A Lattice Spring Model for Extracting Elastic Constants from AFM Nanoindentation Tests

Lorenzo Vaiani and Antonio Boccaccio - Dipartimento di Meccanica, Matematica e Management, Politecnico di Bari, Bari (Italy)

AIM OF THE WORK

In the present work, **Atomic Force Microscopy** (AFM) **nanoindentations** on human **mesenchymal stem cells** (MSCs) were studied and simulated. AFM is an experimental technique typically adopted for biological studies, which consists in moving a **spherical**, **conical** or **pyramidal probe** positioned at the tip of a **microcantilever**, into the surface of the material to investigate (Fig. 1), to obtain a **force-indentation report curve** [1].

In order to retrieve the **elastic constants** of the investigated materials, an **extraction phase** is required, starting from the AFM report curve.

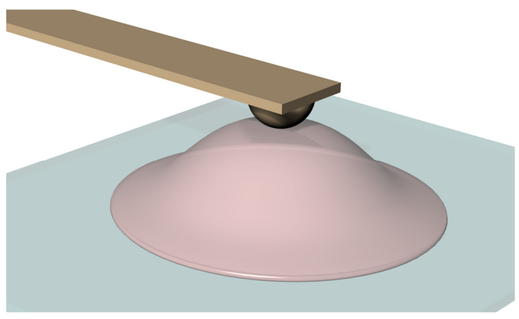

Fig. 1 – Scheme of the AFM experimental setup

Commonly used methods for the extraction procedure are: **Hertz Contact Theory**, **Finite Element Method** (FEM) [2] or **hybrid/particle methods** [3].

Many problems emerge by using the Hertz Theory, the hypotheses of which are often not entirely met, or the Finite Element Method, when dealing with large local deformations. The main focus of the present work was to overcome the **approximations** arising from the application of the Hertz Theory or the **convergence issues** in FEM, with the aim of providing a reliable procedure for extracting a set of elastic parameters by operating on a single AFM report curve. The extraction of **Young's moduli** of **subcellular components** of ten MSCs was then performed through a **Coarse-Grained Lattice Spring Model** (CG-LSM). LSM represents a class of lagrangian methods for simulating mechanical behavior of soft materials, based on the discretization of a deformable system into a **series of particles** interconnected by **spring/damper elements**. It results in a framework that is relatively **easy to implement** through common programming languages, thanks to the **simple physical laws** relating the displacement of masses to the reaction forces exerted by the spring network during deformation. This method is **not as accurate** as FEM approaches, but it offers a great advantage: a **fast and stable** formulation for **large deformations** even in case of **geometrical nonlinearities** and **topological changes**.

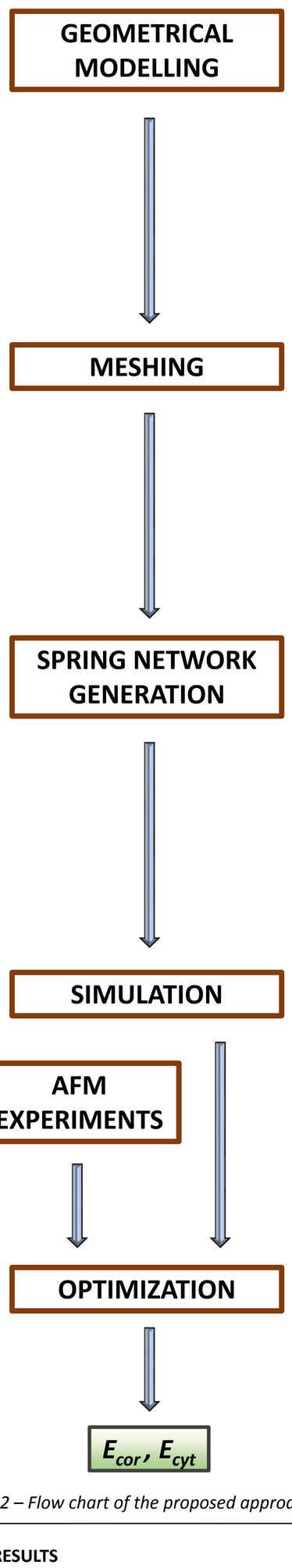

Fig.2 – Flow chart of the proposed approach

GEOMETRICAL MODELLING

The first phase of the proposed procedure (Fig. 2), is the geometrical modelling. In Rhinoceros/Grasshopper (Robert McNeel & Associates, WA), a three-dimensional **CAD model** of a quarter of a mesenchymal stem cell sample was generated at first as a fully revolved surface, as shown in Fig. 3, with a diameter D = 50 µm and an overall height H = 13 µm. The internal structures were simplified and modelled as a set of **subcellular components**: the **cortex**, the **cytoskeleton**, the **nucleus** and the **stress fibers arrangement**, necessary for performing the **adhesion** of the cell to an external substrate [1].

