Ph.D. Annual report
Third Year

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Cycle: XXXVI
Year: 2022-2023
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Research and Activity:

Objective:

Cells are the basic units of life and are subjected to various mechanical forces in their environment. Understanding how cells sense and respond to these mechanical stimuli, such as compression, tension, and shear, is essential, as these mechanics are responsible for cells' shape, motility, differentiation, division, and gene expression[1-4]. Understandings of these mechanical properties have various implications in tissue engineering, drug screening, and evaluation[5], and diagnosis of diseases and their treatments [6]. Despite cells, nuclei are challenging to be assessed by the AFM probe. One solution could be to extract the nuclei and study them in isolation, removing the contribution of the upper cellular compartments that come into contact with the AFM probe. However, the isolation of the nuclei can affect their mechanical properties due to the loss of connections between the nucleus and cytoskeleton via nuclear pores in its native microenvironment inside the cell. This research aims to find the best strategy and technique to solve this puzzle and later apply the technique to cells affected by laminopathies.

Experiment:

In the last years, I measured the mechanical properties of the cells' nuclei in the intact form inside the cells in addition to the isolated form (Figure 1, a, b) using two different AFM probes: standard sharp AFM tips and polystyrene beads with a diameter of 10µm. Our studies show that the nuclei are stiffer in their physiological condition inside the cell (Figure 1, c, d).

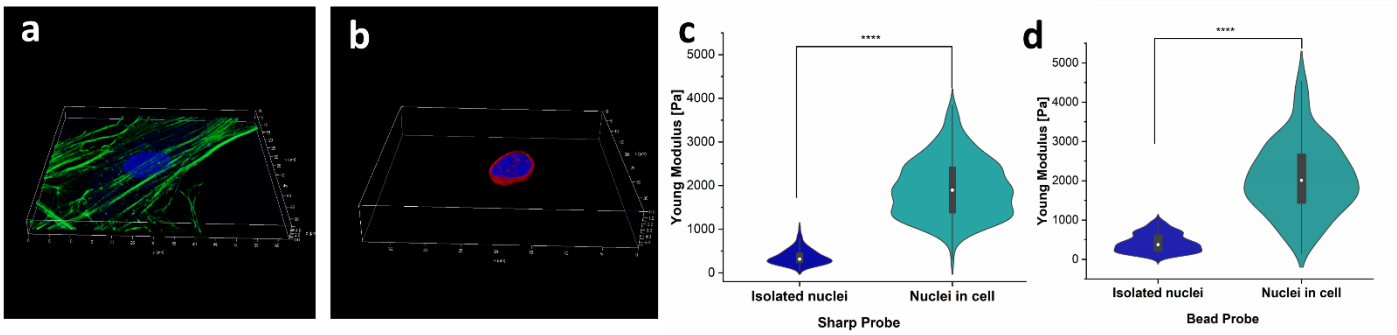

Figure 1: a) Two-color confocal images of human skin fibroblast cells labeled with Phalloidin FITC for actin (green) and Hoechst for DNA (blue); the intact cell shows both signals, green and blue. b) Fixed isolated nuclei, labeled with the previous two fluorescent probes plus an additional anti-Pan Lamin antibody that binds the Lamin A Lamin B in the nuclear membrane. The images show the isolated nuclei without the cytosol around them, and the nuclear lamina preserved around them. c) Yong's modulus of elasticity measured by AFM pyramidal probe. d) Young's modulus of elasticity measured by Bead probe.

To determine which of these cases, between the nuclei in living cells or isolated nuclei, is providing more realistic results, we used a novel technique called Brillouin Microscopy (BM). This technique based on light-matter interaction offers a non-contact and label-free approach to address the previous problems. It can be considered an innovative method of assessing the material's elasticity at the microscale with a full 3D capability [4]. For this reason, I have done a period of study in the CRN in Perugia at the laboratory of Dr. Silvia Caponi, an expert in this field. Our investigations depict the same change in the nuclear stiffness in living cells (Figure 2). It means that the isolation procedure is affecting the nuclei stiffness and causes it to soften after extraction from its natural microenvironment. It is noteworthy that, while Brillouin can be more favorable in studying the nucleus inside the living

cell, having its physiological condition providing a non-contact technique, AFM shows more sensitivity to the changes in the micro-environment of the nucleus. Therefore, combining experimental observation of both techniques could offer significant benefits for addressing mechanobiological questions in a more thorough manner.

