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The Force Sensing Bio-Microscope: An Efficient Tool for Cells Mechanotransduction Studies

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Abstract— **This paper deals with the development of an open design platform for explorative cells mechanotransduction investigation. The produced setup combines SPM techniques and advanced robotics approaches allowing to carry out both prolonged observations and spatial measurements on biological samples. As a result, enhanced force probing method based on scanning microscopy techniques and advanced robotics/automation approaches are integrated in this device. Visual and force feedback control are used to achieve automatic data acquisition and monitoring process when high skills are required. Preliminary *in vitro* experiments on human promyelocytic leukemia cells (NB4) are conducted in order to demonstrate the viability of the proposed design. Some relevant mechanical cell properties are extracted such as elasticity and viscosity parameters.**

Index Terms— **Cells mechanotransduction, Micro and nanoforce measurement, Non-linear elastic properties of cells.**

I. Introduction

Mechanotransduction is a cell process which converts mechanical stimuli into biochemical signals. Since most cells are sensitive to mechanical disturbance, the resulting response to mechanical inputs is determinant in governing their behavior [1]-[3]. It is crucial to consider how external mechanical stimuli are transmitted into the cell. Many researches have been devoted to understand the mechanotransduction mechanism. Despites theses efforts, only a few studies leads to efficient models who predict forces transduction to biochemical signals. Due to the complex cell behavior as well as the complex interactions involved in such a process, mechanotransduction is subjected to many assumptions. Despites this apparent complexity, it has however been shown that cells stimulated are activated by similar mechanisms at the molecular level.

Understanding the mechanotransduction basis, first requires, accurate knowledge of the magnitude and the distribution of forces sensed by the cell in their environment. Moreover, mechanical characterization of the cell properties is also required in order to correlate biological and mechanical behaviors. Actually, due to the structural complexity of cells (such as the deformable cytoskeleton formed by a three dimensional intercellular network of interconnected biopolymers), detecting modifications of cells mechanical properties can give additional knowledge on the way the cell reacts to mechanical stimuli.

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The rest of the paper is organized as follows. Section III is devoted to motivations of the present work. The produced experimental setup is presented in section IV. Section V is focused on the *in vitro* experiments conducted on human promyelocytic leukemia cells (NB4) in order to demonstrate the viability and effectiveness of the developed device. Finally, conclusion and planned future work on the cell mechanotransduction are introduced in section VI.

II. Motivations of the works

Up to date, several robotics and microrobotics experimental setups have been developed to identify the control mechanisms of both individual cells and tissues mechanical responses [4]-[8]. Among these systems, the most promising ones involves Scanning Probe Microscopy (SPM) techniques for nanoscale. The significant research involving AFM makes possible the measuring of relevant cells mechanical properties (Young's modulus, bulk modulus, surface roughness, cell adhesion...) at the microscale [9]-[11] or to investigate cell adhesion and molecules involved in receptor-ligand interactions at the nanoscale. However, most of these studies have been focused and restricted to specific fields of the mechanotransduction since experiments are carried out with standard Atomic Force Microscopes. Despite these fruitful results and the recent advances on biotechnologies, some of the involved bio-chemical and bio-physical mechanisms at both micro and nanoscale aren't completely modeled yet (polymerization of microtubles, ECM activation...). Accordingly, developments yielding to experimental devices with enhanced force probing features and including efficient tracking techniques for mechanotransduction studies are desirable. Effective design of experimental setups for mechanotransduction studies should address some important issues. In fact, elementary biological functions and mechanical properties of biological cells are widely affected by the experimental conditions (temperature, humidity...). In order to be relevant, experiments need to be conducted on *in vitro* conditions. Moreover, the time needed to convert mechanical stimuli into bio-chemical signals which induce cell morphological modification greatly varies from a cell to an other one in homogeneous cell population. As this time can be expressed in hours as well in days, kinematics of cells deformations should be monitored continuously. Furthermore, even if the time needed for cell morphological modification is expressed in hours(or even more), some transient mechanotransduction interactions, such as depolymerization of actin fibers, can occur in a few microseconds, which imposes specific design of the final prototype. Finally, both strength gradient and du-

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ration of mechanical disturbances applied to the cell should also be considered. In fact, the cells response to external mechanical stimuli can dramatically change depending on the forces amplitude and rate. Accurate spatial positioning and force control features are then needed.