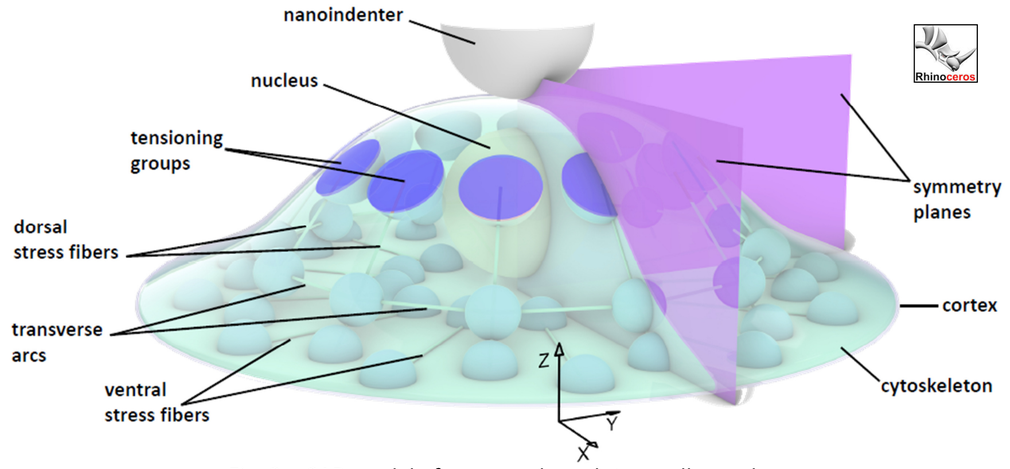

Fig. 3 – CAD model of a mesenchymal stem cell sample

MESHING

Starting from the solid model, a **tetrahedral mesh** (Fig. 4) was generated by using the open-source meshing software **Gmsh** (version 4.5.2). The element size was set to 0.350 µm, with no internal growth.

High **size homogeneity** and **isotropy** are essential requirements throughout the meshing phase, in order to obtain consistent quantitative results from a LSM.

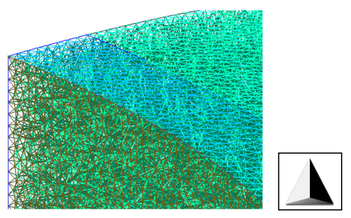

Fig. 4 – Isotropic tetrahedral mesh

SPRING NETWORK GENERATION

The tetrahedral mesh generated in the previous phase was converted to a **spring network** through the **isolation** of **mesh edges** in Rhinoceros and Grasshopper. Then, the definition of several **functional groups of springs** corresponding to the **main subcellular components** essential for the simulation was performed, as illustrated in Fig. 5 and Fig. 6. The **tensioning groups** were defined to identify the springs where to apply the load exerted by the stress fibers, the **contact group** was utilized to identify the springs that will enter in contact with the nanoindenter, the **cytoskeleton**, the **nucleus** and the **cortex groups** identify the springs occupying, respectively, the volumes of these anatomical cell components. The total number of springs was 887605, leading to a spring density of 320 spr/µm³. The average rest length was 0.345 µm, which resulted in a suitable resolution for the indentation region.

