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Elastic Properties Exploration of *In Vitro* Cultured Microscopic Cells based on Haptic Sensing

M. Boukallel, M. Girot, S. Régnier

Laboratoire de Robotique de Paris,
Université Pierre et Marie Çurie
18 route du Panorama - 92265 Fontenay aux Roses, France
Phone: +33 146 548935, Fax: +33 146 547299, E–mail: boukallel,girot,regnier@robot.jussieu.fr.

Abstract – *This paper deals with the design of a micro-force sensing device for biomechanical characterization of biological samples. This device combines (SPM) techniques and advanced robotics approaches and allows to carry out* in vitro *prolonged observations as well as biomechanical characterization experiments. Elastic properties of biological samples are reflected to the macroscale during the mechanical characterization process by means of a haptic sensing device. Non-linear elasticity theory formalism is used in order to achieve realistic elastic rendering. Mechanical characterization experiments are conducted on human tumoral Epithilial Hella cells in order to demonstrate the efficiency and viability of the proposed system.*

Keywords – *Haptic sensing, Micro and nano-force measurement, Non-linear elastic properties of cells,* In vitro *mechanical cell characterization.*

I. INTRODUCTION

It is now well established that variety of cells are sensitive to mechanical disturbances from their environment. The response of cells to mechanical inputs is critical in governing cell behavior, not only in cell culture, but also in extension to the physiology of whole organisms as well. This process by which cells convert mechanical stimuli into biochemical signals is called mechanotransduction. Because cells are mechanically coupled to their environment, changes in the extracellular matrix (ECM) or cell mechanics can dramatically change the cell behavior. For example, hepatocytes, mammary epithelial cells, capillary endothelial cells and fibroblasts in culture can be switched from a growth state to a differentiated, non-proliferating state by modifying the stiffness or adhesivity of the ECM. Because of the strong in vitro and in vivo evidence that cell mechanics governs cell behavior, it is not surprising that many different human diseases may arise from abnormalities in the mechanical environment surrounding cells or the ability of cells to properly respond to these forces. For example it is well adopted that tumors are stiffer than normal tissue, as they are frequently detected through physical palpation. In cancer metastasis, tumor cells must dramatically change their physical interactions with surrounding cells and ECM in order to break away, begin migrating, invade blood vessels, extravasate, and grow at distant sites [2]-[4]. This is one of many examples on how cells in a pathological state stop to obey normal biophysical rules, with serious detrimental consequences.
The treatments of many diseases depend in part upon targeting mechanical processes. Understanding cells mechanotransduction will not only provide a more complete understanding of cell behavior, but will also establish new opportunities for increasing the overall efficiency of treatment of pathologies which have a basis in physical perturbations. Thus, the ability to study accurately both mechanical response and their inferences on individual cells is a key component in understanding the complex biological behavior in their environment (growth, development, auto-repair, ...).

Up to date, several robotics and microrobotics experimental setups have been developed to identify the control mechanisms of both individual cell and tissues mechanical responses [5]-[11]. Among these systems, the most promising ones involves Scanning Probe Microscopy (SPM) techniques for nanoscale. The significant research involving AFM make possible the measuring of relevant cells mechanical properties (Young's modulus, bulk modulus, surface roughness, cell adhesion, ...) at the microscale or to investigate cell adhesion and molecules involved in receptor-ligand interactions at the nanoscale [12]-[16]. However, most of these studies have not been performed in biological clean room environment. Since elementary biological functions and mechanical properties of biological cells are widely affected by the experimental conditions, identified properties and behaviors may not be relevant. As the reaction of the biological samples to stress vary greatly in a given lapse time, it is important to monitor the cell response continuously in an *in vitro* environment. Studies involving AFM techniques for biomedical applications are mainly focused on the accurate determination of the cell mechanical response. Seldom are the studies on mechanotransduction which are extended to *in vitro* tactile sensing of the cell response. Tactile sensing can be a promising solution when combined to traditional biochemical

