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## Coarse-grained elastic network modelling: A fast and stable numerical tool to characterize mesenchymal stem cells subjected to AFM nanoindentation measurements

L. Vaiani, E. Migliorini, E.A. A Cavalcanti-Adam, A.E. E Uva, M. Fiorentino, M. Gattullo, V.M. M Manghisi, A. Boccaccio

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2 **Coarse-Grained Elastic Network Modelling:**  
3 **a Fast and Stable Numerical Tool to Characterize**  
4 **Mesenchymal Stem Cells Subjected to AFM**  
5 **Nanoindentation Measurements**

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34 **ABSTRACT**

35 The knowledge of the mechanical properties is the starting point to study the mechanobiology of  
36 mesenchymal stem cells and to understand the relationships linking biophysical stimuli to the cellular  
37 differentiation process. In experimental biology, Atomic Force Microscopy (AFM) is a common  
38 technique for measuring these mechanical properties.

39 In this paper we present an alternative approach for extracting common mechanical parameters, such  
40 as the Young's modulus of cell components, starting from AFM nanoindentation measurements  
41 conducted on human mesenchymal stem cells. In a virtual environment, a geometrical model of a  
42 stem cell was converted in a highly deformable Coarse-Grained Elastic Network Model (CG-ENM)  
43 to reproduce the real AFM experiment and retrieve the related force-indentation curve. An ad-hoc  
44 optimization algorithm perturbed the local stiffness values of the springs, subdivided in several  
45 functional regions, until the computed force-indentation curve replicated the experimental one. After  
46 this curve matching, the extraction of global Young's moduli was performed for different stem cell  
47 samples. The algorithm was capable to distinguish the material properties of different subcellular  
48 components such as the cell cortex and the cytoskeleton. The numerical results predicted with the  
49 elastic network model were then compared to those obtained from hertzian contact theory and Finite  
50 Element Method (FEM) for the same case studies, showing an optimal agreement and a highly  
51 reduced computational cost.

52 The proposed simulation flow seems to be an accurate, fast and stable method for understanding the  
53 mechanical behavior of soft biological materials, even for subcellular levels of detail. Moreover, the  
54 elastic network modelling allows shortening the computational times to approximately 33% of the  
55 time required by a traditional FEM simulation performed using elements with size comparable to that  
56 of springs.

57 *Keywords:* Elastic Network Model, Atomic Force Microscopy, Cell Material Characterization,  
58 Meshless Methods

## 59 **1. Introduction**

60 Stem cells are undifferentiated cells capable of differentiation towards specific cell  
61 phenotypes. The control of stem cells biology through mechanical factors remains a poorly  
62 understood topic and represents one of the main objectives of mechanobiology [1]. Mechanobiology  
63 is an interdisciplinary research area that integrates engineering and biology, exploiting the best  
64 computational and experimental techniques to investigate the interactions between external  
65 mechanical stimuli and internal adaptive response of living cells [2] and to understand the  
66 relationships linking forces acting on a cell and biophysical responses [3–5]. Computational  
67 mechanobiological models were recently developed to design biomaterials for bone tissue  
68 engineering applications [6–8].

69 The starting point to investigate stem cell mechanobiology is the assessment of the mechanical  
70 properties and deformation characteristics of the cell and its subcellular components. One of the most  
71 commonly adopted techniques to measure these properties is the Atomic Force Microscopy (AFM)  
72 that is widely used in experimental biology for mechanical characterization of whole living cells [9–  
73 11] as well as for subcellular components [12,13], and also for biological tissues in general [14–18].  
74 It essentially consists in moving a spherical, conical or pyramidal nanoindenter fixed at the tip of a  
75 flexible cantilever of known stiffness into the surface of the material to investigate [19,20]. The tip  
76 displacement is detected and, based on the cantilever stiffness, is converted into force, in order to  
77 obtain a force-indentation curve that is strictly related to the stiffness of the material under  
78 examination. The effects of the AFM tip geometry and of the stress state acting on the nanoindented  
79 material were investigated in previous studies [21,22]. A common practice for assessing elastic  
80 properties of a biomaterial starting from force-indentation AFM curves exploits the hertzian contact  
81 theory [20,23]. The main hypotheses of this theory, in the particular case of a spherical indenter, are  
82 the following: (i) the strains in the nanoindented material are infinitesimal, (ii) the contact area is  
83 small, (iii) the nanoindented material has a linear elastic and uniform behavior, (iv) the nanoindented

84 material presents a perfectly flat surface with infinite dimensions, (v) the indenter is perfectly  
85 spherical. In a real AFM tip to living cell contact not all those conditions are satisfied and is  
86 impossible to distinguish, by using this theoretical framework, the single contributions to the overall  
87 stiffness of the several subcellular components [11,24], acting as a series-system composed by  
88 different stiffness values, but only retrieve an overall cell stiffness as a weighted average of the  
89 subcellular components stiffnesses.

90         Nowadays computer simulations represent a powerful tool for studying physical phenomena  
91 presenting real conditions that are not reproducible in a laboratory [25]. A common numerical  
92 technique suitable for modelling deformable objects subjected to loads and constraints is the Finite  
93 Element Method (FEM) [26,27]. The main issues encountered in the finite element analysis of soft  
94 biological materials are the following: (i) the numerical convergence is problematic and often missing  
95 even for small imposed displacements due to very low Young's moduli; (ii) the large local distortions  
96 induced in the mesh and the consequent need for numerical stabilization functions; (iii) the high  
97 computational resources required for modelling complex structures. The main goal of this study is to  
98 overcome the problems related to the large deformations and distortions imposed on mesh-based  
99 models in simulations of soft biological materials. A meshless approach is therefore proposed,  
100 characterized by much more compliant topologies, that have no continuity constraints on  
101 displacement fields and related deformations [28]. Some examples of meshless modelling of living  
102 cells are presented in the review paper by Ye et al. (2016) [29] where several particle-based  
103 simulations of red blood cells interacting with a viscous fluid flow are compared. Very interesting is  
104 also the work of Vassaux and Milan (2017) [30], who studied the effect of the curvature of substrate  
105 during the adhesion of stem cells through a particle-based approach.

106         In this study, AFM nanoindentation measurements were first conducted on human  
107 mesenchymal stem cells. Then, a Coarse-Grained Elastic Network Modelling (CG-ENM) [31–33]  
108 was used to represent the stem cell and its subcellular components and to simulate the nanoindentation  
109 process. The pre-tensioning stress state acting on the cell due to the action of stress fibers that anchor

110 the cell to the substrate, was also simulated and the Young's moduli of different subcellular  
111 components, such as cytoskeleton and cortex, were extracted. The results obtained with the elastic  
112 network model were then compared to those computed with the Hertz contact theory and the Finite  
113 Element Method (FEM), showing a good agreement. We found that the proposed approach generally  
114 leads to a stable numerical modelling even for large deformations and to significantly smaller  
115 computational costs than those typically required in mesh-based numerical tools.

116

## 117 **2. Materials and methods**

### 118 **2.1 Study overview**

119 Aiming at extracting a set of global Young's moduli, a physical-mathematical scheme has  
120 been proposed (Fig. 1), implementing a reversed approach with respect to the classical  
121 characterization of new materials performed through tensile tests. In detail, the workflow includes  
122 the following steps and procedures (Fig. 1):

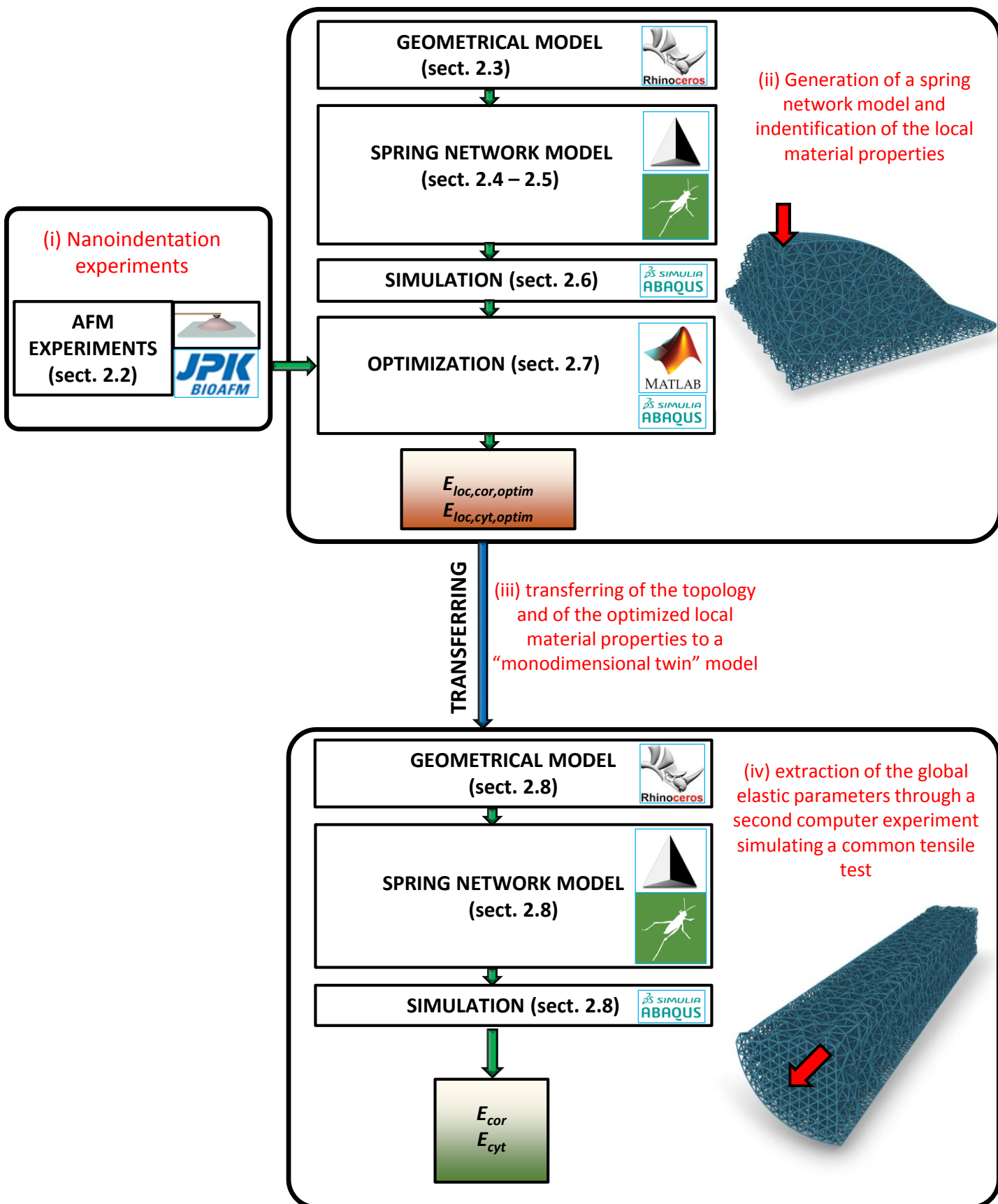
123 (i) nanoindentation experiments;

124 (ii) generation of a spring network model simulating the nanoindentation process of a stem  
125 cell and identification of the optimal local material properties through numerical fitting of real force-  
126 indentation curves;

127 (iii) transferring of the topology and of the optimized local material properties of the spring  
128 network model to a "monodimensional twin" model;

129 (iv) extraction of the global elastic parameters through a second computer experiment  
130 simulating a common tensile test.

131 The last step allowed the comparison of the obtained global Young's moduli to the tabular  
132 data available in the literature. Each block illustrated in Fig. 1 was extensively described in the  
133 following sections.



**Fig. 1.** Scheme of the workflow implemented to extract the stem cells mechanical properties.

## 134 2.2 AFM experiments

135 For AFM nanoindentation measurements, mesenchymal stem cells (MSCs) from human  
136 primary material were used, donated by the Professor Ho group from the Department of Medicine V,  
137 Heidelberg University and isolated as described in a previous study [34].

138 After seeding of the mononuclear cell fraction evolving colonies were separated and MSC  
139 further expanded in plastic culture flasks. Cells were cultured in a sub-confluent monolayer in growth  
140 medium consisting of DMEM-LG supplemented with MCDB201 (40% (v/v), Sigma), L-glutamine  
141 (2 mM, Sigma), penicillin/streptomycin (100 U/mL, Lonza), insulin transferrin selenium (1% (v/v),  
142 Sigma), linoleic acid albumin from bovine serum albumin (1% (v/v), Sigma), dexamethasone (10 nm,  
143 Sigma), L-ascorbic acid-2-phosphate (0.1 mM, Sigma), PDGF subunit B (PDGF-BB), epidermal  
144 growth factor (EGF) (both 10 ng/mL; PreproTech, Rocky Hill, NJ, USA) and FCS (2% (v/v),  
145 HyClone, GE Healthcare). Medium was changed twice per week and early passages 3-5 were used.

146 MSCs were detached with Accutase (Sigma Aldrich) from the flask and plated on glass cover  
147 slips (24×24 mm<sup>2</sup>; Menzel Gläser, Braunschweig, Germany), previously coated with 1 mg/mL of  
148 Fibronectin (Sigma) diluted in PBS. 20 nM of bone morphogenetic protein 2 (purchased from R&D  
149 Systems Inc. Minneapolis, MN, USA) has been added to the media to induce osteogenic  
150 differentiation of MSCs. The differentiation media was replaced once during the first week and just  
151 before the experiment.

152 The cell indentation experiment was realized with an AFM (Nano Wizard, JPK Instruments,  
153 Berlin) combined with an inverted optical microscope (Zeiss Axiovert 200). Glass cover slips were  
154 mounted onto a stage (Biocell™ JPK) in differentiation medium, maintaining the temperature at 37  
155 °C. Chromium/gold-coated cantilevers (CP-qp-SCONTBSG from sQUBE) with a colloidal probe  
156 (radius  $R = 5 \mu\text{m}$ ) made of borosilicate glass were used with a nominal spring constant  $k$  between  $k$   
157  $= 0.006 - 0.015 \text{ N/m}$ , resonance frequency between 8 – 13 kHz and length around 125  $\mu\text{m}$ .  
158 Nanoindentation was performed by lowering the AFM tip onto the cell surface. After the contact with  
159 the cell, the AFM tip exerts an indentation force  $F = k \cdot D$ , being  $D$  the cantilever deflection, which is



160 registered for each position ( $Z$ ) taken by the piezoelectric translator. The indentation ( $d$ ) of the cell  
161 can hence be computed as  $d = Z - D$ .

162 The stiffness of MSCs was tested one week after osteogenic induction via osteogenic media.  
163 Indentation curves were obtained along the cell body crossing the cellular soma. The AFM was set  
164 to have a maximal indentation value no larger than 200 nm to obtain forces applied by the cantilever  
165 to the sample always less than 5 nN. For the calculation of the cellular mechanical properties, we  
166 averaged five curves taken at the highest point of the cell (corresponding to the soma) and we repeated  
167 the measurements on ten different mesenchymal stem cells. In detail, before computing the average  
168 value, the five curves were aligned in correspondence of the point where the AFM tip comes in contact  
169 with the cell. The function *interp1* available in Matlab was utilized to determine, for each  
170 experimental curve, the value of indentation force at specific indentation depths (query points). Then,  
171 the average force-indentation curve was determined, for each of the ten cells, by simply computing  
172 the average value of force in correspondence of these indentation depths and was implemented in the  
173 optimization algorithm described below in the Section 2.7.