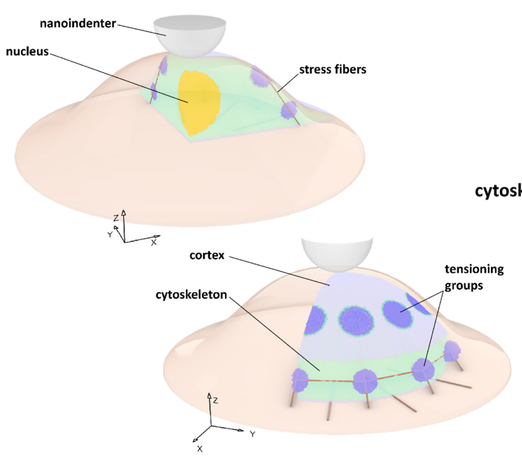

Fig. 6 – Subcellular components

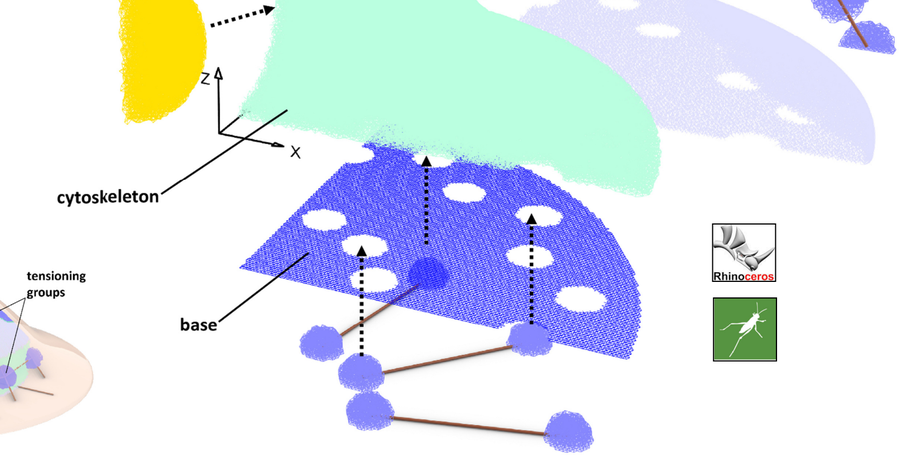

Fig. 5 – Exploded view of the functional groups of the spring network

SIMULATION

In Simulia Abaqus FEA (Dassault Systèmes), the **nanoindenter** was modelled as an **analytical rigid** spherical surface presenting a radius R = 5 µm. A **frictionless contact condition** between the nanoindenter and the cell was set.

The **deformable MSC** was modelled as a network of **truss elements**, having a cross section A = 1 µm² and an axial stiffness defined as $E_{loc}A/L_0$ ($E_{loc,cor}$ for cortex and $E_{loc,cyt}$ for cytoskeleton). **Spherical hinges** in connection nodes ensured high **structural compliance** during deformation, as expected for soft biological materials.

Boundary conditions

Initial step: two orthogonal symmetry planes and an encastre constraint for the MSC lower plane (for simulating adhesion to a flat substrate) were defined.

Step 1 – tensioning: a concentrated load of 10 nN along stress fibers directions was defined for each tensioning group (the magnitude of the tensioning field has an influence on local Young's moduli).

Step 2 – indentation: an imposed displacement of 200 nm was set for the rigid nanoindenter. All these conditions are synthesized in Fig. 7.

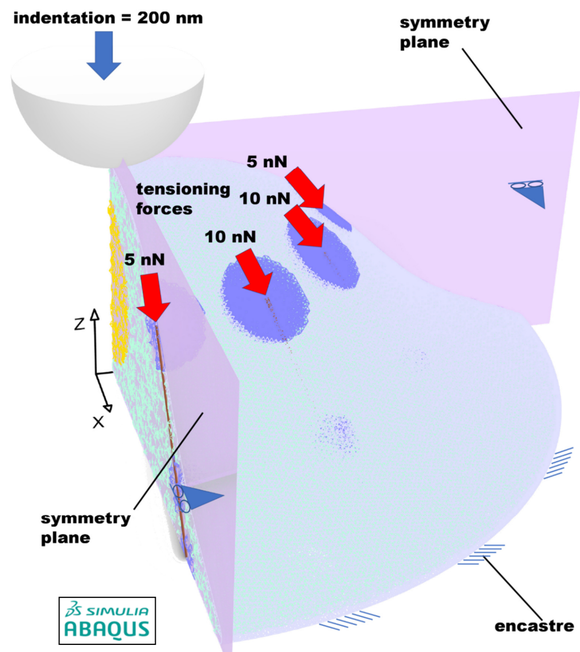

Fig. 7 – Simulation setup and boundary conditions

OPTIMIZATION

An **iterative force-displacement curve fitting** (Fig. 8) between the experimental curves and the curves obtained as output from the LSM simulation were performed in Matlab (The MathWorks, Inc., MA), in order to compute the two unknown Young's moduli: $E_{loc,cor}$ (for cortex) and $E_{loc,cyt}$ (for cytoskeleton). After this **curve matching** the local values were converted to **global values** of Young's moduli, by means of calibration functions.

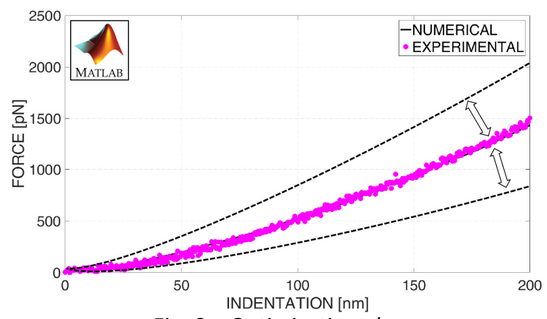

Fig. 8 – Optimization phase

RESULTS

The results of the proposed CG-LSM simulations, FEM and Hertz Contact Theory for the same case studies were analyzed and presented in Table 1.

