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Modeling Soft Contact Mechanism of Biological Cells Using an Atomic Force Bio-Microscope

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***Abstract*— The development of a mechanical force sensing device system based on force/vision feedback control for exploring *in vitro* the contact mechanics of human adherent cervix Epithelial Hela cells is presented in this paper. The design of the prototype combines Scanning Probe Microscopy (SPM) techniques with advanced robotics approaches. Some important issues in the design process, such as *in vitro* environment constraints and calibration of the force sensing probe are also addressed in this paper. The system is then used for accurate and non-destructive mechanical characterization based on soft contact interactions on biological samples. Finally, some mechanical properties of the studied biological samples are estimated using two appropriate models describing the contact mechanism taking into account adhesion forces.**

I. Introduction

Nowadays robotics and microrobotics techniques play an important role for exploring the cell mechanical behaviour. The ability to study accurately the mechanical behaviour of individual cells is a key component in understanding the elementary biological functions that various biological cells exhibit. Furthermore, the individual mechanical cell characterization is a major step towards the knowledge and understanding the behavior of the complex mechanism tissues. Up to date, several experimental setups have been developed to identify the control mechanisms of the cell mechanical response [1]- [6]. Among these robotics and microrobotics systems, the most promising ones involves Scanning Probe Microscopy (SPM) techniques for nanoscale. These techniques have the potential to give an accurate quantitative information about local forces and contact mechanics. The Atomic Force Microscope (AFM) has become a commonly used tool to measure mechanical properties of the biological samples [7]- [11]. In these studies, a flexible cantilever with low spring constant and an atomic sharp tip is brought in the vicinity of the biological sample. Deflection of the cantilever as a result of the mechanism interaction between the microindenter and the sample is monitored by a split photodiode and the use of a laser beam reflected on the back of the cantilever. The significant research involving AFM make possible the measuring of relevant cells mechanical properties (Young's modulus, bulk modulus, surface roughness, ...). However, most of these studies have not been performed in biological clean room environment. Since elementary biological functions and mechanical properties of the major biological cells are widely affected by the experimental conditions, identified properties may not be relevant. In fact, due to the structural complexity of various adherent cells (such as the deformable cytoskeleton formed by a three dimensional intercellular network of interconnected filamentous biopolymers), significant differences on the cell mechanical response can be observed. Moreover, since the reaction of the biological samples to stress vary greatly in a given lapse time, it is important to monitor the characterization process continuously in an *in vitro* environment.
Moreover, some problems are commonly associated with the use of standard commercial cantilever with a sharp tip for the soft sample mechanical characterization. The nanometer size dimensions of tip can cause important local strains which are higher than elastic domain. Furthermore, depending on the magnitude of the applied force over the soft samples, both cantilever tip and the samples can be easily damaged so that the local strain applied in the indented area becomes changed. To overcome these problems, Mahaffy et al. [12] suggest to attach a micro-sphere at the cantilever tip. However, besides the necessity of dexterous manipulations for accurate placement of the micro-sphere, this method needs to use well-know materials (geometry, mechanical properties, ...).

It must also be emphasized that the force measured by the cantilever is calculated by simple analytical formula (via Hooke law) which express the force according to both the deflection and the spring constant of the lever. Consequently, the accuracy of the force-displacement data collected by the AFM is greatly depending on the accurate knowledge of the spring constant since the deflection of the cantilever can be detected accurately by optical laser methods. Several authors have noted that the spring constant provided by cantilever manufacturers are incorrect. These significant errors are mainly due to the difficulty of controlling accurately their thickness during the microfabrication process. Many efforts have been devoted in order to eliminate the necessity to know the cantilever thickness for the spring constant calibration process. As a result, various techniques have been developed

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and published, based on cantilever static or dynamic flexural deflection measurements [13]. The issue of the spring constant calibration using an accurate determination of the cantilever thickness is addressed in this paper. We use the dynamical frequency response method for the thickness determination. As this method is quite accurate, the spring constant calibration is done according to the dimensions of the cantilever.