174

### 175 **2.3 Geometrical modelling**

176 Stem cells belong to the category of eukaryotic cells and are characterized by a complex  
177 internal structure. The subcellular components that were hypothesized to be included in the stem cell  
178 model are: the nucleus, the cytoskeleton, a network of interconnected filaments [35], and the cell  
179 cortex. Stress fibers and focal adhesions, that guarantee the adhesion of the cell to the substrate where  
180 it lies, were also modelled. Focal adhesions are transmembrane receptors that establish the adhesion  
181 between the ventral side of the cell and the extracellular matrix [36–38]. Stress fibers are contractile  
182 bundles of actomyosin subjected to tensile stress, mechanically interacting with focal adhesions and  
183 additional cellular structures [39] – such as the neighboring stress fibers and the cell cortex – and  
184 exerting on them a traction force of 10 nN [40,41]. More in detail, three main types of stress fibers

185 exist, distributed in different regions and characterized by different lengths, from 10 to 100  $\mu\text{m}$ : dorsal  
186 stress fibers, ventral stress fibers and transverse arcs [42].

187 The CAD model of the cell was developed in the Rhinoceros CAD software environment  
188 (Version 6, McNeel) integrated with the Grasshopper plugin. The main geometry is essentially a  
189 revolved surface (Fig. 2), with an overall size of 50  $\mu\text{m}$  for diameter and 13  $\mu\text{m}$  for height (Fig. 2c),  
190 which is consistent with the typical size of mesenchymal stem cells [43]. In order to reduce the  
191 computational effort and increase the resolution at the same time, only a quarter of the whole cell was  
192 modelled. Following previous studies [30,44,45], nine stress fibers were modelled in the quarter  
193 model: three arcs, three dorsal (i.e. two entire and one comprised of two halves) and three ventral  
194 stress fibers. This quarter model includes the following components (Fig. 2b):

195 (i) the nucleus, represented by a sphere quarter of radius 5  $\mu\text{m}$ , with the center placed at 6  $\mu\text{m}$   
196 from the bottom surface;

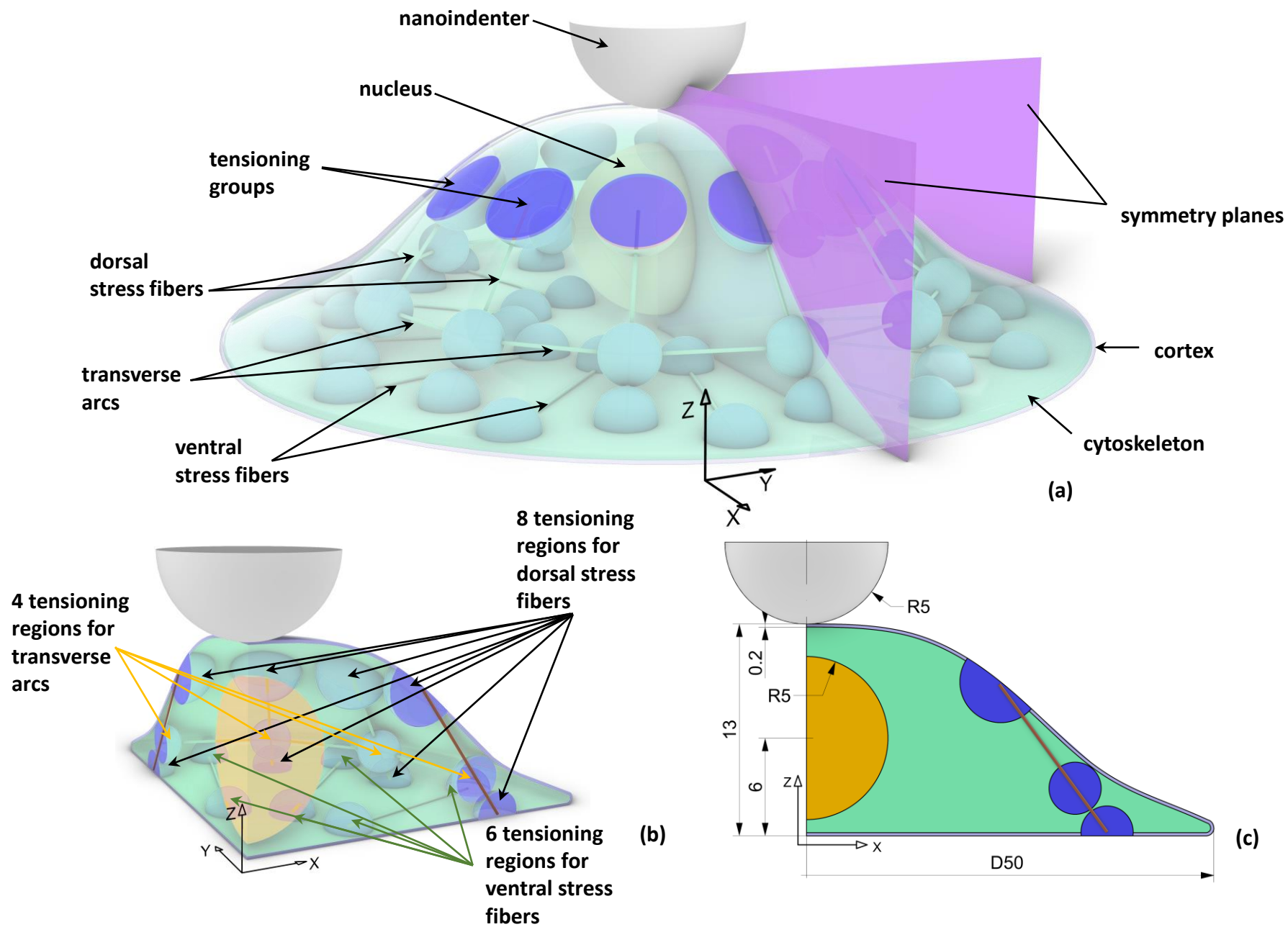
197 (ii) 8 tensioning regions for dorsal stress fibers, 4 located in the medium-upper part of the cell  
198 cortex (representing the cell regions where the dorsal stress fibers apply traction forces in downward  
199 direction) and 4 at the bottom of the cell (representing the regions where the dorsal stress fibers are  
200 connected to the focal adhesions) (Fig. 2b);

201 (iii) 4 tensioning regions for transverse arcs, located in the lower part of the cell cortex and  
202 connected to the dorsal stress fibers (Fig. 2b);

203 (iv) 6 tensioning regions at the bottom of the cell where the ventral stress fibers are connected  
204 to the focal adhesions (Fig. 2b);

205 (v) the cortex region that was separated from the cytoskeleton, thus obtaining a 200 nm thick  
206 membrane enclosing the whole cell (Fig. 2c). The value of the cell cortex thickness used in the model  
207 is consistent with that hypothesized in previous studies [11,19];

208 (vi) the cytoskeleton, resulting as the volume obtained by subtracting, from the initial volume  
209 of the whole cell, the previous volumes.



**Fig. 2.** Exploiting the symmetry properties of the entire cell geometry (a), just one-quarter model was considered (b). (c) Principal dimensions in micrometers of the model used in the study.

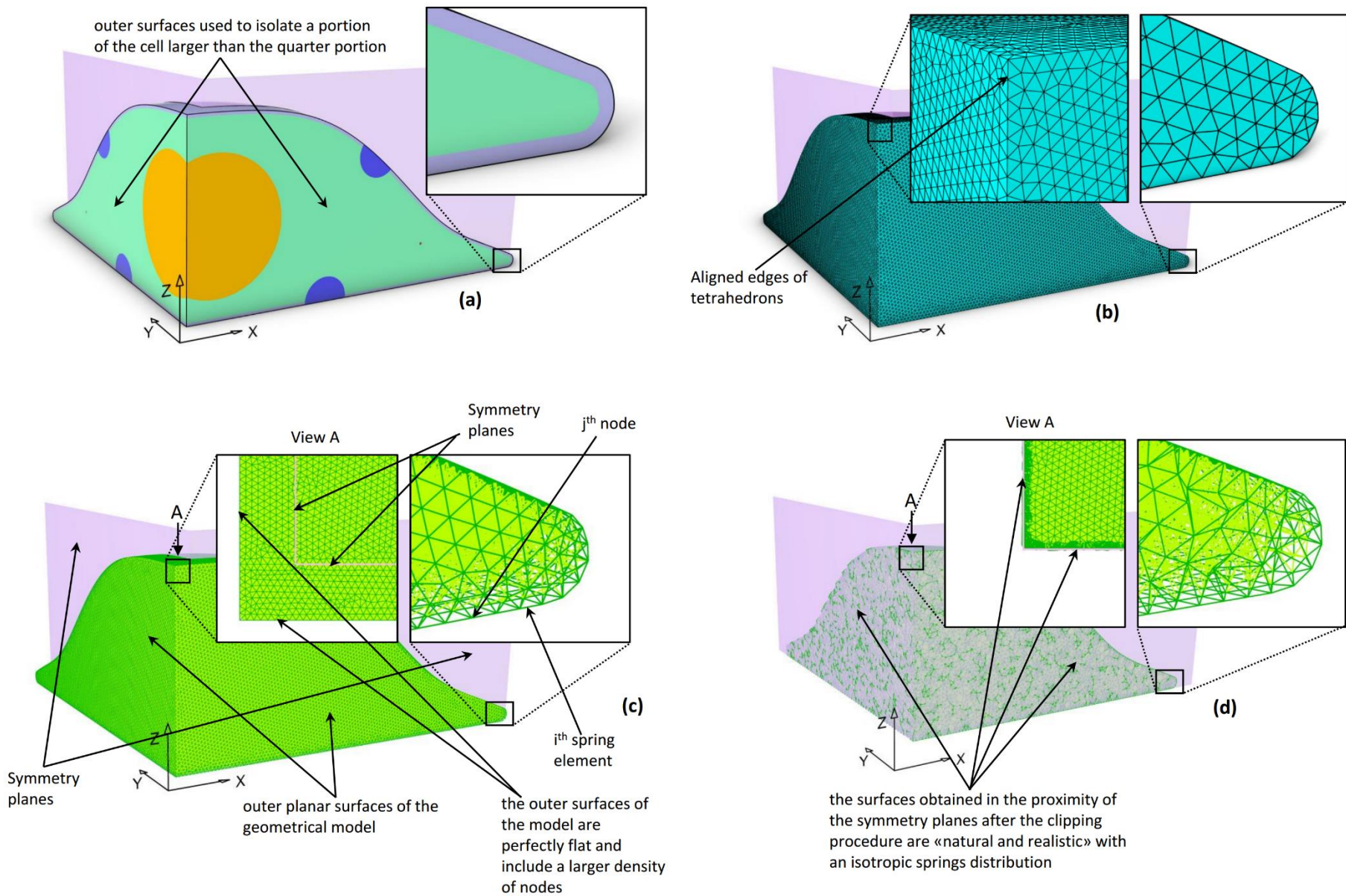
210 The strategy of using tensioning regions allowed to distribute the traction force (which is a  
211 relatively intense force, having the same order of magnitude as the force exerted by the AFM tip on  
212 the whole cell) exerted by stress fibers on large-dimensioned regions, thus avoiding numerical issues.  
213 In fact, the application of the whole traction force on a single point would produce a deformation so  
214 large that any computational tool (including finite element method and coarse-grained elastic network  
215 model) would inevitably undergo non-convergence problems. Tensioning regions not only allow  
216 solving numerical issues, but also reproduce in a reasonably correct way the physics and the biology  
217 of the cell, where internal loads do not act on point-like regions with limited dimensions but on  
218 relatively wide regions, whose actual extensions are proportional to the force exerted to them [46–  
219 48].

220

## 221 **2.4 Spring network generation**

222 The mathematical/physical approach used to model the stem cell, treated as a deformable  
223 object, is a coarse-grained elastic network modelling [31]. It consists of a uniform network of  
224 interconnected springs characterized by a single stiffness magnitude for the elements inside every  
225 single functional region, implementing an internal force field acting between pairs of coarse-grained  
226 lumped masses representing groups of atoms or molecules. The mutual connections between the  
227 different spring elements are spherical joints, thus enabling the maximum structural compliance,  
228 peculiar of a soft biological material.

229 To implement this framework, the CAD model (Fig. 3a) was exported from Rhinoceros and  
230 given in input to the open source meshing software Gmsh (version 4.5.2). A tetrahedral volumetric  
231 mesh was then generated (Fig. 3b), appropriately selecting a refined Delaunay grid algorithm as a  
232 discretization rule, aiming at maximizing uniformity of spring lengths and isotropy in behavior. The  
233 average element size was set equal to  $0.350\ \mu\text{m}$  which is a sufficiently small and adequate dimension  
234 to satisfactorily solve the problem of variable contact between the nanoindenter and the upper region  
235 of the cell cortex.



**Fig. 3.** The CAD model (a) generated in Rhinoceros was given in input to the open source meshing software Gmsh (version 4.5.2) where a tetrahedral volumetric mesh was generated (b). The tetrahedral mesh was then converted into a spring network model (c). To generate the quarter model, a portion of the whole cell larger than the quarter portion was initially considered (a-c). A clipping procedure was finally executed (d) to isolate the portion of the spring network model exactly corresponding to the quarter of the cell. The aligned edges of tetrahedrons shown in (b) have been obtained at the intersection of the planes delimiting the model outer surface. The clipping procedure successively carried out, allows having not aligned edges (and hence not aligned springs) on the axis along which the nanoindenter acts and consequently, an isotropic behavior of the model.

236 The next operation was the transformation of the tetrahedral mesh into a spring network model  
237 (Fig. 3c). To do this, we converted the edges of the tetrahedrons generated in the meshing phase into  
238 springs and the vertices of the tetrahedrons into nodes where springs are interconnected. The  
239 information associated to nodes position and spring connectivity was processed by an ad hoc  
240 algorithm coded in the Grasshopper plugin, for the generation of the spring network into the CAD  
241 environment. The result of the conversion process is a pattern of springs arranged in a tetrahedral  
242 structure according to a refined Delaunay topology (Fig. 3c). The total number of springs was 887605  
243 and the average rest length of springs was 0.345  $\mu\text{m}$ .