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approach to investigate the complex mechanical behavior of cells in their environment and the mechanisms by which biochemical signals are transmitted. Reflect forces as well as physiological changes of cells to the macroscale can lead to a better understanding of the mechanotransduction process. This issue has been addressed in this paper by the developed Force Sensing Bio-Microscope (FSBM) system. This device combines SPM techniques and advanced robotics approaches allowing to carry out *in vitro* prolonged observations on biological samples. A haptic sensing device with 1 DOF is used for investigate either elasticity, elasticity hysteresis or viscoelasticity behaviors. Mechanical characterization experiments are conducted on human tumoral Epithilial Hela cells in order to demonstrate the efficiency and viability of the proposed system.

II. EXPERIMENTAL SETUP OVERVIEW

The *FSBM* experimental setup provides suitable conditions for study in controlled environment so that the biological cells can be kept several hours in living state by using a cage incubator. Moreover, the mechanical measurement process can be done on the biological sample on an extended lapse of time. Figure 1 shows the overview of the developed *FSBM* device. The *FSBM* is composed mainly with four units: the mechanical sensing unit which performs detection, positioning and sensing features, the imaging/grabbing unit for imaging features, the clean room *in vitro* unit which allows experiments to be conducted in biological environment and the haptic device for the realistic sensing of biological elastic properties. Two computers (master and slave) are used to control the units. The slave computer is assigned to control the mechanical sensing, the imaging and the clean room units while the master computer is dedicated for the haptic device. The slave computer is connected to the master computer using a UDP communication. A user definable interface based on MATLAB® Communication Toolbox is developed to allows efficient flow communication. The bilateral configuration of the connection between the master and the slave computers is presented in figure 2.

A. Mechanical sensing unit

The *FSBM* mechanical sensing unit is based on the detection of the deflection of a cantilever by an optical technique. A four quadrant photodiode with internal amplifiers associated to a low power collimated laser diode (wavelength 650 nm) are used in order to perform both axial and lateral nanoNewtons forces measurements. The total sensing area of the photodiode is 7 mm^2 with a spectral response from 400 to 1100 nm. The optical path of the Gaussian laser beam is optimized using a pair of mirrors and a aspheric condenser glass lens. Hence, a sensitive and accurate detection device is produced for the aim of our study. The sensitivity of the optical detection device is 5 $mV/\mu m$.

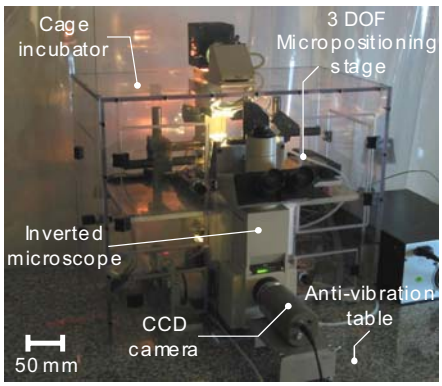

Fig. 1. The *FSBM* experimental setup overview.

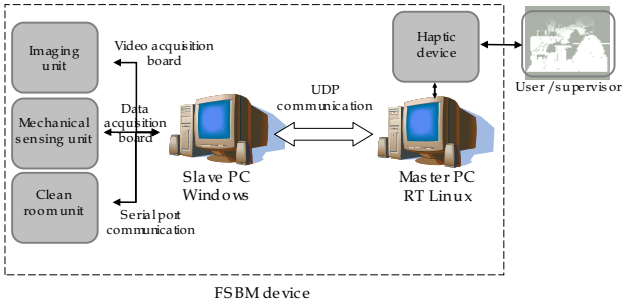

Fig. 2. Block diagram of the *FSBM* device.