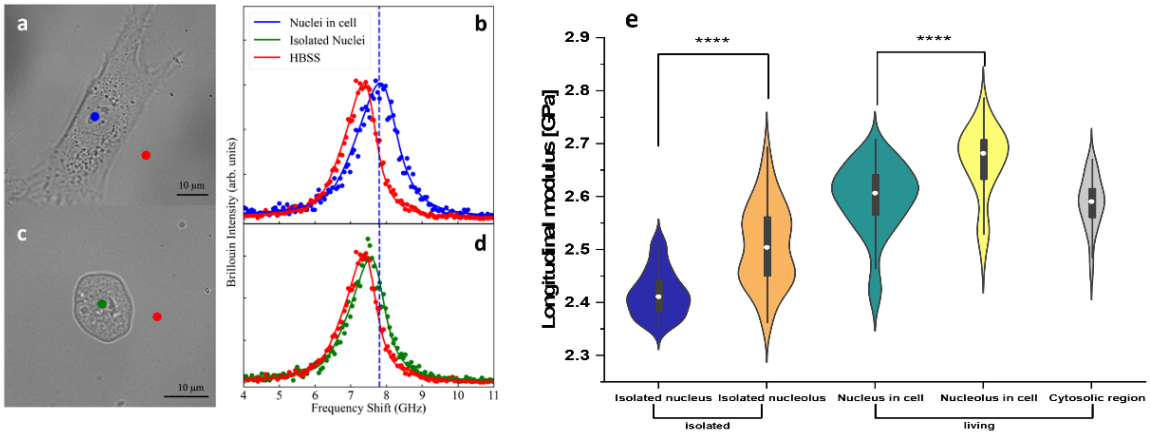

Figure 2: a)Phase contrast optical microscopy image of two different points of measurement of the nucleus in living cell, blue dot, and the buffer with red dot b) Brillouin shift of the nucleus in living cell in respect to the buffer marked by the dashed line c) isolated nucleus points of measurement in green inside the nucleus and in red in buffer d) frequency shift of the isolated nucleus in respect to the buffer f) the longitudinal modulus of all the 4 measured points, isolated nucleus, isolated nucleolus, nucleus region of living cells, nucleolus region of living cells, and additionally the cytosolic region.

Diseased Progeria cells:

As the final goal in this research, we applied the same technique of measurements by AFM on the model diseased cells of Hutchinson Gilford Progeria Syndrome (HGPS). In the progeria cells the proteins in the nuclear lamia are overexpressed. This rare alteration of proteins causes a disease that induces faster aging in the patients. We carried out experiments on the nuclear region of living cells and isolated nuclei of control cells as well as HGPS ones. We performed the experiments both with the micro-bead probes and the sharp probe of the same characteristic of the previous experiments on the fibroblast skin cells. Our finding shows that with the micro-bead probe on the nuclear region of living cells, there is no significant difference between the control cells and HGPS cells while the isolated nuclei shows a small increase in the elasticity of the HGPS nuclei. After repeating the measurements with the sharp probe which is more sensitive to the localized differences the results were more promising. Despite on the living cells, where the difference was still insignificant, on the isolated nuclei a significant increase was observed (Figure 3).

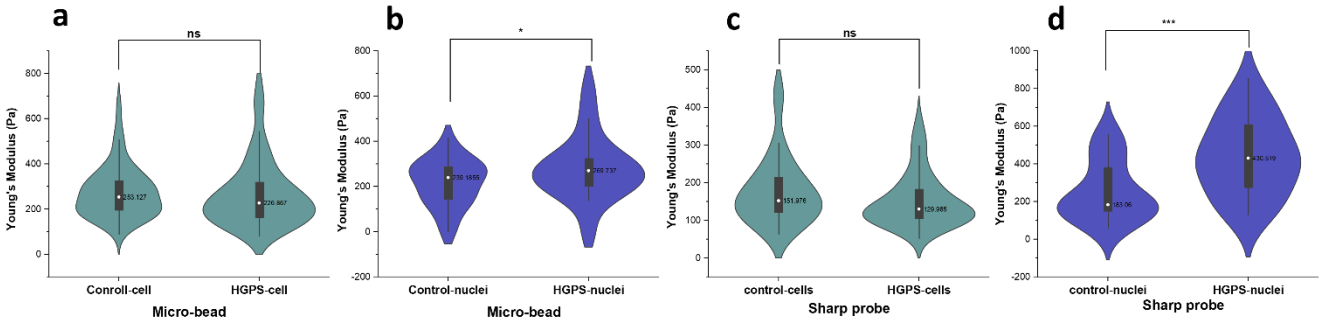

Figure 3: The Young's modulus of elasticity on the control and HGPS cells a) micro-bead probe on cells b) Microbead probe on isolated nuclei c) sharp probe on cells d) sharp probe on isolated nuclei.

Publications:

Kerdegari, Sajedeh et al. "Insights in Cell Biomechanics through Atomic Force Microscopy." Materials 16.8 (2023): 2980.

Schools and Conferences:

- Focus On Microscopy conference, April 02-05, 2023, Porto, Portugal (Poster)
- European Biophysical Societies' Association (EBSA), July31-August4, 2023, Sweden (Oral Presentation)
- FisMat, September 4-8, 2023, Milano, Italy (Oral Presentation)
- Erice school of biophysics, October 2023, Sicily, Italy

1. Abuwarda, H. and M.M. Pathak, *Mechanobiology of neural development.* Current opinion in cell biology, 2020. **66**: p. 104-111.
2. Beicker, K., et al., *Vertical light sheet enhanced side-view imaging for AFM cell mechanics studies.* Scientific reports, 2018. **8**(1): p. 1-12.
3. Rigato, A., *Characterization of cell mechanics with atomic force microscopy: Mechanical mapping and high-speed microrheology*. 2015, Aix-Marseille.
4. Prevedel, R., et al., *Brillouin microscopy: an emerging tool for mechanobiology.* Nature methods, 2019. **16**(10): p. 969-977.
5. Krishnan, R., et al., *Cellular biomechanics in drug screening and evaluation: mechanopharmacology.* Trends in pharmacological sciences, 2016. **37**(2): p. 87-100.
6. Yu, W., et al., *Nanocytology as a potential biomarker for cancer.* Biomark Med, 2017. **11**(3): p. 213-216.