244 To generate the quarter model, a portion of the whole cell larger than the quarter portion was  
245 initially considered (Figs. 3a-c). This portion was meshed and converted into the spring network  
246 model. A clipping procedure was finally executed (Fig. 3d) to isolate the portion of the spring network  
247 exactly corresponding to the quarter of the cell. With this strategy, we solved two main problems that  
248 arise when the quarter model is built directly using cutting planes exactly corresponding to the  
249 symmetry planes of the cell. The first problem is related to the isotropy of the model: the refined  
250 Delaunay algorithm, selected to build the tetrahedral mesh, populates the outer planar surfaces of the  
251 geometrical model with a quite regular triangular tessellation and so the density of nodes on these  
252 surfaces will be higher than that of any other plane crossing the model. This implies that if the outer  
253 planes of the geometrical model correspond to the symmetry planes, on those planes a larger density  
254 of nodes and hence a larger density of springs will result, and also this local distribution of springs  
255 will be perfectly planar, which contrasts the hypothesis of isotropy of the spring network. The second  
256 problem is related to the structural response of the nanoindented material. If the outer planes of the  
257 geometrical model correspond to the symmetry planes of the cell, at the intersection between the two  
258 planes, the refined Delaunay algorithm will build tetrahedrons with one of the edges aligned to the  
259 central axis around which the revolution of the cell model takes place. These edges will be converted  
260 into springs and due to their alignment with the central axis, which is also the direction along which  
261 the nanoindenter acts, the structural response of the cell model to the nanoindentation process will

262 mostly depend on those aligned springs. However, this contrasts the hypothesis of isotropic  
263 mechanical behavior of the model. The above-mentioned problems are overcome and solved with the  
264 described clipping procedures that allow, definitely, leaving a quarter of the cell with an isotropic and  
265 a more “natural and realistic” springs distribution, especially in the regions close to the symmetry  
266 planes.

267

## 268 **2.5 Definition of spring groups**

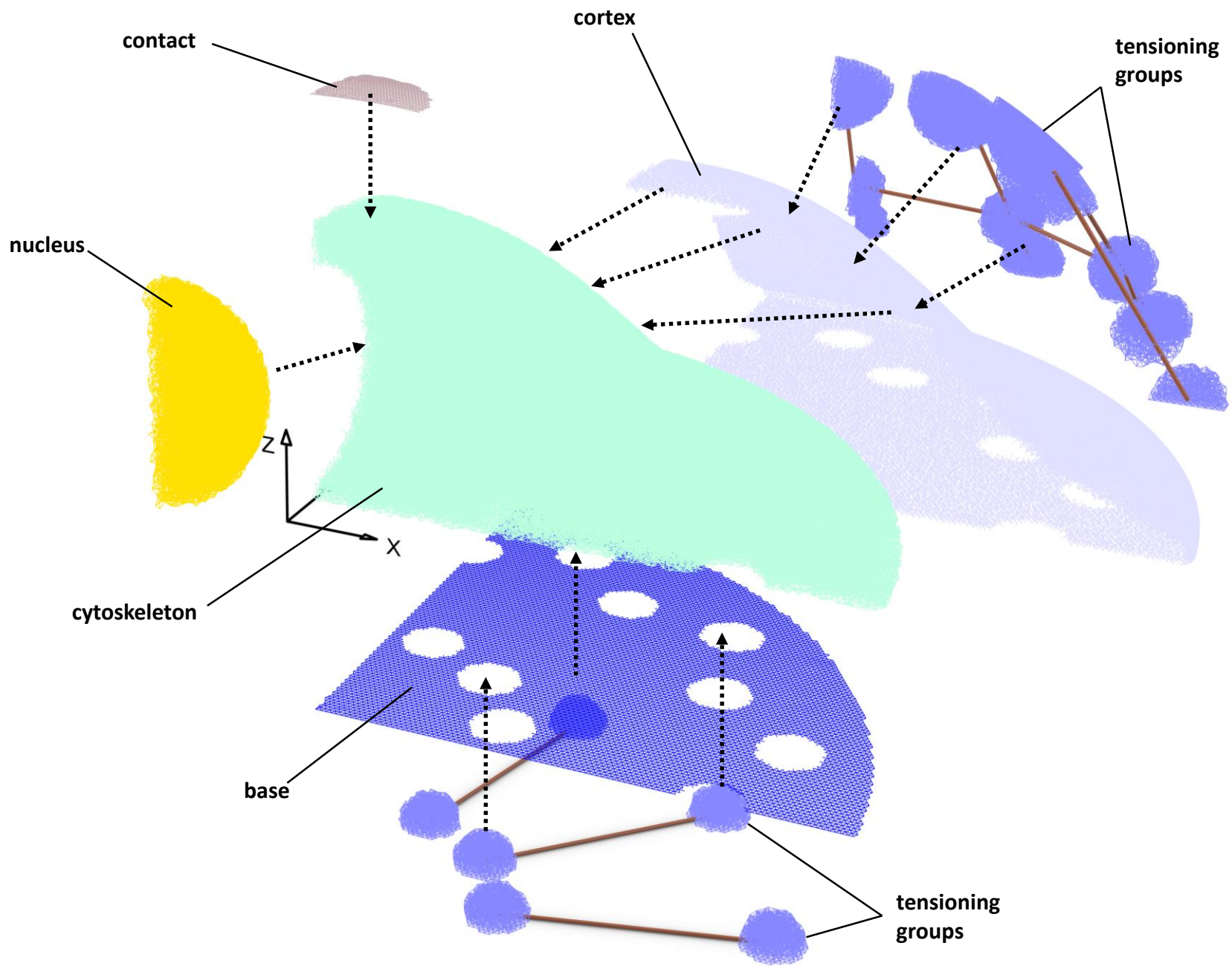
269 The next phase was the definition, into the CAD environment, of different spring groups  
270 functional to the subsequent simulation (Fig. 4). The spring groups that were defined are the  
271 following. (i) Nucleus – the central spherical region characterized by higher stiffness compared with  
272 that of the cytoskeleton. (ii) Tensioning groups – the regions of the cytoskeleton pulled downwards  
273 or upwards (in case of dorsal stress fibers) or pulled together (in case of transverse arcs). (iii) Cortex  
274 – the external surface surrounding the whole cell, characterized by a constant thickness. (iv) Base –  
275 the lowest layer of the cell, representing the flat surface adherent to a substrate. (v) Contact – the  
276 portion of the cell cortex that enters in contact with the spherical indenter. (vi) Free – the remaining  
277 unconstrained region mainly corresponding to the cytoskeleton, affected by the boundary conditions  
278 imposed by adjacent groups, always interconnected to it.

279

## 280 **2.6 Simulation**

281 All the geometrical entities composing the spring network model of the cell were then  
282 imported in Abaqus (version 6.14, Dassault Systèmes) where a spherical nanoindenter with a 5  $\mu\text{m}$   
283 radius – which is the same radius of the nanoindenter used in the experiments – was created and  
284 modelled as a rigid part aligned to the revolution axis of the cell model.

285 The springs were modelled as truss elements, i.e. two-node elastic rods with a constant cross  
286 section  $A$ , set equal to  $1 \mu\text{m}^2$ , and a rest length  $L_0$  equal to the average dimension of the edges of the  
287 previously meshed tetrahedrons. The springs are capable of generating internal reaction forces acting



**Fig. 4.** Spring groups defined in the model.

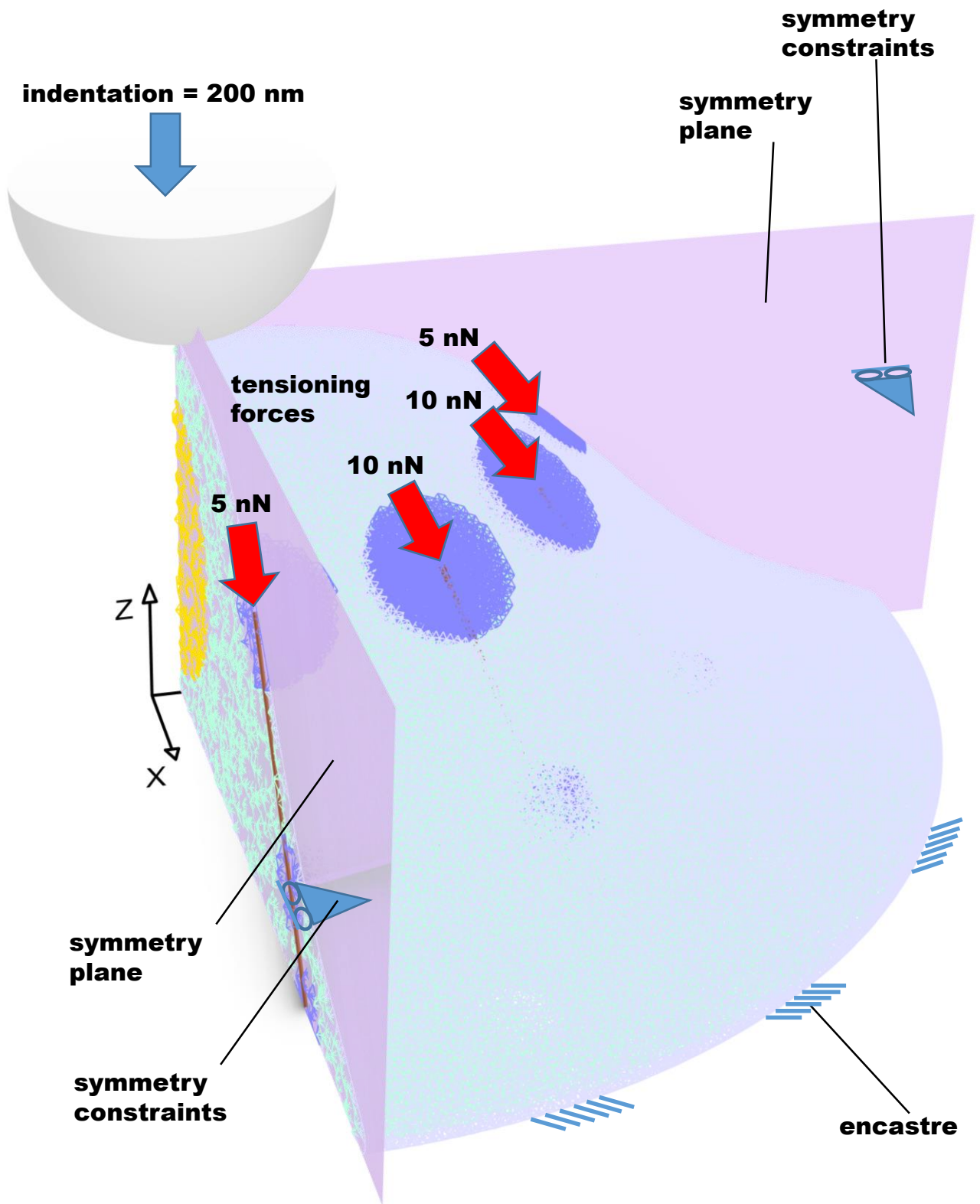


288 only along the axis connecting the nodes. As the truss elements are connected one each other with  
289 spherical joints, that constraint only the relative positions, leaving free the relative rotations, no  
290 bending stiffness has been defined for this kind of elements. The extensional spring stiffness  $E_{loc}A/L_0$   
291 was defined by setting a local Young's modulus, one for cytoskeleton springs  $E_{loc,cyt}$  and one for  
292 cortex springs  $E_{loc,cor}$ , assuming for the nucleus region a local modulus  $E_{loc,nuc}$  one order of magnitude  
293 higher than the average stiffness of the cytoskeleton.  $E_{loc,cor}$  and  $E_{loc,cyt}$  were determined via the  
294 optimization algorithm outlined in the next section, that iteratively compares the numerical force-  
295 indentation curve computed in the simulation with the same curve obtained in the nanoindentation  
296 measurements previously described. The choice of setting the value of the material properties for the  
297 nucleus and not to optimize it derives from the fact that, being the nucleus rather distant from the  
298 nanoindenter tip, the system is not sufficiently sensitive to “capture” the structural response of the  
299 nucleus and therefore to analyze its mechanical behavior. The same hypothesis was followed in a  
300 previous study [11].

301 The contact between the nanoindenter and the upper region of the cell was assumed as  
302 frictionless and was solved by implementing the “hard contact” algorithm available in Abaqus.

303 The simulation included two different steps: tensioning of the stress fibers and indentation,  
304 consisting in the rigid translation of the spherical indenter into the cell surface. Both steps were treated  
305 on a physical-mathematical level as incremental static problems, thus excluding all dynamic  
306 contributions (inertia, time-dependent effects, dissipations, etc.). The condition of “nonlinear  
307 geometry” was enabled in every simulation, in order to correctly map the large local deformation  
308 field in the contact region.

309 The boundary and loading conditions acting on the model were the following (Fig. 5). (i) No  
310 displacement or rotation allowed for the base layer of the cell (encastre) for reproducing adhesion.  
311 (ii) 10 nN [40,41,46] forces imposed on every single tensioning group for stress fibers (for the  
312 tensioning groups crossing the symmetry planes a 5 nN force was applied in order to achieve a 10 nN  
313 force when mirroring the quarter model). The overall force in every group was distributed as 25% in



**Fig. 5.** Boundary and loading conditions imposed to the cell in the simulation environment. For the sake of clarity only the forces acting on the visible tensioning groups are indicated and not those acting on the internal ones.

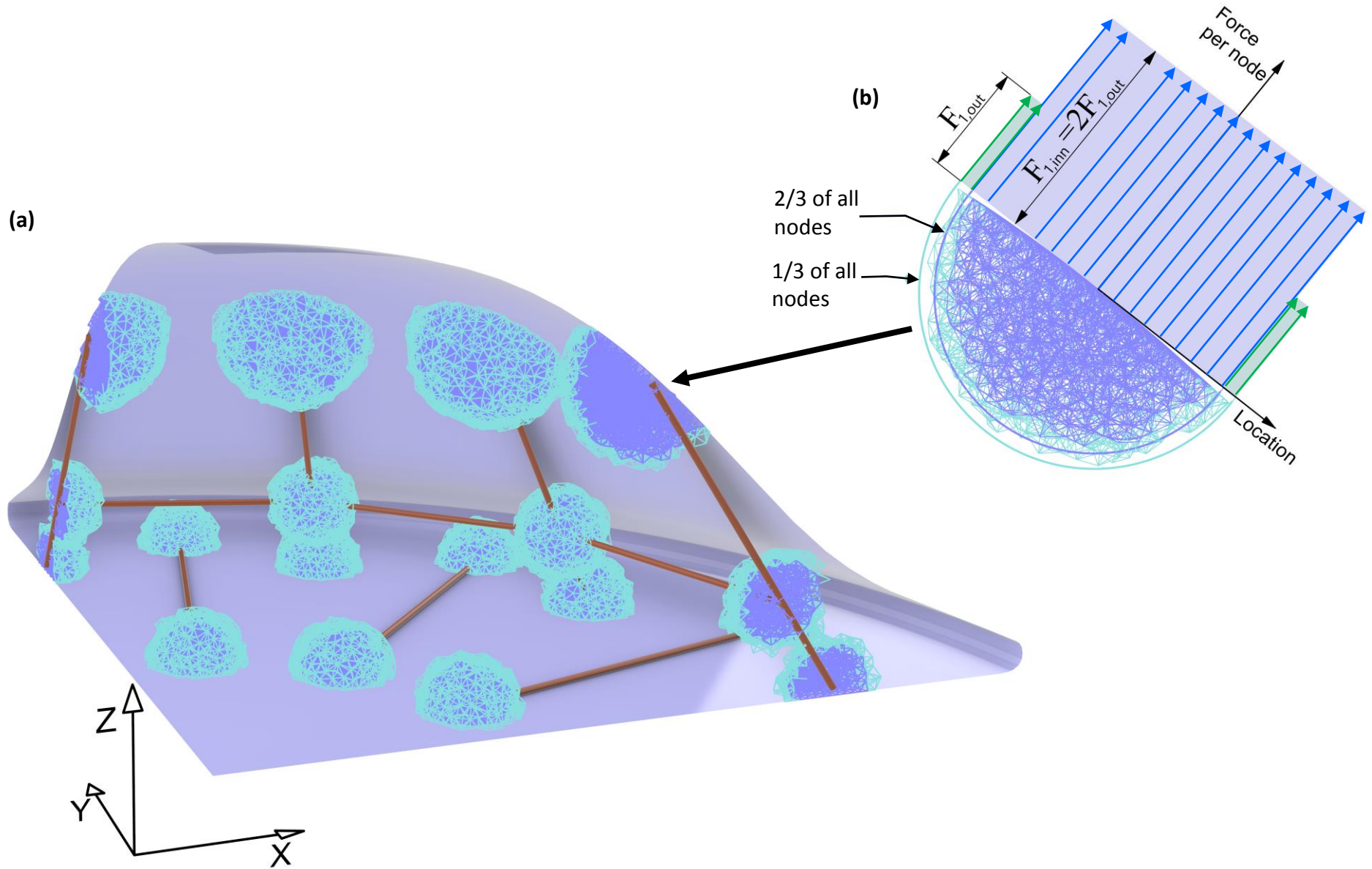
314 an outer shell and 75% in an inner half-sphere, in order to realize a sort of force gradient when moving  
315 from the highly-tensioned central region to the unloaded free elements neighboring the tensioning  
316 groups [46]. In order to implement such a condition, the force per node in the inner half-sphere region  
317  $F_{1,inn}$  was set equal to  $2F_{1,out}$ , the latter being the force per node for the nodes in the outer shell region  
318 (Fig. 6). With this strategy, a non-natural and non-physical sudden step-shaped magnitude change of  
319 the force acting on the tensioning groups was avoided, thus realizing a gradient for the force per node  
320 [46–48]. (iii) A 200 nm displacement imposed to the rigid spherical indenter in the second step. (iv)  
321 Symmetry constraints imposed on the symmetry planes delimiting the model.