Another difficulty is associated with the mechanical characterization of biological sample using sharp cantilever. Usually, the spectroscopy curves collected with the AFM are used in conjunction with an appropriate analytical model to estimate Young's modulus, friction, wear and other material properties. According to the literature, the Hertzian model which describes the relationship between force and indentation is the commonly approach used for fitting the experimental data. Also, two major assumptions are made : linear elasticity and infinite sample thickness. Some authors have showed that in the case of soft contact mechanism, models derived from linear elasticity can lead to significant errors. Moreover, due to the imperfections of the tip radius of curvature an unknown contact region results between the probe and the sample. Consequently, uncertainties are introduced for choosing the appropriate fitting analytical model. It has been also shown that depending on the applied force and the sample's thickness, large errors may result when using infinite thickness models [14] [15]. The authors compute force-displacement curves for finite sample thickness to show that, for soft and thin samples the error in the estimated elasticity modulus can be an order of magnitude. Costa and Yin [16] have also showed using finite element modeling that linear elasticity derived models lead to significant errors in case of sharp pyramidal tips.

In our opinion, the cell mechanical characterization in *in vitro* environment using tipless cantilever seems to be a promising solution. As studies involving such cantilevers are less prone to problems associated to sharp tip cantilever, enhanced non destructive cell mechanical characterization should be achieved.For this purpose, a force bio-microscope system has been developed which combines SPM techniques and advanced robotics approaches. A tipless chemically inert cantilever is used in this study. The spring constant calibration using an accurate determination of the cantilever thickness is addressed in this paper. We use a dynamical frequency response method for the spring constant cantilever calibration. Both cell mechanical properties and contact mechanism are modeled with appropriate models taking in account adhesion forces. More precisely, the JKR (Johnson, Kendall and Roberts) and the DMT (Derjaguin, Muller and Toporov) contact theories are used to estimate both Young's modulus and the contact area resulting from the mechanical characterization process. In order to demonstrate the accuracy of the JKR and the DMT models in the case of soft contact mechanism, the estimated force-deformation curves are compared with the one predicted by the Hertz theory.

II. Experimental setup overview

The automated Force Bio-Microscope *FBM* device is an hybrid AFM microscope associating both scanning microscopy approach and biological environment constraints. The *FBM* consists mainly in 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 a biological environment.

The *FBM* experimental setup provides suitable environmental conditions so the biological cells can be kept several hours in living state by the use of a cage incubator. Therefore, the mechanical experiments can be done over a long duration.
A master computer is used to drive the *FBM* in automatic operating mode based on force/vision referenced control. Data acquisition process between the master computer and the *FBM* are achieved using two specialized PCI cards. A user-definable graphical interface is developed in order to make configuration of the automatic cell mechanical characterization process easier. To avoid undesired mechanical vibrations during the cell characterization process, the *FBM* experimental setup is installed on an anti-vibration table. The overall configuration of the *FBM* and the different working components are shown in figure 1.

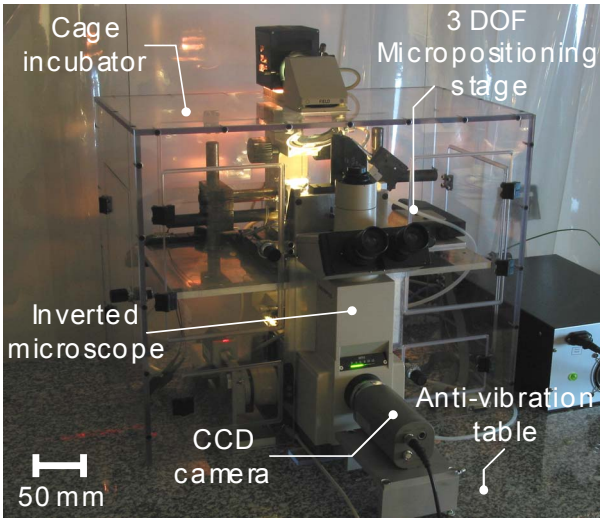

Fig. 1. The *FBM* experimental setup overview.