A low spring constant (0.2 N/m) uncoated tipless silicon cantilever is used as probe for the cell mechanical characterization. The lever is 450 μm long, 90 μm large and 2 μm thick. The sample to be studied is accurately positioned below the cantilever by a 3 DOF's (x,y and z) micropositioning encoded stage with a submicrometer resolution (0.1 μm). The kinematic features of the micropositioning stage allows to achieve accurate mechanical measurements in a workspace of 25 x 25 x 25 mm^3 with good repeatability.

B. Imaging/grabbing unit

The *FSBM* imaging/grabbing unit consists of an inverted microscope (Olympus IMT-2) with Nikon 10x and 20x objectives. A phase contrast device is mounted on the microscope for precise contrast operation. The inverted microscope is fitted out with a CCD camera (754x488 pixels resolution). Using a frame grabber and a specialized imaging PCI (Matrox Imaging®) device associated to the CCD camera, automatic mechanical characterization based on image features tracking is achieved.

C. *Clean room* in vitro *unit*

The *FSBM* is equipped with a controlled heating module which maintains temperature on the cage incubator at 37 ^oC using a single thermocouple. The cage incubator ensures a temperature stability within the 0.1 ^oC. A mixed stream composed by a 5% CO_2 and humidified air is fed into a small incubating chamber containing the biological samples, avoiding in

TABLE I

HAPTIC DEVICE PARAMETER DATA

Parameter	Numerical value
Maximum current input	2.15 A
Nominal voltage	40 Volts
Inductance resistance	2 Ohms
Maximum torque Γ_m	0.11 Nm
Maximum speed	8200 tr/mn
Angular range	infinite
Angular resolution	1/5000 tr
Efficiency	86 %
Radius of the Aluminum disc	35 mm
Maximum tangential force	2.86 N

this way condensation on the cage walls that could damage the mechanical parts of the microscope and the micropositioning stages. The whole system including the *FSBM* is placed in a positive pressure clean room to protect the biological environment.

D. Haptic device

The haptic device consists of Maxon® DC motor coupled with an accurate encoder. The device performs two functions : accurate placement of the cantilever above the sample (z direction) and elastic and visco-elstic haptic rendering. The outstanding technical features of the the DC motor and the components working with allow effective haptic rendering at the macroscale. Besides the compact design, the device presents a linear behavior between both the voltage/speed and the load/speed. Figure **??** presents the overview of the haptic device. A low mass inertia Aluminum ergonomic disc is fixed to the motor spindle in order to perform efficient manipulation and sensing tasks. The table I summarizes the relevant technical features of the DC motor and the encoder.

III. NON-LINEAR ADAPTATIVE SPRING (NAS) FOR ELASTIC AND ELASTIC HYSTERESIS SENSING

Since the mechanical response of soft elastic material (load/deformation) does not obey to linear relationship (Hooke's law), they can be modeled as a non-linear spring. A typical load-elongation diagram for soft tissue is shown in figure 3. We can note that loading and unloading occur on different load-deformation paths. As this diagram can be segmented to linear areas, the mechanical behavior can be modeled as n serial linear springs with spring constants K_1, K_2, ..., K_n.

During the load path, the strain energy E_{si} stored by each linear spring is expressed as the area below the curve and

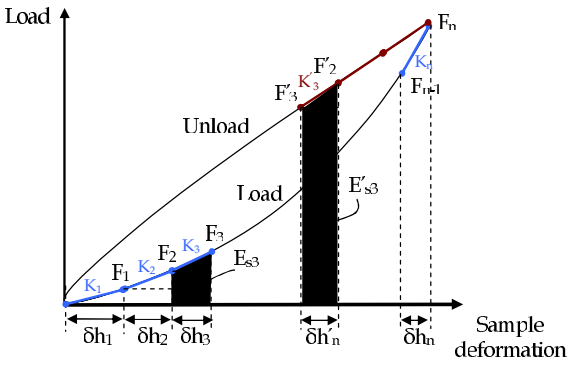

Fig. 3. A typical load-elongation diagram for soft tissue.