322 The measurement units used in the simulation were  $\mu\text{m}$  for linear dimensions and nN for  
323 forces; this combination has proved to be optimal to ensure the stability of the solver, thus avoiding  
324 under/overflow issues.

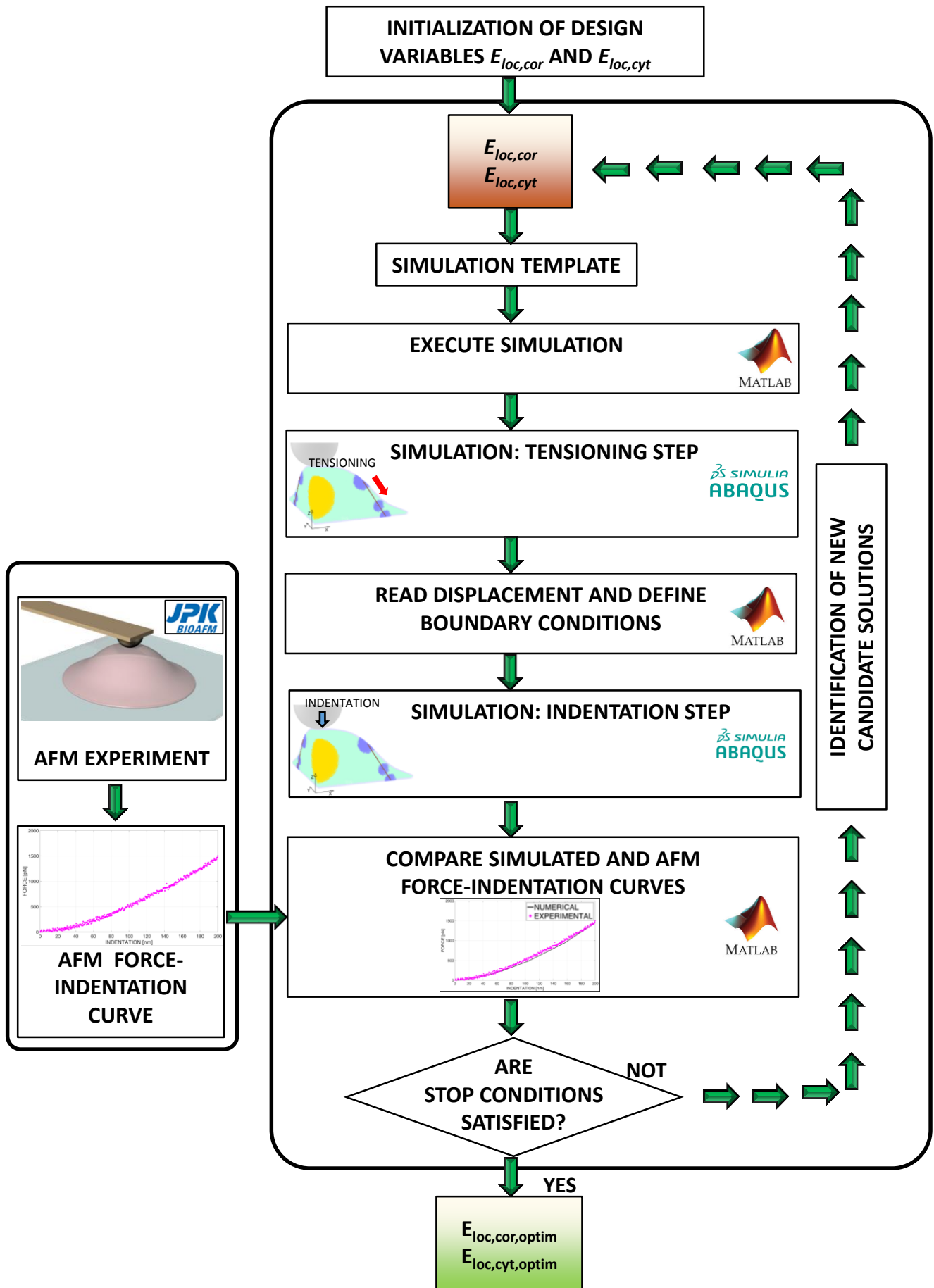
325

## 326 **2.7 Optimization**

327 An optimization algorithm was written in Matlab environment for iteratively comparing the  
328 force-indentation curves obtained as experimental AFM reports to those predicted in the numerical  
329 simulations (Fig. 7). In detail, in the optimization algorithm, the *fmincon* tool available in the  
330 optimization toolbox of Matlab, was implemented, that is devoted to find the minimum of constrained  
331 nonlinear multivariable functions. Starting from guess values for  $E_{loc,cor}$  and  $E_{loc,cyt}$ , defining the local  
332 spring stiffnesses for cortex and cytoskeleton respectively and perturbing them, different resulting  
333 curves were obtained as output from the numerical solver. Following a previous study [12], for each  
334 cell sample, optimization runs were started from five different sets of material properties previously  
335 randomly generated. Thanks to this strategy and to the large range of variability chosen for material  
336 parameters, the whole search space was covered, thus increasing the probability of finding the global  
337 optimum. The algorithm iterated this cycle until the least squared difference between experimental  
338 and numerical force-indentation curves was less than a pre-defined numerical threshold. The optimal  
339 values of  $E_{loc,cyt,optim}$  – for cytoskeleton – and of  $E_{loc,cor,optim}$  – for cortex – were then given in input to



**Fig. 6.** The overall force in every group (a) was distributed as 25% in an outer shell and 75% in an inner half-sphere (b), in order to realize a sort of force gradient when moving from the highly-tensioned central region to the unloaded free elements neighboring the tensioning group.



**Fig. 7.** Scheme of the optimization algorithm implemented to determine the optimal local material properties for cytoskeleton and cortex.

340 a “monodimensional twin” model (sect. 2.8), that aimed at converting them in global Young’s moduli,  
341 comparable with those presented in classical engineering and scientific literature for materials.

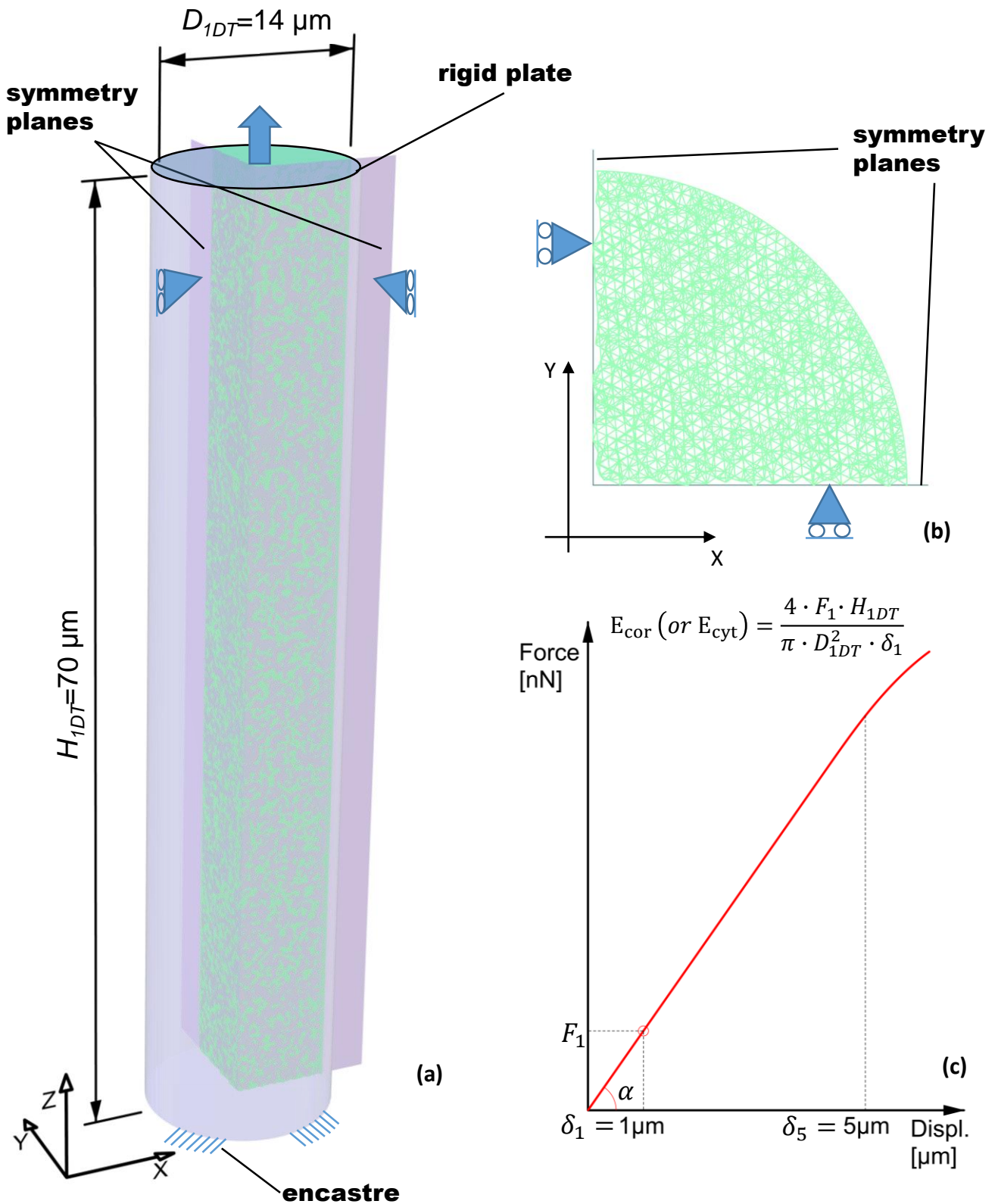
342 The optimization flow above described was executed to calculate the elastic parameters  
343  $E_{loc,cyt,optim}$  and  $E_{loc,cor,optim}$  for ten different stem cell samples. In detail, for each nanoindented cell  
344 sample, five curves were experimentally retrieved by measuring force and displacement on the rigid  
345 nanoindenter. The optimization algorithm utilized as a reference experimental curve for each  
346 investigated stem cell sample, the curve averaged over the five measurements carried out on each  
347 single cell.

348

## 349 **2.8 Extraction of mechanical properties from the monodimensional twin model**

350 The same procedure previously described to build the model of the cell was replicated for a  
351 “monodimensional twin” model, i.e. a cylinder of approximately the same volume of the cell,  
352 presenting an overall height  $H_{IDT} = 70 \mu\text{m}$ , a diameter  $D_{IDT} = 14 \mu\text{m}$  and characterized, according to  
353 the UNI EN 10002-1 standard (Metallic materials - Tensile Testing) by an aspect ratio of height :  
354 diameter = 5 : 1. This cylinder was utilized to extract the global Young's moduli of cytoskeleton and  
355 cortex (Fig. 8). This second model presented the same geometrical (same spring network topology,  
356 cross sections and rest lengths) and structural (same local spring stiffnesses) characteristics of the  
357 cell. Also in this case, exploiting the symmetry of the problem, just one-quarter of the cylindrical  
358 volume was modelled and symmetry constraints were applied to the nodes lying on the symmetry  
359 planes. As in the cell model, outer planes of the geometrical model different from the symmetry  
360 planes were utilized to isolate a portion of the cylindrical volume larger than the quarter portion.  
361 Successively, a clipping procedure was utilized to isolate the portion of the spring network exactly  
362 delimited by the symmetry planes (Fig. 8). With this strategy, we guaranteed the isotropic structural  
363 response of the monodimensional twin model.

364 For each of the ten stem cell samples, two optimal values were determined through the  
365 optimization algorithm above described (sect. 2.7):  $E_{loc,cyt,optim}$ , for the cytoskeleton and  $E_{loc,cor,optim}$



**Fig. 8.** (a) Simulation setup for the monodimensional twin model (dimensions in micrometers); (b) Magnified cross sectional view of the quarter model; (c) Procedure for the extraction of global Young's moduli.