A. Mechanical sensing unit

The *FBM* mechanical sensing unit is based on the detection of the flexural 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 force measurements. The total sensing area of the photodiode is 7 mm^2 with 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 $mV/\mu m$.

A low spring constant (k=0.2 N/m from manufacturer data) uncoated tipless silicon cantilever is used as probe for the cell mechanical characterization. The cantilever 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 (x,y and z) micropositioning encoded stage with 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 a good repeatability. The configuration of the mechanical sensing unit including the optical detection device is 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.

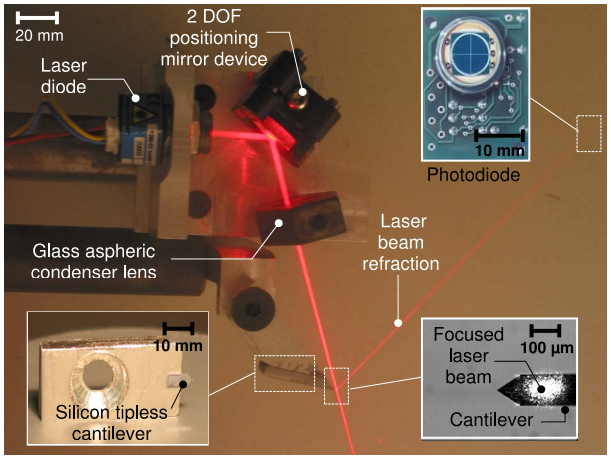

Fig. 2. The mechanical sensing unit.

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

B. *Imaging/grabbing unit*

The *FBM* imaging/grabbing unit consists of an inverted microscope (Olympus IMT-2) with Nikon 10x and 20x objectives. A phase contrast lens is also available 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 vision feedback control with image tracking is developed.

C. *Clean room* in vitro *unit*

The incubating system is formed with a controlled heating module which maintains temperature on the cage incubator at 37 ^oC using a single thermocouple probe. 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 that could damage the mechanical parts of the microscope and the micropositioning stage. The whole system including the *FBM* is placed in a positive pressure clean room to protect the biological environment.

III. Cantilever spring constant calibration

The length and width of the cantilever are measured by an optical method using a glass calibrated micro-array. Figure 3 shows the calibration process under the microscope. The obtained values for length and width are in good agreement with those of the manufacturer. Knowing all the dimensions of the cantilever, the spring constant is then calculated by a static method.

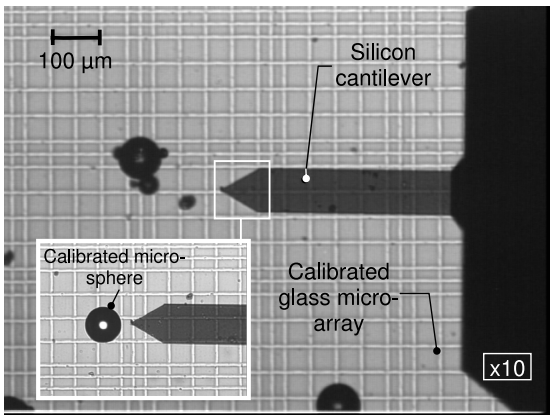

Fig. 3. Cantilever length and width measurements, $L = 450\mu m$ and $l = 90\mu m$.

A. *Frequency response method for the determination of cantilever's thickness*

Let us consider a cantilever of uniform section S, density ρ, Young's modulus $E^{'}$, and inertial moment I. Each point of the cantilever should validate the classic wave equation for a beam in vibration, under the hypothesis of undamped system:

$$\rho S \frac{\partial^2 v(x,t)}{\partial t^2} + E^{'} I \frac{\partial^4 v(x,t)}{\partial x^4} = 0 \qquad (1)$$

where $v(x,t)$ is the instantaneous deformation of the beam depending on time and position.