calculated as

For i=1

$$E_{s1} = E_1 = \frac{1}{2}K_1\delta h_1^2 \tag{1}$$

Where δh_1 is the deformation resulting for the applied variation load δF_1

For i=2

$$E_{s2} = \frac{1}{2}K_2\delta h_2^2 + \delta h_2 F_1 \tag{2}$$

For i=3

$$E_{s3} = \frac{1}{2}K_3\delta h_3^2 + \delta h_3 F_2 \tag{3}$$

For i=n

$$E_{sn} = \frac{1}{2}K_n\delta h_n^2 + \delta h_n F_{n-1} \tag{4}$$

Then, the total strain energy E_{Ti} stored by the non-linear spring for a given deformation δh_i is the sum of energies stored by each linear springs as

For i=1

$$E_{T1} = E_{s1} = \frac{1}{2}K_1\delta h_1^2 \tag{5}$$

For i=2

$$E_{T2} = E_{s2} + E_{s1} = \frac{1}{2}K_1\delta h_1^2 + \frac{1}{2}K_2\delta h_2^2 + \delta h_2 F_1 \tag{6}$$

For i=3

$$E_{T3} = E_{s3} + E_{s2} + E_{s1} = \tag{7}$$

$$\frac{1}{2}K_1\delta h_1^2 + \frac{1}{2}\delta h_2(K_2\delta h_2 + 2F_1) + \frac{1}{2}\delta h_3(K_3\delta h_3 + 2F_2) \tag{8}$$

For i=n

$$E_{Tn} = \frac{1}{2}K_1\delta h_1^2 + \frac{1}{2}\sum_{2}^{n}(\delta h_i(K_i\delta h_i + 2F_{i-1})) \tag{9}$$

As energy formulas are discretized, software implementation becomes easier. Strain energy equations and their iterative calculations are implemented in the slave computer based on

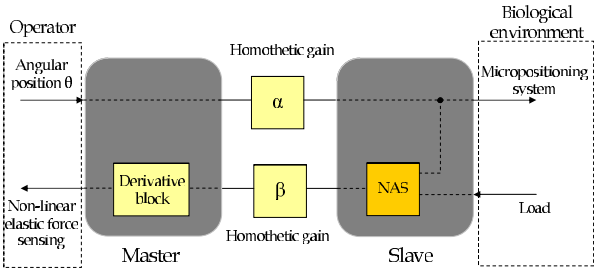

Fig. 4. Overview of the Master/Slave bilateral coupling scheme.

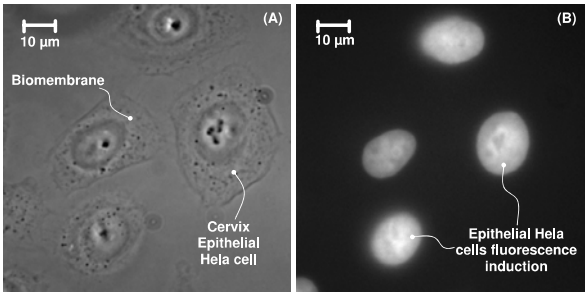

Fig. 5. (A) Magnified image of the cervix Epithelial Hela cells obtained with an 63x objective. (B) The cervix Epithelial Hela cells morphology observed by fluorescence techniques.

Matlab® Simulink Toolbox. Figure 4 shows the overview of the adopted Master/Slave bilateral coupling scheme. The homothetic gains α and β are determined during the calibration process. The appropriate values of the homothetic gains in our case are $\alpha = 5\ 10^{-4}\ \mu m/rd$ and $\beta = 1500\ N/J$.