366 for the cortex. These quantities were then used for defining the stiffness of the springs constituting  
367 the monodimensional twin model and hence a virtual tensile test to extract a typical force-  
368 displacement curve was performed. In the simulations, constraints preventing all the displacements  
369 were imposed to the nodes lying on a flat base of the cylinder, while a displacement of  $\delta_5 = 5 \mu\text{m}$  was  
370 imposed to the nodes lying on the other flat base via a rigid plate that was utilized to easily retrieve  
371 the value of the reaction force. Therefore, for each cell sample, two force-displacement curves were  
372 traced, one was obtained by assigning to the springs the Young's modulus  $E_{loc, cyt, optim}$  (for the  
373 cytoskeleton), the other by assigning the Young's modulus  $E_{loc, cor, optim}$  (for the cortex). As in the  
374 common experimental practice, the slope of the curve was extracted for the calculation of the resulting  
375 global Young's moduli  $E_{cor}$  and  $E_{cyt}$ , for cortex and for cytoskeleton, respectively. The value of force  
376  $F_l$  registered for the displacement  $\delta_l = 1 \mu\text{m}$  was utilized to determine the global Young's modulus  
377 as (Fig. 8).

$$378 \quad E_{cor} \text{ (or } E_{cyt}) = \frac{4 \cdot F_1 \cdot H_{1DT}}{\pi \cdot D_{1DT}^2 \cdot \delta_1} \quad (1)$$

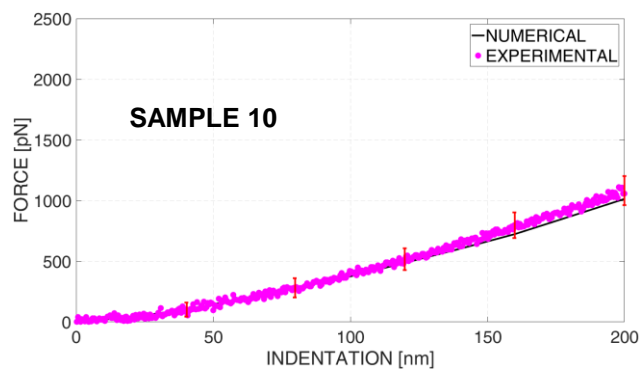
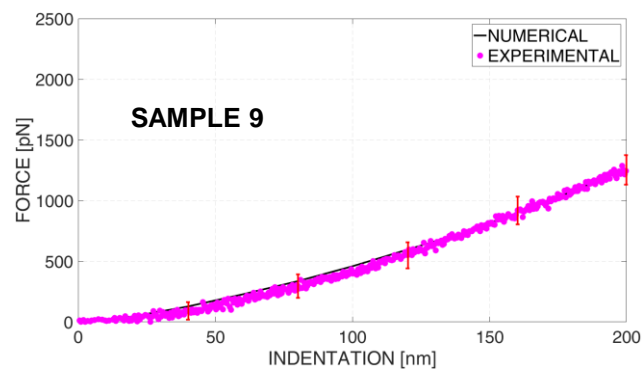
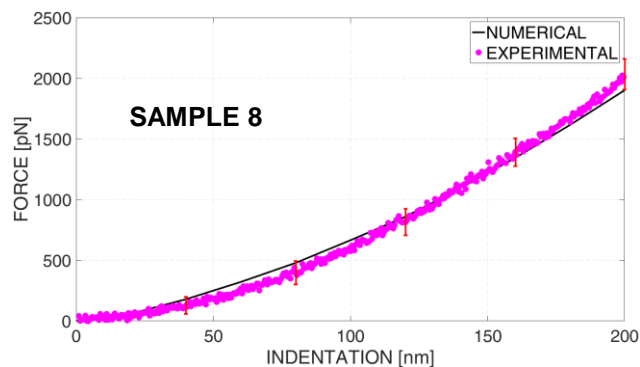
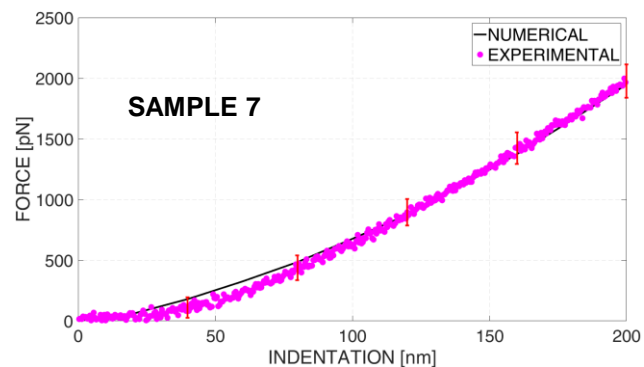
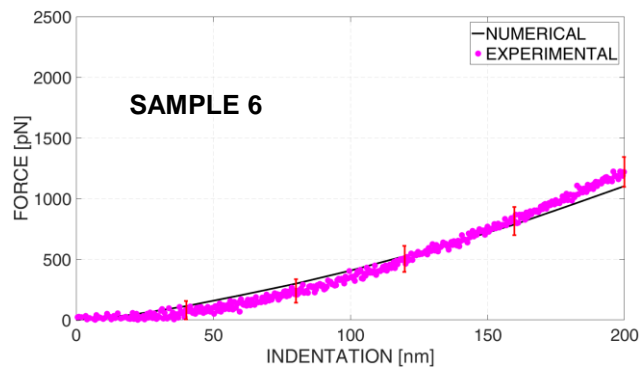
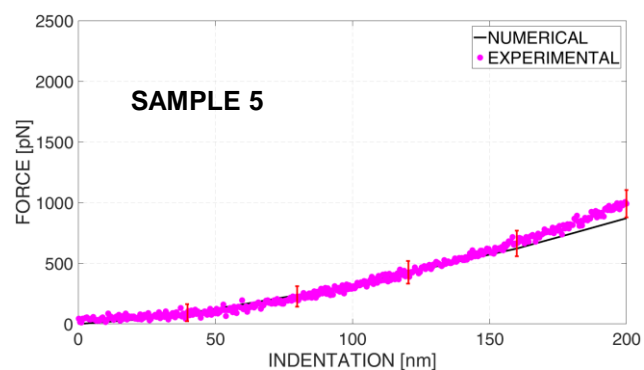
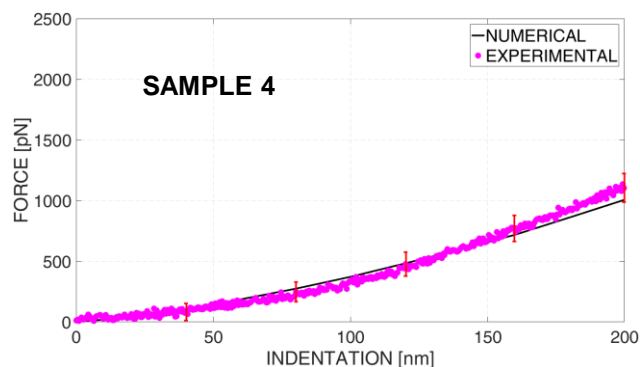
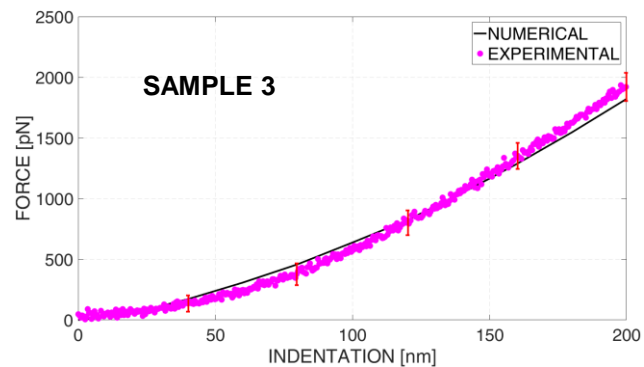
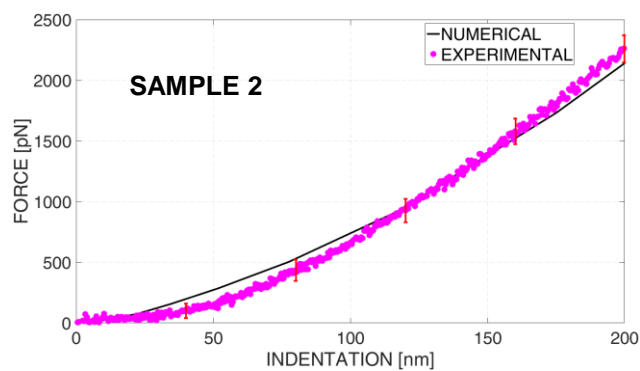
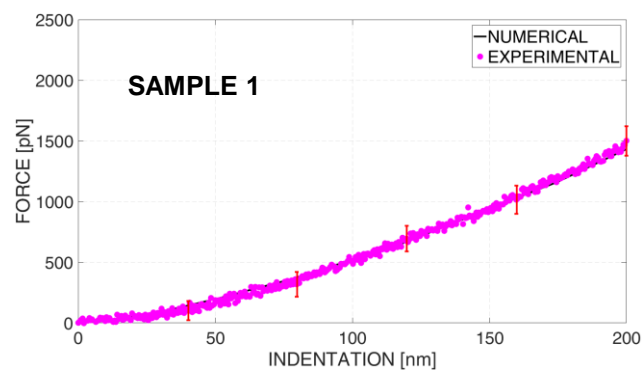
379

### 380 **3. Results and Discussion**

381 The average experimental force-indentation curves were compared to those predicted  
382 numerically (as described in Sect. 2.7) and the optimization algorithm changed many times the elastic  
383 parameters of the spring network model of the stem cell, until the difference between the two curves  
384 was minimized (Table 1). The rather high values of the correlation coefficient  $R^2$ , used as a metric  
385 for the detail level of the curve fitting, could be considered as a proof of the reasonably good adequacy  
386 of the model to reproduce the physics of the problem (Fig. 9, Table 2). Error bars were also  
387 superimposed to the average experimental force-indentation curves to show the dispersion of data at  
388 different indentation depths (Fig. 9).

389 Through the procedure described in Sect. 2.8, the global Young's moduli for cortex and  
390 cytoskeleton were then extracted from the monodimensional twin models and compared to those





**Fig. 9.** Experimental force-indentation curves registered for the ten stem cell samples and related numerical curves predicted via the optimization algorithm. Error bars were superimposed to the average experimental force-indentation curves to show the dispersion of data at different indentation depths.

391 calculated in a previous study [24] implementing both a FEM model based on the Arruda-Boyce  
 392 hyperelastic constitutive law [23,49] and an analytical model based on the Hertz contact theory [50]  
 393 (Table 3).

394 **Table 1.** Optimal local material properties predicted via the optimization algorithm.

Sample N.	Coarse-Grained Elastic Network	
	$E_{loc,cyt,optim}$ [Pa]	$E_{loc,cor,optim}$ [Pa]
Sample 1	160	508
Sample 2	252	490
Sample 3	208	504
Sample 4	110	470
Sample 5	95	415
Sample 6	127	485
Sample 7	230	480
Sample 8	221	507
Sample 9	140	490
Sample 10	120	470

395  
 396 **Table 2.** Correlation coefficient  $R^2$  values related to the experimental-numerical curve fitting,  
 397 computed for the proposed coarse-grained elastic network model versus the same coefficient values  
 398 computed for the FEM model [24].

Sample N.	$R^2$	
	Coarse-Grained Elastic Network	FEM
Sample 1	0,9962	0,9825
Sample 2	0,9836	0,9979
Sample 3	0,9862	0,9984
Sample 4	0,9776	0,9920
Sample 5	0,9733	0,9947
Sample 6	0,9746	0,9959
Sample 7	0,9929	0,9936
Sample 8	0,9893	0,9963
Sample 9	0,9952	0,9833
Sample 10	0,9949	0,9737

399

400 **Table 3.** Global material properties computed with the proposed coarse-grained elastic network  
 401 model compared to those predicted via the FEM method and the Hertz contact theory [24] (applied  
 402 to the experimental force-indentation data).

Sample N.	Coarse-Grained Elastic Network		Finite Element Method, Arruda-Boyce		Hertzian Contact Theory
	$E_{\text{cyt}}$ [Pa]	$E_{\text{cor}}$ [Pa]	$E_{\text{cyt}}$ [Pa]	$E_{\text{cor}}$ [Pa]	$E_{\text{Hertz}}$ [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

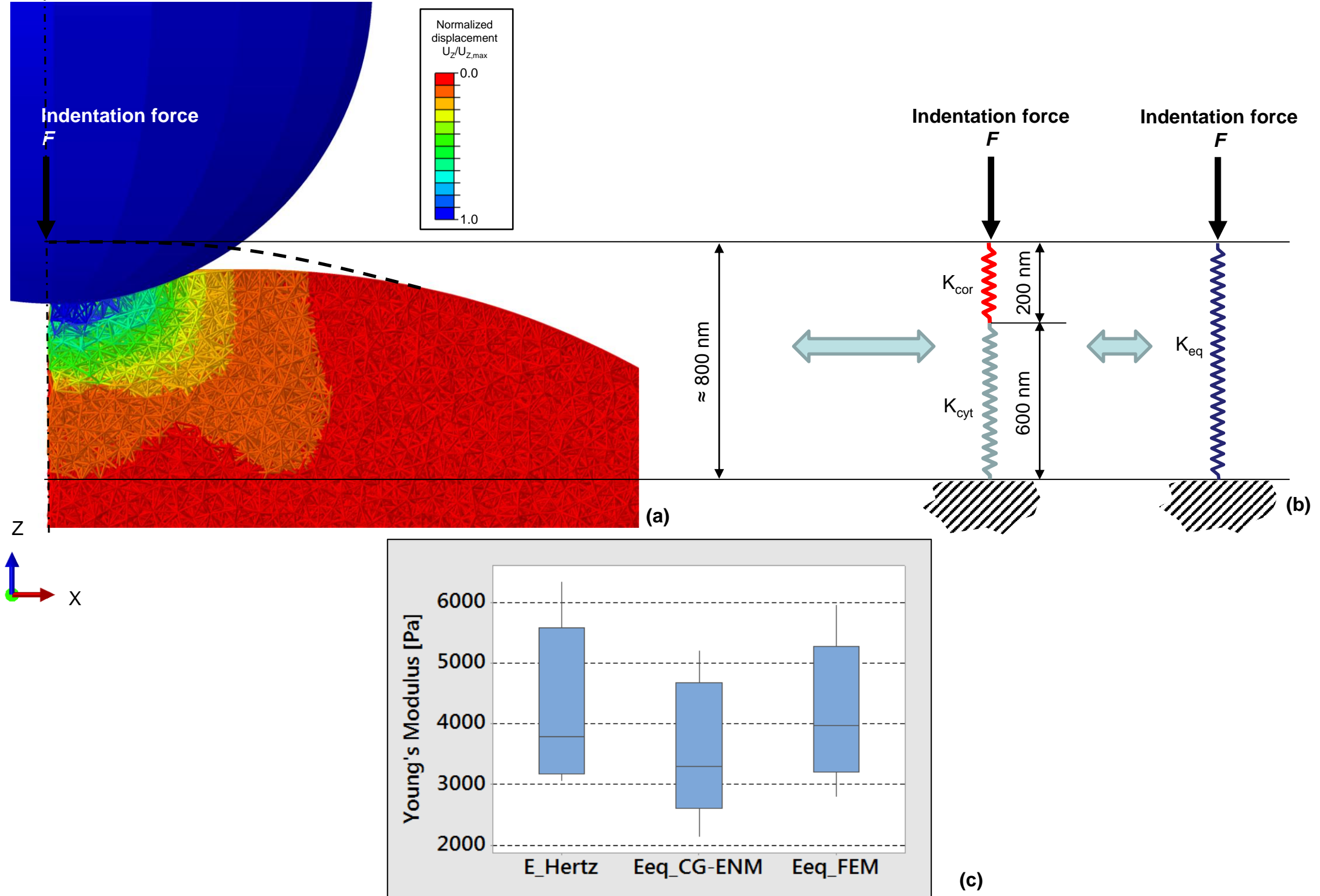
403  
 404 In detail, the values of  $E_{\text{cyt}}$  and  $E_{\text{cor}}$  predicted through the Finite Element Method and listed in  
 405 Table 3, were computed starting from the optimal hyperelastic parameters proper of the Arruda-  
 406 Boyce law, i.e. the shear moduli  $\mu_{8\text{chain},\text{cyt}}$  (for cytoskeleton), and  $\mu_{8\text{chain},\text{cor}}$  (for cortex) according to  
 407 the following relationships:  $E_{\text{cor}} = 2(1+\nu)\mu_{8\text{chain},\text{cor}}$ ,  $E_{\text{cyt}} = 2(1+\nu)\mu_{8\text{chain},\text{cyt}}$  where  $\nu$ , the Poisson's ratio,  
 408 was set equal to 0.4999 to account for material incompressibility [12,19].