The system of boundary equations (the fixed end of the cantilever must have zero displacement and zero rotation and the free end cannot have a bending moment or a shearing force) applied to the position equation 1, accepts a solution only if the determinant is zero, which is equivalent to:

$$1 + \cos\mu \cosh\mu = 0 \tag{2}$$

with $\mu = \left[\omega^2 \frac{\rho S}{E' I}\right]^{1/4} L$, equation 2 gives a condition on μ to be respected, which defines the eigen frequency of the system.

With solutions of equation 2, if the length, and the experimental eigen frequency of the cantilever are known, the mean value of the thickness can easily be calculated by the following equation:

$$< h >= \frac{1}{N} \sum_{i=1}^{N} \omega_i \frac{L^2}{\mu_i^2} \sqrt{\frac{12\rho}{E'}} \tag{3}$$

with N the number of the measured eigen frequency.

All the results for different modes (experimental results of mode 3 are unexploitable, because some mechanical parts of the microscope start resonating) are summarized in the following table:

	$Modes\ number$			
	1	2	4	
μ	1.875	4.693	10.995	theory
$f(kHz)$	12.63	82.4	446	measured
$h(\mu m)$	1.516	1.579	1.557	estimated
$\langle h \rangle (\mu m)$	1.550			

In our case, the use of eigen frequency to determine the last dimension of the cantilever improves the accuracy, in comparison to the optical method, by a factor of 100. Moreover, this method can be achieved before each experimentation. Actually, the useful life of the cantilevers is very short (they can only be used once because of biological environment conditions), and the calibration process needs to be repeated at every cantilever exchange.

B. Static approach for the spring constant cantilever determination

Knowing the dimensions of the cantilever and its material properties, the spring constant of a rectangular cantilever is given by $k = 3E^{'} I/L^3$, with the inertia momentum $I = lh^3/12$. The difference of k (0.187 N/m, 0.211 N/m, 0.202 N/m) can be explained by the error on the measured eigen frequency, but also because the estimated thickness is a mean value of each mode. Actually, the variation of the thickness all along the cantilever affects differently the eigen frequency of all the modes.

The variation of the value of k are weak and acceptable, the logarithmic error is about 3.7%, with a contribution of the thickness for this error of 1.9%. In comparison with the spring constant announced by the manufacturer, the mean value is near for this batch $\langle k \rangle \simeq 0.20(N/m)$, but the uncertainty is deep lower (3.7% instead of 90%).

IV. *In vitro* MECHANICAL CHARACTERIZATION EXPERIMENTS

Experiments presented in this paper are focused on the mechanical stress response of the Epithelial Hela *(EpH)* cells under controlled environment. In order to be valid, some assumptions are made in this regard: (i) the biological samples are assimilated to a perfect elastic spherical material; (ii) the liquid enclosed by the biomembrane of the Epithelial Hela cells is incompressible; (iii) the surfaces are assumed frictionless so that only normal forces are transmitted; (iv) the osmotic influence on volume modification is neglected; (v) the stress is considered to be perpendicular to the contact area between the cantilever and the cell.

A. Biological samples preparation and morphology

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. The cervix *(EpH)* cells can be assimilated morphologically to an elliptical cells with a thin surrounding biomembrane which has two functions : ensuring both protection of the cytoplasm and adhesion on the subtract (cf. figure 4).

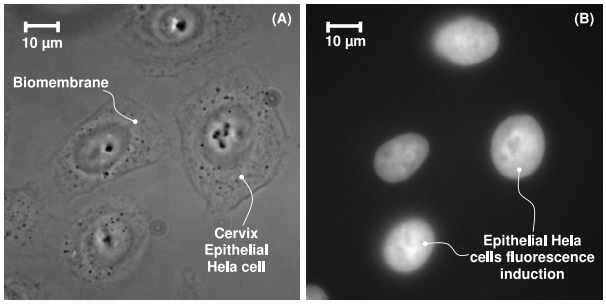

Fig. 4. (A) Magnified image of the cervix Epithelial Hela cells obtained with an 63x objective.(B) The cervix Epithelial Hela cells morphology observed by fluorescence techniques.

