Confocal microscope was employed to image the samples. In the sample of the whole cell, we could see the nuclei in blue and the rest of the cell in red color associated to DAPI and MitoTracker respectively, while in the extracted nuclei only blue DAPI could be observed.

AFM measurement:
Mechanical properties of the cell were studied by AFM. However, heterogeneous sample properties of the complex biological system require the use of larger probes to integrate properties over larger areas. For example, micrometer-sized spheres can be attached to an AFM cantilever [2]. In my case beads of 5µm in diameter were attached to tipless cantilevers of 0.03N/m spring constant. Force indentation was done on top of the nucleus of each cell. To guide the probe to a specific location on the cell where the nucleus exists, an inverted optical microscope was used.
All the AFM measurements were carried out on samples in buffer solution, as well as maintaining the temperature at 37°C, for no more than 2 hours to keep the physiological condition for living cells. Indentation performed on each single cell with the 5nN force and Force-Distance curves were recorded.
To obtain Young's elastic modulus (E) of the nuclei from the F-D curves, we need to know the contact area of the sample and tip which is dependent on the indentation depth, probe geometry, speed, sample properties like adhesion, etc. Therefore, one needs a model to consider and calculate the required parameters. Different models can be employed, the most famous and widely used ones are Hertzian group: Hertz 1881, Sneddon 1965, Johnson, Kendall, &Roberts (JKR) 1971, Derjaguin, Muller, & Toporov, (DMT) 1975. Nuclei, such as adherent cells and biological tissues, are particularly ill-posed to fulfil all the assumptions of any contact mechanics model described so far. In particular, cells have a limited thickness, they are not homogeneous and often also not isotropic [3]. For these reasons, the mechanical properties of the cell nuclei are going to be studied with a modified model to find the best fit of the F-D curve that can describe the nucleus better. In the first attempt, I use the Sneddon model to calculate the Young's modulus of my samples. In this second year, new models will be tested.

Courses and Exams:

• Optical Microscopy at the nanoscale, Alberto Diaspro	Unige	Pass
• Biosensing, Elena Angeli, Ornella Cavalleri, Marco Salerno	Unige	Pass
• Fluorescence Super-Resolution Microscopy, Giuseppe Vicedomini	IIT	Pass
• Advanced Optical Fluorescence Microscopy Methods, Paolo Bianchini	IIT	Pass
• Atomic Force Spectroscopy, Annalisa Relini	Unige	Exam to be taken
• Microscopic and Spectroscopic Techniques, Renato Buzio, Andrea Gerbi, Letizia Savio	Unige	Exam to be taken

Schools and Conferences:

- XXV international School of Pure and Applied Biophysics, January 18-22, Venice (online)
- Focus On Microscopy, March 28-31, 2021 (online)
- Functional Imaging Course, October 2021, LCAM, Netherland

References

[1] A. Diaspro and P. Bianchini, "Optical nanoscopy," *Springer,* 2020.

[2] M. K. e. al., "Atomic force microscopy-based mechanobiology," *Nature reviews,* 2019.

[3] N. Gavara, "A beginner's guide to atomic force microscopy probing for cell mechanics," *Wiley Microscopy Research and Technique,* 2016.