409 The proposed coarse-grained elastic network model has some limitations. First, the Poisson's  
 410 ratio is a non-controllable parameter [33], which directly depends on the discretization rule of the  
 411 deformable volume, consisting in a nearly-regular and hence isotropic network of interconnected  
 412 tetrahedrons. Second, the organelles residing into the cytoplasm were considered as a part of it and  
 413 their material properties were "confused" with those of cytoskeleton. Third, even if the proposed  
 414 model is capable of distinguishing, unlike the hertzian contact theory, the material properties of  
 415 different subcellular components, in the current study a clear and direct experimental demonstration  
 416 of the correctness of the model predictions is missing. Ideally, to demonstrate this, nanoindentation

417 measurements should first be carried out on cells, the proposed coarse-grained elastic network model  
418 should then be implemented to determine the material properties of different subcellular components.  
419 At this point, the different subcellular components should be physically isolated, nanoindented and  
420 analyzed, one by one, with the proposed model. If the model works correctly, the material properties  
421 predicted before and after the isolation should be the same. An intriguing procedure recently proposed  
422 [51] that can be adopted to separate the single contributions may consist in treating pharmacologically  
423 and selectively the single subcellular component. One should first nanoindent the cell and retrieve  
424 the force-indentation curve. Based on this curve, the proposed model should be implemented to  
425 determine the mechanical properties of the different subcellular components. At this point, a  
426 pharmacological treatment can be made to alter the mechanical properties of a specific subcellular  
427 component. Obviously, this leads to the alteration of the “global” force-indentation curve. We expect  
428 that by implementing the proposed framework to the altered force-indentation curve, the system will  
429 predict altered mechanical properties for the pharmacologically targeted component and the same  
430 mechanical properties - as those predicted in the cell before the pharmacological treatment - for the  
431 components not targeted. However, due to compensatory effect inside the cell, we believe that the  
432 selectivity of the pharmacological treatment could be an issue to investigate. For instance, the  
433 chromatin condensation in the nucleus with Trichostatin A (TSA) has been targeted in a previous  
434 work but that induced an unpredictable change in the stiffness of the nucleus that is cell-dependent  
435 and a slight stiffening of the cytoplasm as compensatory effect [51]. In the future, it will be interesting  
436 to address these aspects by engineering a synthetic eukaryote cell with a bottom-up approach and  
437 therefore isolating the influence of each component on the mechanical properties of the engineered  
438 cell, as a result of a multi-variable experimental plan. Fourth, hypothesizing to schematize the cell as  
439 a revolved surface, just one-quarter model was considered and symmetry constraints were applied on  
440 the symmetry planes. Indeed, symmetry constraints can give edge effects especially in the case where  
441 the extension of the constrained region is comparable to the extension of the unconstrained region.  
442 However, in the proposed model, the extension of the constrained region is much smaller than that of

443 the unconstrained region. Just to give an idea of this, we can state that the number of nodes where  
444 symmetry constraints act is the 5.97% of the total number of nodes. Preliminary analyses revealed  
445 that the generic force-indentation curve computed with the one-quarter model is practically  
446 overlapped to that predicted with the entire model. For instance, implementing  $E_{\text{cyt}}$  and  $E_{\text{cor}}$  computed  
447 for the cell sample 9 we found, for a 200 nm indentation depth, that the force predicted with the entire  
448 model differ from that predicted with the quarter model by less than 0.6%. This consideration allows  
449 us to conclude that the edge effects related to the use of symmetry constraints are negligible and hence  
450 the predictions of the one-quarter model are overlapping to those obtained with the entire model.

451 However, in spite of these limitations, the predicted displacement fields appear consistent with  
452 the nanoindenter geometry and with the physics of the problem (Fig. 10 (a)) as well as the predictions  
453 of the proposed model are consistent with the experimental results reported in the literature. The  
454 predicted material properties, in fact, are in good agreement with those measured via atomic force  
455 microscopy and Hertz contact theory in other studies [52–54]. Interestingly, the material properties  
456 predicted through the coarse-grained elastic network model are consistent with those computed via  
457 the Finite Element Method and via the Hertz contact theory reported in a previous study [24]. It is  
458 also interesting to observe, and is a further proof of the correctness of the model predictions, that the  
459 values of the Young's modulus  $E_{\text{Hertz}}$  computed with the Hertz contact theory falls – for each of the  
460 ten cell samples investigated and for both, coarse-grained elastic network model and FEM – within  
461 the interval  $[E_{\text{cyt}}, E_{\text{cor}}]$ , and are closer to  $E_{\text{cyt}}$ . This result is consistent with the physics of the problem.  
462 Given the prevalence of the cytoskeleton volume with respect to the cortex volume, it is reasonably  
463 correct that the average material properties predicted by the coarse-grained elastic network model are  
464 closer to those of the most prevalent material. In order to make a coherent statistical comparison  
465 between the sets of Young's moduli obtained with the coarse-grained elastic network model and with  
466 the finite element method, to the values computed via the Hertz contact theory, an equivalent Young's  
467 modulus must be defined for the former cases. To this purpose, the following semi-quantitative  
468 procedure is therefore proposed. The action of the AFM tip on the nanoindented cell can be



**Fig. 10.** (a) Normalized displacement field  $U_z/U_{z,max}$  predicted by the coarse-grained elastic network model (CG-ENM). (b) Scheme of the AFM tip-cell contact adopted to determine the equivalent Young's modulus. (c) Box plot of the equivalent Young's moduli compared with the Young's moduli computed via the Hertz contact theory.

469 schematized, according to a previous study [55], with a force acting on two springs disposed in series  
 470 (Figure 10(b)). Other semi-empirical approaches have been recently proposed to describe the  
 471 mechanics of the double layer on which the nanoindenter acts [56–58]. The first spring, having a  
 472 length  $L_{cor} = 200$  nm (i.e. the thickness of the cell cortex), a cross section  $A_{cor}$  and an elastic constant  
 473  $K_{cor} = \frac{E_{cor} \cdot A_{cor}}{L_{cor}}$ , is representative of the elastic response of the cell cortex; the second spring, having a  
 474 length  $L_{cyt} = 600$  nm (i.e. the height of the cytoskeletal region where non-zero stress values act during  
 475 the 200 nm nanoindentation), a cross section  $A_{cyt}$  and an elastic constant  $K_{cyt} = \frac{E_{cyt} \cdot A_{cyt}}{L_{cyt}}$ , is  
 476 representative of the elastic response of the cytoskeleton. Applying the classical relationships for  
 477 springs disposed in series and following the hypothesis that  $A_{cor} = A_{cyt}$  it can be easily demonstrated  
 478 that the equivalent Young's modulus  $E_{eq}$  of the spring that describes the elastic response of the two  
 479 springs having  $K_{cor}$  and  $K_{cyt}$  as stiffnesses, can be expressed as:

$$480 \quad E_{eq} = (L_{cor} + L_{cyt}) \cdot \frac{E_{cor} \cdot E_{cyt}}{E_{cyt} \cdot L_{cor} + E_{cor} \cdot L_{cyt}} \quad (2)$$

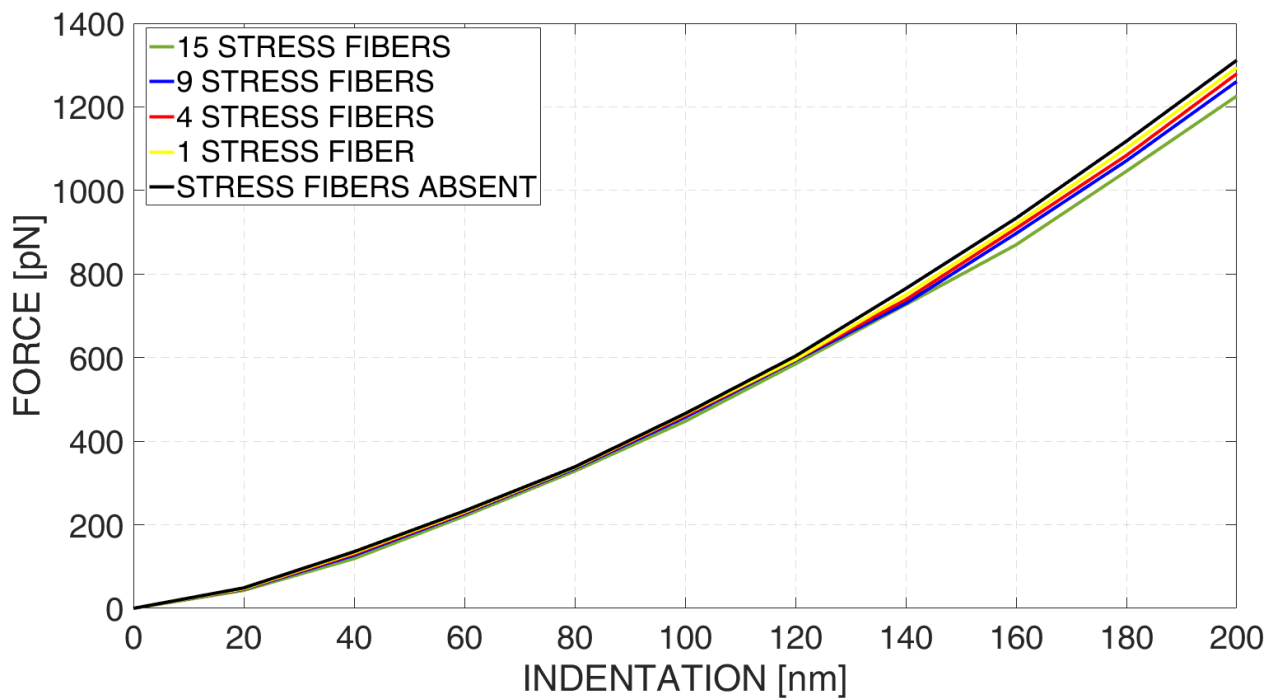
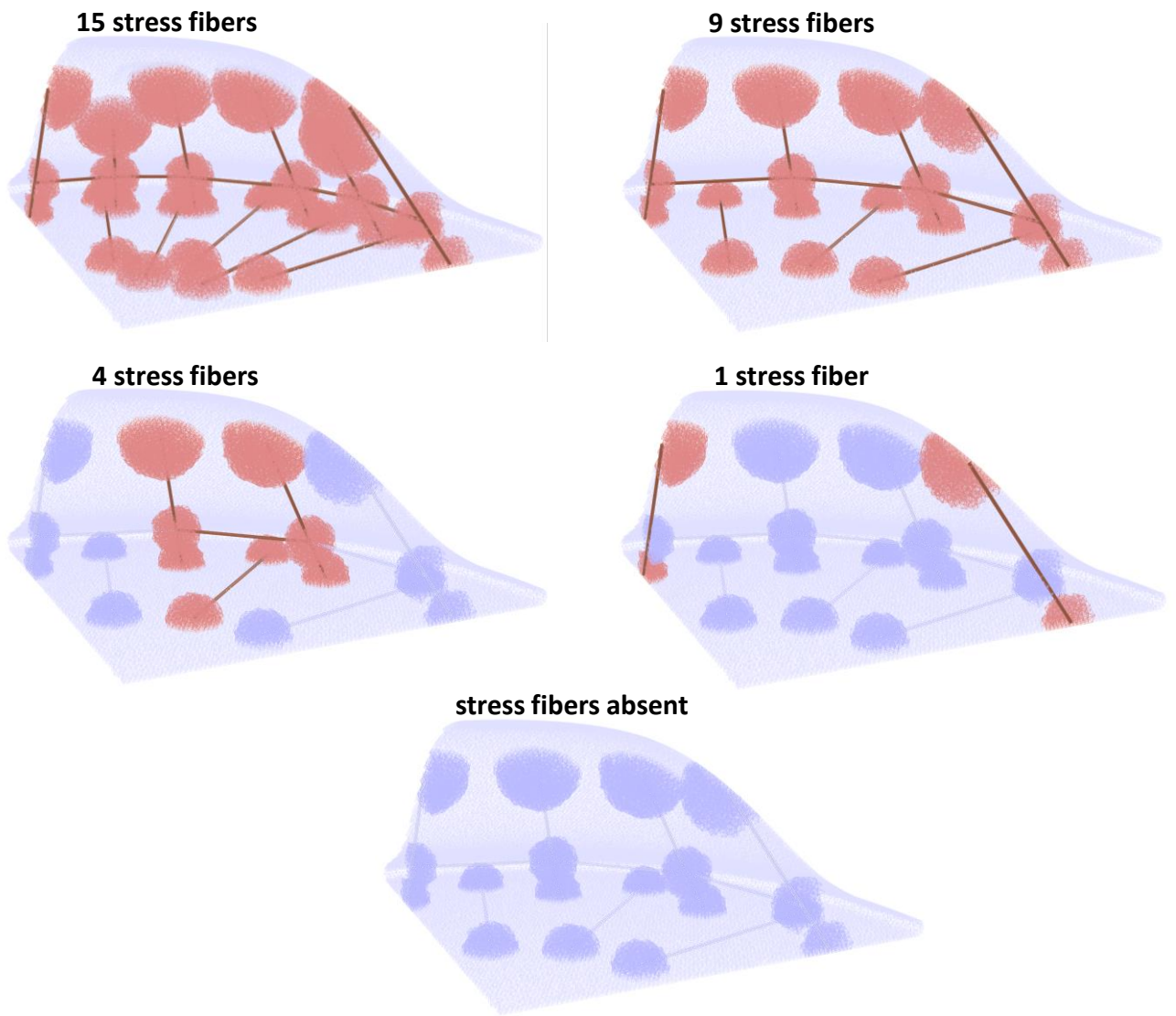
481  
 482 Applying the Eq. (2), for each cell sample, and setting the values  $E_{cor}$  and  $E_{cyt}$  obtained with both, the  
 483 coarse-grained elastic network model (CG-ENM) and the FEM (Table 3), we determined the  
 484 equivalent Young's moduli and compared them with those computed via the Hertz contact theory  
 485 (Fig. 10(c)). To make statistical inferences, we started to enquire whether data followed a normal  
 486 distribution. We used the Shapiro-Wilk normality test, AS R94 algorithm, on all Young's moduli sets  
 487 (i.e. the set of equivalent Young's moduli computed for coarse-grained elastic network model, the set  
 488 of equivalent Young's moduli computed for FEM and the set of Young's moduli computed via the  
 489 Hertz contact theory). All the three sets passed the normality test ( $E_{Hertz}$ :  $W=0.886$ ,  $p\text{-value}=0.155$ ;  
 490  $E_{eq\_CG-ENM}$ :  $W=0.914$ ,  $p\text{-value}=0.312$ ;  $E_{eq\_FEM}$ :  $W=0.917$ ,  $p\text{-value}=0.335$ ). To evaluate the  
 491 homoscedasticity, we applied the Levene test, that revealed that samples were homoscedastic ( $F(2,$   
 492  $27)= 0.467$ ,  $p\text{-value}=0.632$ ). Given these preliminary results, we performed ANOVA to compare the

493 three Young's moduli distributions. It revealed that there is not a statistically significant difference  
494 between the distributions of the equivalent Young's moduli and the Young's moduli obtained with  
495 the Hertz theory ( $F(2, 27)=1.149$ ,  $p\text{-value}=0.332$ ).