B. Mechanical cell response characterization

Figure 5(A) shows the experimental curves of the photodiode output as function of the sample displacement (Δz) performed on both *EpH* cells and hard surface. The single step of the sample displacement is 200 nm and the total displacement is 8 μm. Deformations δ of the *EpH* cells are monitored by calculating the difference between the sample displacement Δz and the cantilever deflection Δd. The non linear elastic behaviour of the *EpH* cells can be seen in the figure 5(B) which presents the sample deformation δ as function of the load force applied by the cantilever.

C. Analytical model for both Young's modulus and contact area estimation

The Young's modulus E as well as the contact area a resulting from the *EpH* cells mechanical characterization

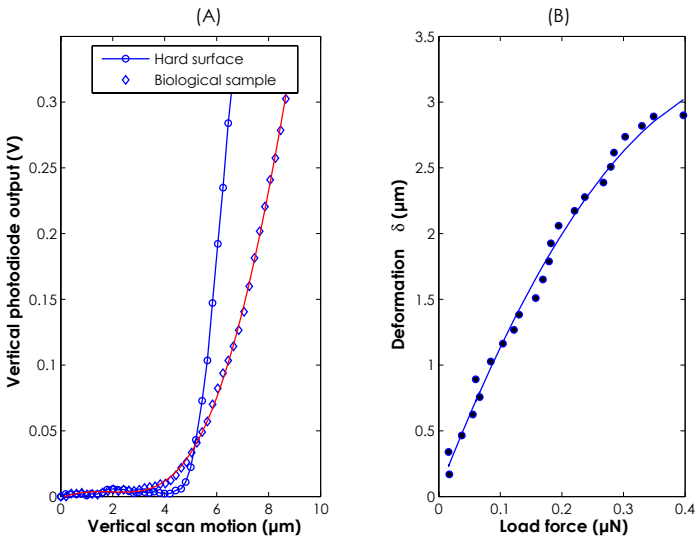

Fig. 5. (A) Experimental data of the photodiode output as function of the sample displacement performed on both *EpH* cells and hard surface. (B) Experimental curve of the sample deformation δ as function of the load force applied by the cantilever.

process are estimated using an appropriate analytical fitted model. Since the Young's modulus can be used to predict the elongation or compression of the biological sample as long as the stress is less than the yield strength of the sample, the chosen models are fitted to sample deformations where elastic linear properties are satisfied. According to figure (B) the quasi linear elastic behaviour is satisfied for load P less then 0.15 μN. Three analytical models are chosen to estimate the Young's modulus and contact area. Thus, the Hertz, the JKR (Johnson, Kendall and Roberts) and the DMT (Derjaguin, Muller and Toporov) models are respectively used.

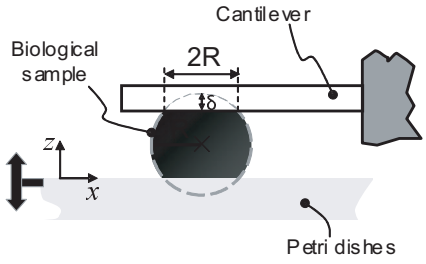

Fig. 6. Mechanical interaction scheme between the silicon tipless cantilever and the biological sample.

Figure 6 presents the mechanical interaction between the silicon tipless cantilever and the biological sample. Noting R the radius of the biological sample (R=5 μm), w the adhesion work and P the load force applied by the cantilever, the contact area a can be expressed respectively according to the Hertz, the JKR and the DMT theories [17]:

$$a^3 = \frac{RP}{K} \quad (4)$$

$$a^3 = \frac{R}{K}(P + 3\pi Rw + \sqrt{6\pi RwP + (3\pi Rw)^2}) \quad (5)$$

$$a^3 = \frac{R}{K}(P + 2\pi Rw) \quad (6)$$

Where K is the effective Young's modulus of the two materials in contact. K is expressed according to either the Hertz, the JKR or the DMT models as:

$$\frac{1}{K} = \frac{3}{4}(\frac{1-\nu^2}{E} + \frac{1-\nu'^2}{E'}) \quad (7)$$

ν and ν' are respectively the Poisson's coefficients of the *EpH* cells (ν=0.5) and the silicon cantilever. The manufacture data gives the Young's modulus of the silicon tipless cantilever and the Poisson's ratio as E'=140 GPa and ν'=0.17.