The issue of cells mechanotransduction, based on effective design of an open instrumentation and control setup, has been addressed in this paper. A Force Sensing Bio-Microscope (FSBM) system is developed for this purpose. This device combines SPM techniques and advanced robotics approaches allowing to carry out both prolonged observations and spatial measurements on biological samples. A cage incubating system is associated to the device in order to maintain all the required environmental conditions for cell culture (temperature, CO_2 and humidity). The FSBM can be run in two modes: teleoperated and automatic modes. The automatic mode are used for measurements when high skills are required. These latter are achieved by means of sensors fusion and active control approaches (visual/force feedback controllers).

III. Experimental setup overview

The FSBM consists mainly of three units: the mechanical sensing unit which performs detection, positioning and sensing features, the imaging/grabbing unit for imaging and cell tracking features, and the clean room *in vitro* unit which allows experiments to be conducted in biological environment. The overall configuration of the FSBM and the different working components are shown in figure 1.

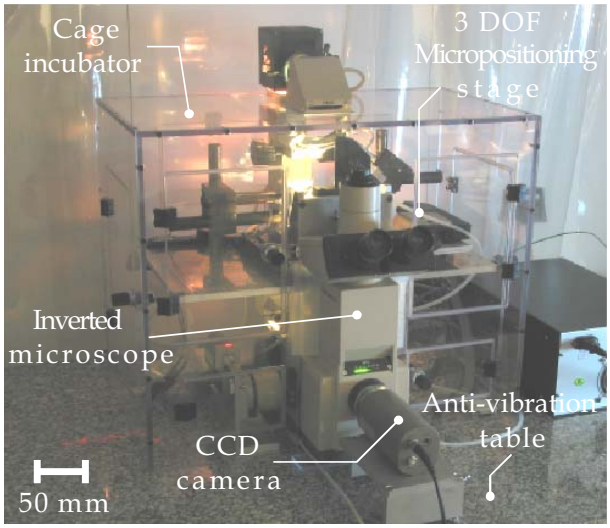

Fig. 1. The FSBM experimental setup overview.

The mechanical sensing unit is based on the measure 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, 1 mW) are used in order to perform both axial and lateral nN 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 an aspheric condenser glass lens. Hence, a sensitive and accurate detection device is produced for the aim of this study. The sensitivity of the optical detection device is 5 $mV/\mu m$.

A low spring constant (0.2 N/m) uncoated tipless silicon cantilever is used as a probe for the cell mechanical characterization. The lever is 450 μm long, 90 μm wide 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 stages with a submicrometer resolution (0.1 μm). The kinematics features of the micropositioning stages allow to achieve accurate mechanical measurements in a workspace of 25 x 25 x 25 mm^3 with a good repeatability. The mechanical sensing unit and the optical detection device are presented in figure 2. A magnified picture of the cantilever with the focused laser beam on its reflective surface is shown in the same figure.

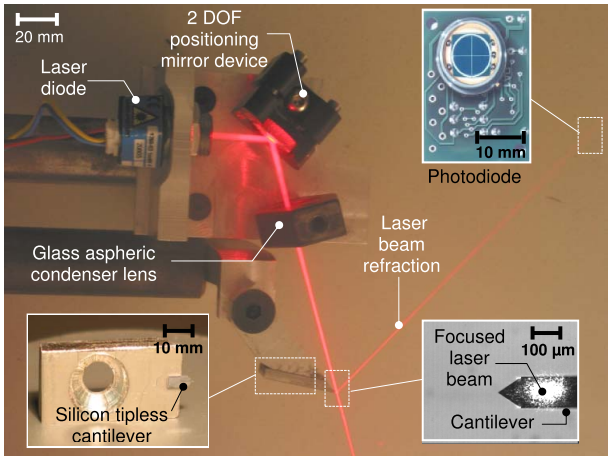

Fig. 2. The mechanical sensing unit.

For the preliminary study, we focused on force feedback control of cantilever flexural deflection. Thus, only the vertical motion of micropositioning stage is servoed. Knowing the vertical position of the micromotors as well as the deflection of the cantilever using the optical detection device, a optimized Proportional and Derivative (PD) controller was designed to ensure optimal control performance. The (PD) terms are optimized using the Ziegler-Nichols method.