496 For all samples, the  $R^2$  values were close to 1 (Table 2), which means that the assumptions  
497 made on discretization topology (refined Delaunay algorithm for tetrahedrons and an adequate high  
498 spring density for the considered deformations) and on spring behavior (linear elastic) were  
499 reasonably correct for this analysis.  $R^2$  values computed for the FEM are slightly higher than those  
500 obtained through the coarse-grained elastic network modelling. However, it is worthy to note that the  
501 correlation coefficient computed with the FEM method derives from an optimization process  
502 performed by managing four design variables, ( $\mu_{8chain}$  for cytoskeleton and cortex, and  $\lambda_L$  for  
503 cytoskeleton and cortex) while that computed with the coarse-grained elastic network model derives  
504 from an optimization process including only two design variables ( $E_{loc,cor}$  and  $E_{loc,cyt}$ ). In other words,  
505 we can conclude that a 2-parameter model (coarse-grained elastic network model) provided a  
506 reasonably good and fast approximation of the results of a 4-parameter model (FEM, Arruda-Boyce).

507 An articulated model of the cell was introduced and described with a reasonable but arbitrary  
508 number of design hypotheses. Preliminary analyses were conducted to evaluate the impact of these  
509 hypotheses on the obtained results. For instance, the quarter model of the cell was hypothesized to  
510 include nine stress fibers. We evaluated how the cell mechanical properties change for a variable  
511 number of stress fibers: fifteen, nine, four, one, or for the case of stress fibers absent. Fig. 11 shows,  
512 for instance, the force-indentation curves obtained for the same material properties (i.e  $E_{loc,cor,optim}$  and  
513  $E_{loc,cyt,optim}$  computed for cell sample 9, Table 1) and for different numbers of stress fibers. It can be  
514 seen that reducing the number of stress fibers leads, for the same nanoindentation depth, to increasing  
515 values of force that the cell opposes to the AFM nanoindenter. This result can be justified with the  
516 "qualitative" argument that the stress fibers create a tensile stress state on the cellular region in contact  
517 with the AFM tip that favors the tip penetration into the cell. As the number of stress fibers decreases,  
518 the entity of the tensile stress acting on the cell decreases too and consequently the nanoindenter





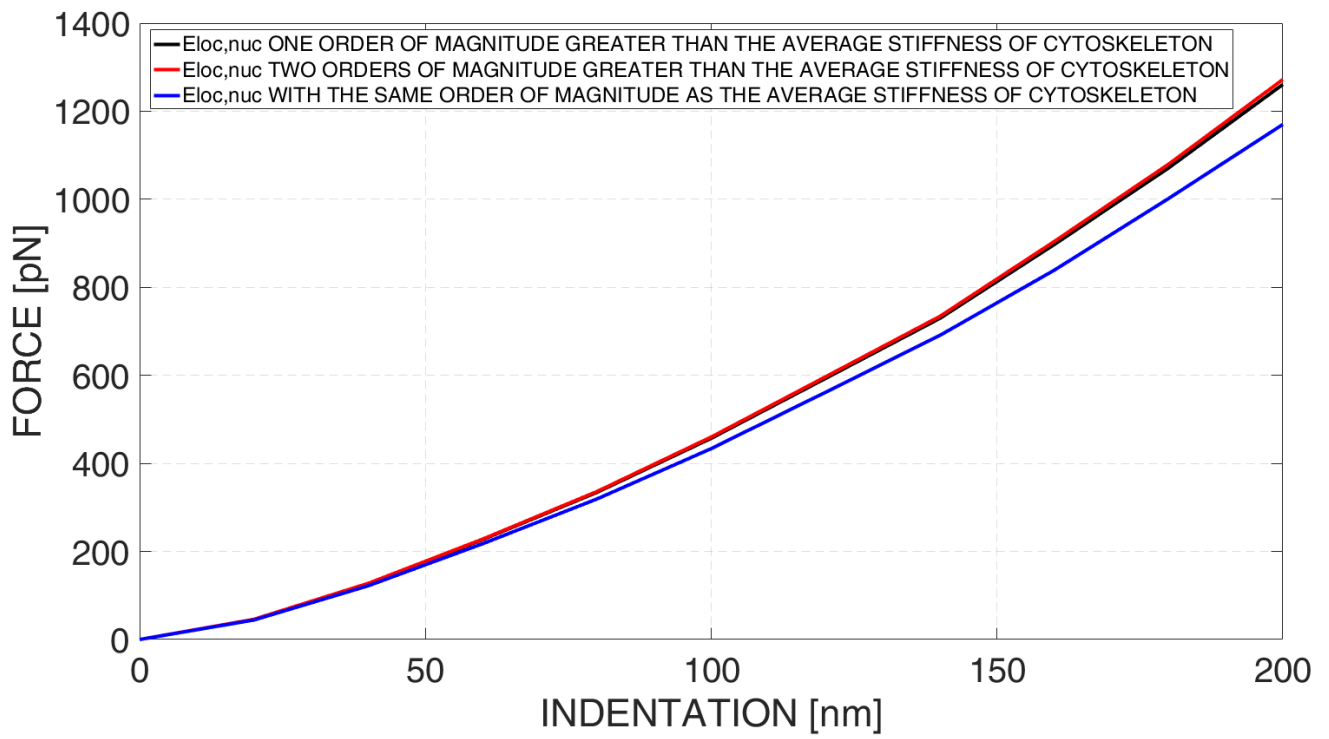
**Fig. 11.** Force-indentation curves computed by the model for the same material properties (i.e.  $E_{loc,cor,optim}$  and  $E_{loc,cyt,optim}$  of sample 9) and for a variable number of stress fibers. The activated (in red) and the disabled (blue) stress fibers simulated in the model are shown in the figures above the diagram.

519 experiences an increasing reaction force that the cell opposes to its penetration. The global material  
 520 properties predicted via the proposed coarse-grained elastic network model decrease for decreasing  
 521 numbers of stress fibers. Table 4 lists, for instance,  $E_{\text{cyt}}$  and  $E_{\text{cor}}$  computed for the cell sample 9.  
 522 However, the percent difference – with respect to the model including nine stress fibers – never  
 523 exceeds 4.03% in the case of the cytoskeleton and 1.00% in the case of the cortex. These results lead  
 524 us to conclude that, in view of a simpler description of the cell, a sort of reasonable result can be  
 525 achieved even without stress fibers. However, the model should consider the effect of stress fibers  
 526 especially in the case of cells including a large number of them.

527 **Table 4.** Global material properties computed for the cell sample 9 and for different  
 528 numbers of stress fibers.

# of stress fibers	Optimal global properties		Percent difference cytoskeleton	Percent difference cortex
	$E_{\text{cyt}}$ [Pa]	$E_{\text{cor}}$ [Pa]	[%]	
15	2594	8759	2.65	-0.97
9	2527	8845	-	-
4	2497	8815	-1.18	-0.34
1	2448	8770	-3.13	-0.85
0	2425	8756	-4.03	-1.00

529  
 530 The nucleus stiffness was set as a constant value and not as a design variable to optimize. Its  
 531 value, according to previous studies [11,59] was set one order of magnitude higher than the average  
 532 stiffness of the cytoskeleton. Nevertheless, recent literature has pointed out that this is not necessarily  
 533 the case [51]. To evaluate the effect of the hypothesized nucleus material properties on the predicted  
 534 values of  $E_{\text{cyt}}$  and  $E_{\text{cor}}$ , preliminary analyses were carried out with different values of  $E_{\text{loc},\text{nuc}}$ . Figure  
 535 12 shows, for instance, the force-indentation curves obtained implementing  $E_{\text{loc},\text{cyt},\text{optim}}$  and  $E_{\text{loc},\text{cor},\text{optim}}$   
 536 predicted for the cell sample 9 and three different values of the nucleus material properties  $E_{\text{loc},\text{nuc}}$ :  
 537 -  $E_{\text{loc},\text{nuc}}$  with the same order of magnitude as the average stiffness of the cytoskeleton;



**Fig. 12.** Force-indentation curves computed by the model for the same material properties (i.e.  $E_{loc,cor,optim}$  and  $E_{loc,cyt,optim}$  computed for sample 9) and for variable values of  $E_{loc,nuc}$ .

538 -  $E_{loc,nuc}$  one order of magnitude higher than the average stiffness of the cytoskeleton (design  
 539 hypothesis);

540 -  $E_{loc,nuc}$  two orders of magnitude higher the average stiffness of the cytoskeleton.

541 The force-indentation curves obtained with  $E_{loc,nuc}$  with one or two orders of magnitude higher than  
 542 the average stiffness of cytoskeleton are practically overlapped. Larger differences can be seen  
 543 instead, in the case of  $E_{loc,nuc}$  with the same order of magnitude as the average stiffness of the  
 544 cytoskeleton. The global material properties predicted with the proposed coarse-grained elastic  
 545 network model increase for decreasing values of  $E_{loc,nuc}$ . Table 5 lists, for instance, the values of  $E_{cyt}$   
 546 and  $E_{cor}$  predicted by the optimization algorithm for the three values of  $E_{loc,nuc}$  as well as the percent  
 547 difference – computed with respect to the model with  $E_{loc,nuc}$  one order of magnitude higher than the  
 548 average stiffness of the cytoskeleton – of the predicted values of  $E_{cyt}$  and  $E_{cor}$ . It can be seen that the  
 549 highest value of this difference was predicted in the case of the cytoskeleton and does not exceed  
 550 5.18%.

551 **Table 5.** Global material properties computed for the cell sample 9, for different nucleus material  
 552 properties  $E_{loc,nuc}$

$E_{loc,nuc}$	Optimal global properties		Percent difference cytoskeleton	Percent difference cortex
	$E_{cyt}$ [Pa]	$E_{cor}$ [Pa]	[%]	
One order of magnitude higher (design hypothesis)	2527	8845	-	-
Two orders of magnitude higher	2523	8833	-0.16	-0.13
Same order of magnitude	2658	8971	5.18	1.42

553  
 554 The global material properties were computed by implementing a monodimensional twin  
 555 model with a cylindrical shape. It would be very interesting knowing how the utilized geometry  
 556 affects the predicted material properties. To investigate this issue, two further (in addition to the main

557 one) monodimensional twin models were built (Fig. 13(a)) with the same aspect ratio 5 : 1 and having  
 558 volumes equal to  $\pm 10\%$  of the original volume. Interestingly, implementing  $E_{loc,cyt,optim}$  and  $E_{loc,cor,optim}$   
 559 predicted for the cell sample 9 and subjecting the models to a tensile test, the obtained stress/strain  
 560 curves were very close one each other (Fig. 13(b) and (c)). The global material properties predicted  
 561 with these two monodimensional twin models differ – with respect to the original monodimensional  
 562 twin model – by very small quantities never exceeding, in absolute value, 0.32% (Table 6).

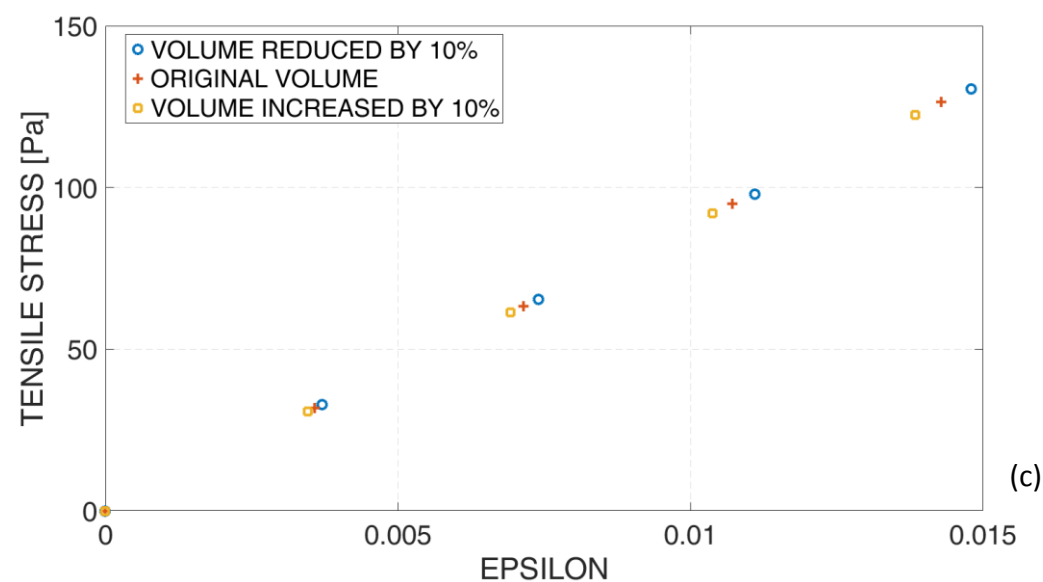
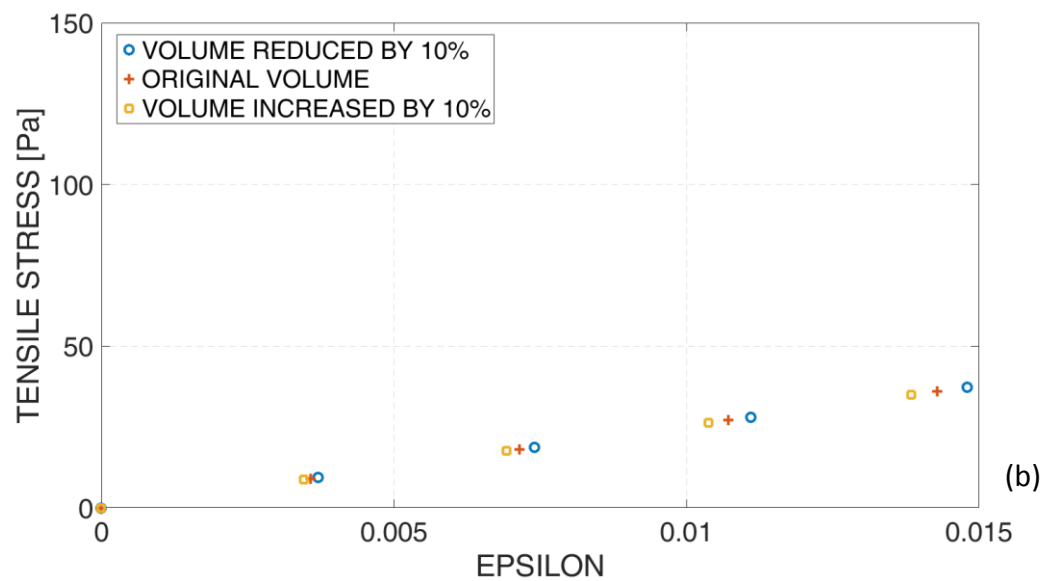
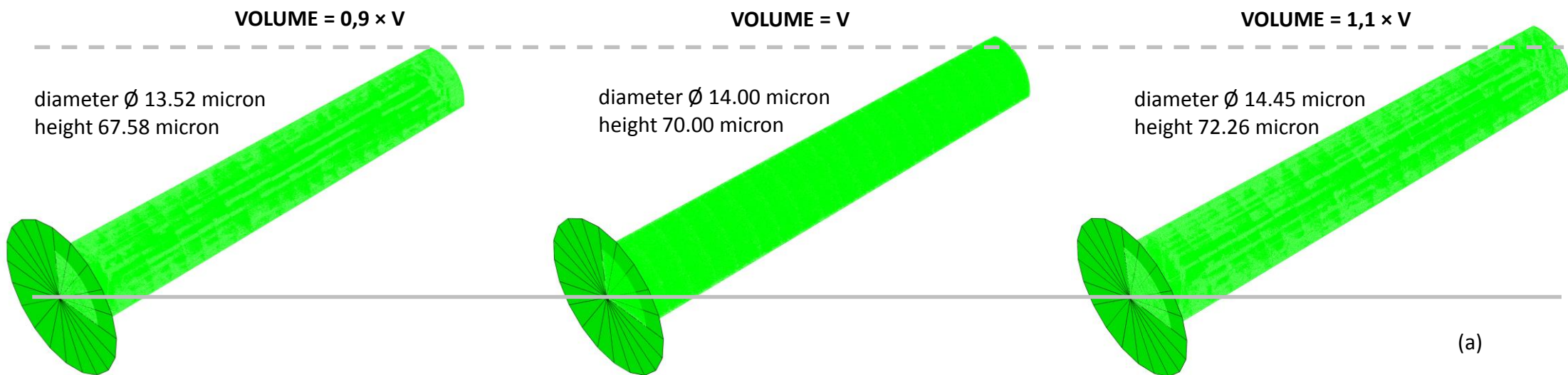
563