Table 1 – Computed Young's moduli

Sample N.	Coarse-grained elastic network		Finite element method, Arruda-Boyce		Hertzian contact theory
	E_{cyt} [Pa]	E_{cor} [Pa]	E_{cyt} [Pa]	E_{cor} [Pa]	E_{Hertz} [Pa]
Sample 1	2911	9216	3519	9754	4110
Sample 2	4578	8898	5290	9687	6350
Sample 3	3771	9144	4462	9671	5200
Sample 4	1999	8352	2575	9738	3195
Sample 5	1722	7531	2258	9645	2800
Sample 6	2286	8751	2901	9570	3410
Sample 7	4152	8666	4638	9807	5550
Sample 8	3988	9161	4551	9729	5680
Sample 9	2527	8845	3129	9555	3455
Sample 10	2166	8484	2640	9618	3050

To perform correct comparisons of LSM and FEM results to the averaged values coming from the Hertz Contact Theory, the double-layered indentation region was mathematically modelled as a spring series system, in order to compute a single equivalent stiffness value. To make statistical inferences, we used ANOVA to compare the Young's moduli values. It resulted that there is not a statistically significant difference between the distributions of the equivalent Young's moduli computed through LSM or FEM and the Young's moduli obtained through the Hertz Contact Theory ($F(2, 27) = 1.149$, p-value = 0.332). It is possible to state that the global values for E_{cyt} and E_{cor} and the equivalent stiffness obtained by means of the **proposed LSM** approach were in **good agreement** to FEM and to the averaged Hertz Contact Theory for all the investigated samples.

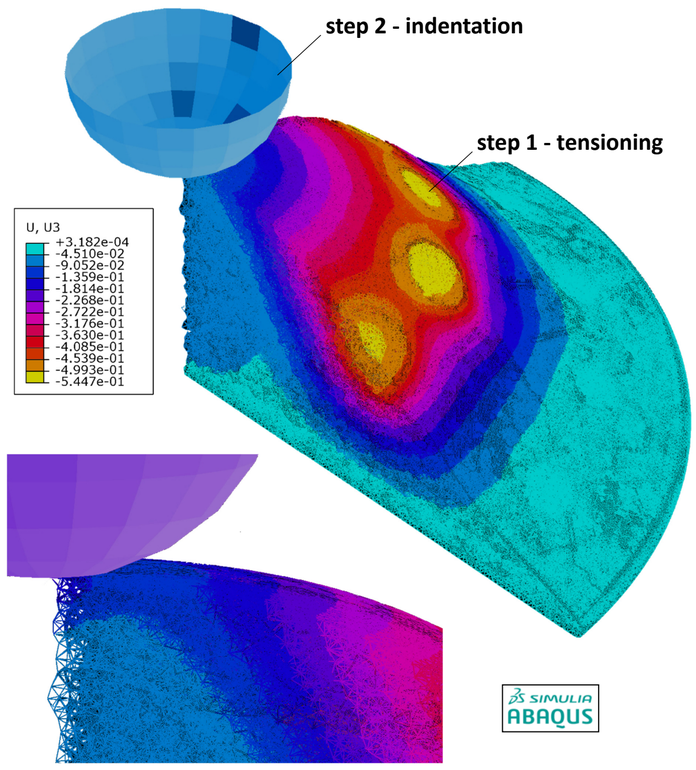

Fig. 9 – Displacement field in vertical direction

The most interesting feature of the proposed approach is the **intrinsic compliance** of the structure, resulting as a suitable solution for **soft biological materials**. The vertical displacement field is illustrated in Fig. 9.

The **time required** by LSM was about **33%** of the same FEM simulations presenting the same average mesh size.

The algorithm for volumetric discretization and the chosen element sizes were adequate for the considered indentation problem.

The magnitude of the **tensioning pre-stress field** can **increase** the values of the predicted elastic moduli of cortex and cytoskeleton **up to 15%** at full pulling.

One experimental strategy that can be adopted to retrieve the elastic constants of the subcellular components consists in physically separating the components and in indenting each of them, but this would inevitably lead to the death of the cell and hence to a change of the material properties. The proposed implementation allows to extract two or more elastic constants proper of different subcellular components from a single AFM report curve.

As a conclusion, the proposed Coarse-Grained Lattice Spring Model, when supported by an interactive **CAD pre-processing**, results in a **simple**, **fast** and **stable** approach for **extracting the elastic parameters** of **subcellular components** in MSCs, and this approach could be scaled and extended to other biological materials.

Main references

[1] E. Migliorini et al. – Nanotechnology, 2021

[2] R. Vargas-Pinto et al. – Biophysical Journal, 2013

[3] M. Vassaux, J.L. Milan – Biomech. and Model. in Mechanobiology, 2017