The biological samples need specific requirements to be kept in live outside the *vivo* conditions, and to carry out prolonged observations. Besides the biological nutrition medium, biological cells need 37 ^oC temperature condition and 5% of CO_2 gas. The incubating system is maded up of a controlled heating module which maintains temperature at 37 ^oC using a single thermocouple probe. The desired temperature of 37 ^oC is reached in 2 hours. 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 this way condensation on

the cage walls which could damage the mechanical parts of the microscope and the micropositioning stages. Temperature control is achieved by means of a configurable PID controller communicating with a water bath via serial port to the master computer. The whole system including the FSBM is placed in a positive pressure clean room to protect the biological environment.

The 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 library package (Matrox Imaging) associated to the CCD camera, automatic mechanical characterization based on image features tracking is achieved. The pixel to real world calibration of the CCD camera is achieved by means of a calibrated glass micro-array as well as calibrated micro-spheres.

IV. Viscoelastic properties determination for Young's modulus estimation

Commonly to evaluate the rigidity of a material, regardless of the geometry of the sample, the Young's modulus is used for rigid body with quasi-static load tests. For solid body there's no influence of the deformation speed on the force displacement curves, but if the solid has plasticity or viscoelastic behavior the Young's modulus estimation is becoming problematic. Due to the complex cell structure such as the multilayer deformable bio-membrane(which enclose cytoplasm, liquid...) some viscous effects during load tests unable the use of classical Young's modulus estimation.

To avoid viscosity effect on the measured force, the time needed by the cell to reach a stagnant state must be previously evaluated. Since the cell relaxation time can be characterized accurately, conventional load experiments on deformable body with visco-elastic properties allows to determine Young's modulus in a quasi-static way.

Without model, experimental determination of the relaxation time should be done for each different value of step deformation, and for each different cell. The result, in addition of a long time of test, is the long mechanical solicitation of the cell before the load test, causing the cell to react to stimuli and to distort results of Young's modulus. One way to solve the problem is to model viscous parameters, and to identify this parameters via a single experiment.

To evaluate viscosity parameters, we choose a Kelvin-Voigt model, figure 3, which consist of a spring and a damping in series for viscous effect, and of a spring in parallel for the rigidity of the cell.

The response to a displacement step input drops exponentially to reach the equilibrium state which characterizes the quasi static rigidity of the system. Based on this model the relaxation time is defined, which is either the time needed to reach equilibrium or the time needed to switch between transient and continuous response.

For Kelvin-Voigt parameters identification, figure 4 shows the response of a NB4 cell of 12 μm height under cyclical

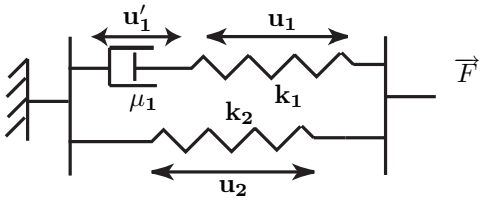

Fig. 3. The Kelvin-Voigt model.

deformation of 5 μm (Kevlin Voigt estimated parameters: $k_1 = 2.6e-3\ N/m$, $k_2 = 2.2e-3\ N/m$, $\mu_1 = 2.59e-4\ Pa.s$). With these parameters, $t_{5\%}$ which is the time for the cell to reach 5% of the final stagnant force, is evaluated, and used as the time to wait before take force into account.

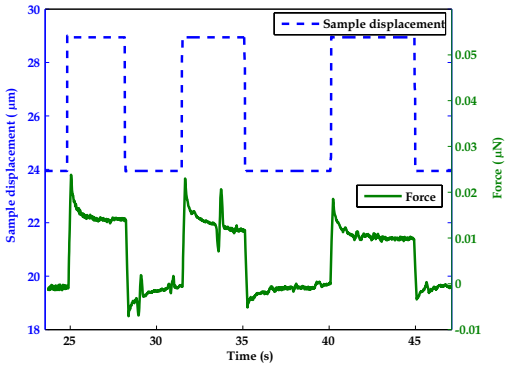

Fig. 4. Response of the *NB4* cell for an Heaviside step of deformation. The sample displacement and the applied force are plotted according to the time.

Note that after evaluating statistically the relaxation time for each test, a $4\ s$ with $1\ s$ of uncertainty is observed. This difference can be explained by the reaction of the cell to mechanical stimuli; as the cell is an active structure, its reaction to stress induces chemical changes such as polymerization and depolymerization of actin fibers which are non reversible for each cyclical deformation. This non-reproducible behavior led us to use a margin of safety on the evaluated relaxation time in order to ensure the end of the viscous effect.