564 **Table 6.** Global material properties computed for the cell sample 9, for different geometries of the  
 565 monodimensional model

<i>Volume</i>	Optimal global properties		Percent difference cytoskeleton	Percent difference cortex
	$E_{cyt}$ [Pa]	$E_{cor}$ [Pa]	[%]	
Original Volume V	2527	8845	-	-
0.9×V	2519	8818	-0.32	-0.31
1.1×V	2529	8853	0.08	0.09

566

567 The most interesting feature of the proposed workflow is the intrinsic compliance of the spring  
 568 network, due to the missing constraints for spring rotations at nodes. This is ensured by modelling  
 569 the spring connections as internal spherical hinges, thus decoupling every pair of springs and not  
 570 allowing bending moment transmission, but only axial forces. From a physical point of view, such a  
 571 model can be regarded as an attempt to a micromechanical representation of weak and non-directional  
 572 bonds in biological materials [30]. The associated mathematical description can generally consist in  
 573 a series of simply linear systems in the form  $Ax=B$  for solving the static problem of a single  
 574 incremental deformation, thus leading to a stable and fast formulation of the whole static problem of  
 575 large deformation. Furthermore, it is not necessary to perform numerical interpolation on a continuum  
 576 volumetric domain to compute expressions for displacement, deformation and other field variables  
 577 as in finite element analysis. To demonstrate these features, as a proof of large deformation capability



**Fig. 13.** (a) Monodimensional twin models utilized to assess the effect of the geometry on the predicted material properties. Stress-strain curves obtained with the models with different geometry implementing  $E_{loc,cyt,optim}$  (b) and  $E_{loc,cor,optim}$  (c) computed for cell sample 9 (Table 1). Only markers are represented (and not lines) to better visualize the very small differences between the three models.

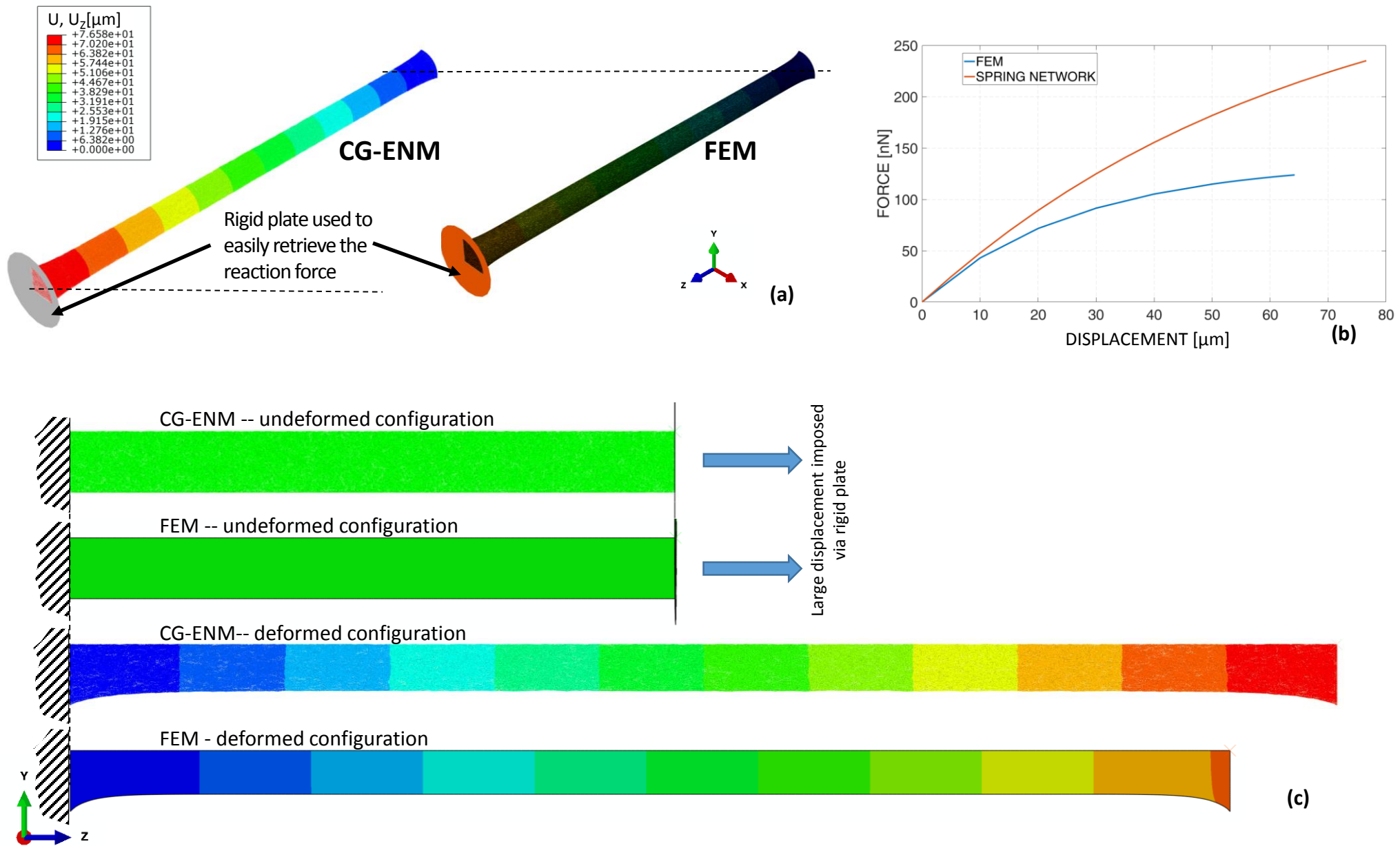
578 in Fig. 14 (a) and (c) are presented undeformed and related deformed configurations for two  
579 monodimensional twin models built, one with the FEM method the other with a spring network. Both  
580 the models were hypothesized to be linear elastic. A flat base of the cylinder was constrained while a  
581 sufficiently large displacement was imposed to the other flat base via a rigid plate to understand until  
582 which extent the two models guarantee numerical convergence. In the FEM model, the value of the  
583 material properties implemented is  $E_{cor}$  determined for sample 1, whereas in the spring network model  
584 the value of  $E_{loc,cor,optim}$  (directly computed by the optimization process for the cell sample 1) was  
585 utilized. Interestingly, the spring network showed larger deformation than FEM until reaching the  
586 numerical non-convergence condition and thus the end of the analysis (Fig. 14b). The two curves  
587 presented the same tangent and were practically overlapped for small values of displacement but  
588 tended to diverge, due to the intrinsic different characteristics of the two models, for very large values  
589 of displacement.

590 Another remarkable result to point out is the comparison between the computation times  
591 necessary to complete the simulation. Generally, the duration of each analysis on a spring network  
592 model is about 1/3 of the time necessary to complete a FEM analysis with a comparable density of  
593 elements.

594

## 595 **4. Conclusions**

596 This paper describes a physical/mathematical method for extracting Young's moduli of a  
597 deformable solid structure. The study focused on nanoindented human mesenchymal stem cells and  
598 reproduced through a coarse-grained elastic network a real AFM experiment. An optimization  
599 algorithm iterated a loop until a satisfactory fit between real and simulated force-indentation curves  
600 was reached. The topological and behavioral characteristics of the simulated cells were then  
601 transferred to a monodimensional twin model and the Young's moduli for subcellular components  
602 were extracted through a virtual tensile test.



**Fig. 14.** (a) 3D view of virtual tensile tests performed through coarse-grained elastic network model (CG-ENM) and FEM. (b) An example of force-displacement curves obtained with CG-ENM compared to that obtained with FEM. (c) Comparison between undeformed and deformed configurations of CG-ENM and FEM models.



603           The resulting Young's moduli showed good agreement with the corresponding moduli  
604 resulting from a previous study, performed using the Finite Element Method, and also presented the  
605 same order of magnitude as the elastic moduli predicted by hertzian contact theory and related  
606 literature.

607           The proposed approach can be considered as a useful and fast tool to study the mechanical  
608 behavior of biological soft materials. As a general rule, in a coarse-grained elastic network model,  
609 the time required to complete the simulation is about 33% of the time required to run the same  
610 simulation with a FEM model presenting the same discretization density for the elements.  
611 Remarkable numerical stability and computational speed make this method comparable to a  
612 traditional FEM analysis and even preferable in the case of large displacements and deformations of  
613 soft materials.

614

## 615 **Declaration of Competing Interest**

616           The authors have declared that no competing interests exist.

617

## 618 **Ethics statement**

619           Bone marrow from healthy donors for allogeneic transplantation was taken after written  
620 consent using guidelines approved by the Ethic Committee on the use of Human subjects at the  
621 Heidelberg University.

622

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628

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- 803
- 804



805 **Figure Legends**

806

807 **Fig. 1.** Scheme of the workflow implemented to extract the stem cells material properties.

808

809 **Fig. 2.** Exploiting the symmetry properties of the entire cell geometry (a), just one-quarter model was  
810 considered (b). (c) Principal dimensions in micrometers of the model used in the study.

811

812 **Fig. 3.** The CAD model (a) generated in Rhinoceros was given in input to the open source meshing  
813 software Gmsh (version 4.5.2) where a tetrahedral volumetric mesh was generated (b). The  
814 tetrahedral mesh was then converted into a spring network model (c). To generate the quarter model,  
815 a portion of the whole cell larger than the quarter portion was initially considered (a-c). A clipping  
816 procedure was finally executed (d) to isolate the portion of the spring network model exactly  
817 corresponding to the quarter of the cell. The aligned edges of tetrahedrons shown in (b) have been  
818 obtained at the intersection of the planes delimiting the model outer surface. The clipping procedure  
819 successively carried out, allows having not aligned edges (and hence not aligned springs) on the axis  
820 along which the nanoindenter acts and consequently, an isotropic behavior of the model.

821

822 **Fig. 4.** Spring groups defined in the model.

823

824 **Fig. 5.** Boundary and loading conditions imposed to the cell in the simulation environment. For the  
825 sake of clarity only the forces acting on the visible tensioning groups are indicated and not those  
826 acting on the internal ones.

827

828 **Fig. 6.** The overall force in every group (a) was distributed as 25% in an outer shell and 75% in an  
829 inner half-sphere (b), in order to realize a sort of force gradient when moving from the highly-  
830 tensioned central region to the unloaded free elements neighboring the tensioning group.

831

832 **Fig. 7.** Scheme of the optimization algorithm implemented to determine the optimal local material  
833 properties for cytoskeleton and cortex.

834

835 **Fig. 8.** (a) Simulation setup for the monodimensional twin model (dimensions in micrometers); (b)  
836 Magnified cross-sectional view of the model; c) Procedure for the extraction of global Young's  
837 moduli.

838

839 **Fig. 9.** Experimental force-indentation curves registered for the ten stem cell samples and related  
840 numerical curves predicted via the optimization algorithm. Error bars were superimposed to the  
841 average experimental force-indentation curves to show the dispersion of data at different indentation  
842 depths.

843

844 **Fig. 10.** (a) Normalized displacement field  $U_z/U_{zmax}$  predicted by the coarse-grained elastic network  
845 model (CG-ENM). (b) Scheme of the AFM tip-cell contact adopted to determine the equivalent  
846 Young's modulus. (c) Box plot of the equivalent Young's moduli compared with the Young's moduli  
847 computed via the Hertz contact theory.

848

849 **Fig. 11.** Force-indentation curves computed by the model for the same material properties (i.e.  
850  $E_{loc,cor,optim}$  and  $E_{loc,eyt,optim}$  of sample 9) and for a variable number of stress fibers. The activated (in  
851 red) and the disabled (blue) stress fibers simulated in the model are shown in the figures above the  
852 diagram.

853 **Fig. 12.** Force-indentation curves computed by the model for the same material properties (i.e.  
854  $E_{loc,cor,optim}$  and  $E_{loc,cyt,optim}$  computed for sample 9) and for variable values of  $E_{loc,nuc}$ .

855

856 **Fig. 13.** (a) Monodimensional twin models utilized to assess the effect of the geometry on the  
857 predicted material properties. Stress-strain curves obtained with the models with different geometry  
858 implementing  $E_{loc,cyt,optim}$  (b) and  $E_{loc,cor,optim}$  (c) computed for cell sample 9 (Table 1). Only markers  
859 are represented (and not lines) to better visualize the very small differences between the three models.

860

861 **Fig. 14.** (a) 3D view of virtual tensile tests performed through coarse-grained elastic network model  
862 (CG-ENM) and FEM. (b) An example of force-displacement curves obtained with CG-ENM  
863 compared to that obtained with FEM. (c) Comparison between undeformed and deformed  
864 configurations of CG-ENM and FEM models.

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