The JKR and the DMT theories suggest that adhesion work w can be expressed by two ways according to the pull-off force P_{off} needed to overcome adhesion forces as [17]:

$$P_{off} = \frac{3}{2}\pi Rw \quad (JKR) \quad (8)$$

$$P_{off} = 2\pi Rw \quad (DMT) \quad (9)$$

As the pull-off force P_{off} is accurately measured using the *FBM* ($P_{off} \simeq 20\ nN$), the adhesion work w is introduced in equations 6 and 6 to estimate the contact area a.

The deformation δ of the elastic body is expressed respectively using the Hertz, the JKR and the DMT analytical models as [17]:

$$\delta_{Hertz} = \delta_{DMT} = \frac{a^2}{R} \quad (10)$$

$$\delta_{JKR} = \frac{a^2}{R} - \sqrt{\frac{8\pi wa}{3K}} \quad (11)$$

Figure 7 (A) shows the estimation of the biological sample deformation δ as function of the simulated load force P using the Hertz, JKR and DMT theories. These analytical results are compared to the experimental measurements performed with the *FBM* and presented in IV-B. The *EpH* cells Young's modulus E is estimated using the biological sample deformation δ and the contact area a obtained by the different modeling approach. Figure 7 (B) shows the estimated stress $\sigma = \frac{P}{a}$ as function of the estimated strain $\varepsilon = \frac{\delta}{2R}$ using the Hertz, JKR and DMT theories. Since linear elastic deformation are satisfied, the Young's modulus E of the studied biological sample can be determined by calculating the slope of the obtained curves (σ=$E\varepsilon$). These results emphasize, in our case, that the Hertz model is not appropriate for estimation of contact mechanism in the case of soft materials at the microscale. Since adhesion forces are not considered, large errors are observed between the experimental data and the predicted force-deformation curves (in order of 0.2 μm of magnitude).

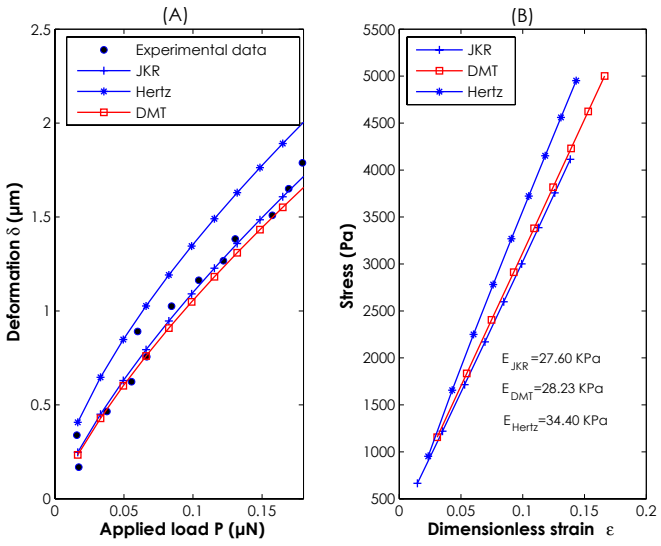

Fig. 7. (A) Estimation of the biological sample deformation δ as function of the simulated load force P using the Hertz, JKR and DMT theories compared to the experimental data.(B) Estimated stress $\sigma = \frac{P}{a}$ as function of the estimated strain $\varepsilon = \frac{\delta}{2R}$ using the Hertz, JKR and DMT theories.

We have observed small deviation between the JKR and the DMT models for estimating the force-deformation curve. Moreover, the estimated Young's modulus E_{JKR}=27.60 KPa, E_{DMT}=28.23 KPa of the studied biological sample are in a good agreement with the literature. .