V. Analytical model for Young's modulus estimation

To ensure a biological environment, previous tests have been performed on the temperature, humidity and CO_2 conditions. The lack of humidity leads to slow evaporation of the medium which decreases the level of liquid in the Petri dishes, changing the optical path of the laser beam and the force calibration in less than one hour. Without 5% of carbon dioxide, the change of pH in the medium causes the cell apoptosis. Finally, the change of temperature by turning off the heating module, decreases significantly the mechanical response of the cell in less than two minutes. By ensuring stable biological environment, the load tests are constant for several hours, and cyclical load results are the same during all the experiment.

The figure 5A presents the applied force on the cell as a function of the sample displacement. This curve gives a first

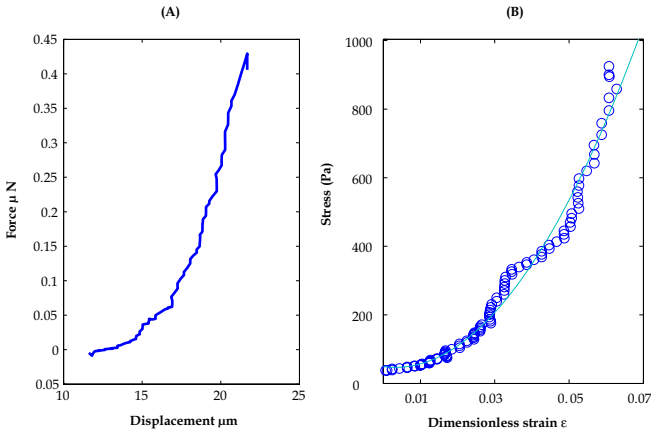

Fig. 5. (A) Experimental curve showing the applied force as a function of the sample displacement. (B) Estimated stress σ as function of the estimated strain ϵ using the JKR theory.

approximation of the stiffness of the cell and is depending on both, the amplitude of the sample displacement, and the geometry of the cell (the size of the cell affect the gradient of the curves which is linked to the stiffness of the cell). Usually, to be relevant the stiffness estimation of a material should be done according to a 3D strain model. For this purpose, we locally consider the cell as a perfect sphere, on which the tipless cantilever, considered as a perfect solid plan, applies forces or displacements on the cell. As adhesion phenomena is preponderant at the micrometer scale, we choose to use a JKR (Johnson, Kendall and Roberts) model [12].

The JKR model is based on linear isotropic 3D strain Hertz model with a correction for the adhesion phenomena, and is particularly suitable for soft solid, large contact radius, and high adhesion energy which is in a good agreement with the cells studies. With this model the contact area for the stress calculation is easily estimated. Figure 5B shows the dimensionless strain as a function of the stress. Because the limit of the hypothesis of homogeneous sphere for the cell is reached for large strains ($\epsilon > 0.03$), and lead to deviation between experimental results and the predicted linear behavior, the Young's modulus calculated for $\epsilon < 0.03$, is estimated to $3.2kPa$.

VI. Conclusion and Future Work

In this paper, an experimental device for explorative mechanotransduction studies is developed. The FSBM combines enhanced force probing method based on scanning microscopy techniques with advanced robotics and automation approaches. An incubating system is integrated to the device in order to perform experiments in biological conditions. The device can be driven in two modes: a teleoperated and automatic modes. In the automatic mode, visual and force tracking control are used and yielded to reliable automatic data acquisition and monitoring process. In order to demonstrate the viability and effectiveness of the proposed setup, experiments on human promyelocytic leukemia cells (NB4) are conducted. Some relevant mechanical cell properties are extracted such as elasticity and viscosity parameters. Kinematic cell deformations are modeled using an appropriate analytical model taking into account adhesion at the microscale.

Current work involves modeling transient bio-chemical and bio-physical interactions of the cells mechanotransduction. As the produced device is an open design platform, we work toward increasing their capabilities. For example a fluorescence unit will be integrated to the microscope as additional visualization and monitoring device. Some important issues will be also addressed in cells mechanotransduction modeling such as kinematic cell deformation and cell migration taking into account friction, osmotic influence, and nonlinear elastic behavior parameters. Finally, we investigate tactile sensing capabilities and try to find effective design and control solutions. When these solutions are combined to traditional biotechnologies approaches, they allow to investigate the complex mechanical behavior of cells in their environment and the mechanisms by which bio-chemical signals are transmitted.

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