For the unload path the same approach is adopted. If we note $K_i^{'}$ and $\delta h_i^{'}$ the ith constant spring and the sample deformation, thus, the total strain energy $E_{Ti}^{'}$ is expressed as

$$E_{Tn}^{'} = \frac{1}{2}K_1^{'}\delta h_1^{'2} + \frac{1}{2}\sum_{2}^{n}\left(\delta h_i^{'}(K_i^{'}\delta h_i^{'} + 2F_{i-1}^{'})\right) \quad (10)$$

IV. EXPERIMENTAL VALIDATION OF THE NAS MODEL

Haptic sensing experiments are conducted on human tumoral Epithilial Hela cell (EpH) based on the Non-linear Adaptative Spring (NAS) model. The cells are chosen since they are adherent and present an interesting soft elastic behavior. The *EpH* cells can be assimilated morphologically to elliptical cells with a thin surrounding biomembrane. In the present study, the average dimensions of the biological sample is 10 μm long, 9 μm large and 6 μm height (cf. figure 5). The Epithelial Hela cells *(EpH)* are prepared on Petri dishes with specific culture medium formed by Dulbecco's Modified Eagle's Medium (DMEM) with high glucose and L-glutamine components and 10 % of foetal bovine serum.

Figure 6(A) shows the biological sample displacement carried out by a human operator using the haptic device. Displacements less than 15 μm are achieved during this experiment. The cell deformation δh is monitored by calculating the difference between the vertical position of the sample and the cantilever deflection. Figure 6(B) shows the derivative form of the sample deformation δh as function of time experiment. According to this figure, the studied biological sample exhibits the same deformation amplitude whatever the load-deformation path (load and unload). Figure 6(C) reveals the elastic behavior of the biological sample as well as the elastic

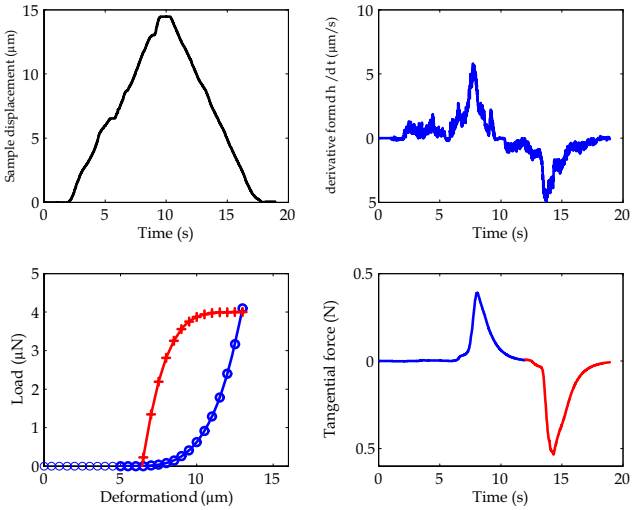

Fig. 6. (A) Biological sample displacement carried out by a human operator interface. (B) Derivative form of the sample deformation as function of time experiment. (C) Elastic behavior of the *EpH* cells. (D) Tangential forces sensed by the human operator.

hysteresis. This curve is obtained using the discretized strain energy formulas presented in the last section. In opposition to the sample deformation diagram (cf. figure 6(B)), this figure emphasize the difference between the load and unload paths. We can note that the total strain energy for the same sample deformation are bigger in the case of the unload path than the load path. The tangential forces sensed by the human operator during the experiment is presented in figure 6(D). Since the elastic behavior of the biological sample is disymmetric, the operator senses different amplitude forces for the load and unload paths. Hence, the forces sensed are more important in the case of the unload path since the total stored strain energy is bigger.

V. CONCLUSION

This paper has presented the development of a microforce station with haptic sensing features for *in vitro* mechanical cell characterization. The experimental setup combines Scanning

Probe Microscopy (SPM) techniques with advanced robotics approaches. Mechanical characterization is conducted using the *FSBM* on tumoral human Epithelial Hela adherent cells. These experiments demonstrate the efficiency of the experimental setup developed to explore the non-linear elastic properties of adherent biological samples. Non-linear elasticity theory formalism is used in order to achieve realistic elastic rendering for the macroscale.

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