V. Conclusion

We have presented in this paper the development of a microforce sensing system for the *in vitro* single cell mechanical characterization. The experimental setup combines both SPM techniques to advanced robotics techniques. As the developed system operate in a fully automatic mode based on visual and force feedback control, effective mechanical characterization and reliable data acquisition are achieved. All three units of the Force Bio-Microscope *(FBM)* (force sensing, vision control and *in vitro* environment) are designed, calibrated or configured toward a reliable *in vitro* cell mechanical characterization. Calibration of the tipless cantilever spring constant which perform a non destructive mechanical characterization is addressed in this paper. The developed approach yields to determine the cantilever's thickness. This dynamical approach allows to overcome the main problem of usual calibration methods based on the determination of the geometry of cantilevers.

Mechanical characterization experimentations are conducted using the *FBM* on human adherent cervix Epithelial Hela cells. These experiments demonstrate the efficiency of the developed experimental setup in exploring the mechanical properties of adherent biological sample. The contact mechanism resulting from the cell mechanical characterization process is predicted using two appropriate models (JKR and DMT) taking into account adhesion forces. These predicted mechanical behaviour models are compared to the Hertzian model.

VI. Future work

Current work involves modeling the contact mechanism resulting from the cell mechanical characterization process using a tipless cantilevers taking into account friction, osmotic influence and anisotropic non linear elastic properties. Future work will be focused on exploring the mechanical transduction of living cells in *in vitro* environment conditions. One of the most promising results of this study will be the description of both mechanosenitivity and mechanical transduction mechanisms at the microscale for biological cells or tissue configuration.

References

[1] Y. Sun and B.-J. Nelson, "MEMS for cellular force measurements and molecular detection", Journal of Information Acquisition, vol.1, no. 1, pp. 23-32, January 2004.

[2] K.-J. Van Vliet, G. Bao and S. Suresh, "The biomechanics toolbox: experimental approaches for living cells and biomolecules", Acta Materialia journal, vol. 51, pp. 5881-5905, August 2003.

[3] D.-H. Kim, S. Yun and B. Kim, "Mechanical force response of single living cells using a microrobotic system", in proceedings of the Int. Conference on Robotics and Automation, pp. 5013-5018, 2004.

[4] J. Guck, R. Ananthakrishnan, C.-C. Cunningham and J. Kas, "Stretching biological cells with light", J.Physics:Condens. Matter, vol. 14, pp. 4843-4856, May 2002.

[5] J.-M. Sharp, A.-R. Clapp and R.-B. Dickinson, "Measurement of long-range forces on a single yeast cell using a gradient optical trap and evanescent wave light scattering", Colloids and Surface B:Biointerfaces, vol. 27, pp. 355-364, 2003.

[6] M. Lukkari and P. Kallio, "Multi-purpose impedance-based measurement system to automate microinjection of adherent cells", in proceedings of the Int. Sympos. on Computational Intelligence in Robotics and Automation", pp. 20-26, 2005.

[7] M., Radmacher, "Measuring the elastic properties of biological samples with AFM", IEEE Engineering in Medicine and Biology, pp. 47-57, 1997.

[8] O. Sahin, G. Yaralioglu, R. Grow, S.-F.Zappe, A.Atalar, C.Quate and O. Solgaard, "High-resolution imaging of elastic properties using harmonic cantilevers", Sensors and Actuators A, vol. 114, pp. 183-190, Februrary 2004.

[9] S. Park, K. Costa and G. Ateshian, "Microscale frictional response of bovine articular cartilage from atomic force microscopy", Journal of Biomechanics, vol. 37, 1697-1687, Februrary 2004.

[10] E.-K. Dimitriadis, F. Horkay, J. Maresca, B. Kachar and R.-S. Chadwick, "Determination of elastic moduli of thin layers of soft material using the atomic force microscope", Biophysical Journal, vol. 82, pp. 2798-2810, May 2002.

[11] X. Yao, J. Walter, S. Burke, S. Stewart, M.-h. Jericho, D. Pink, R. Hunter and T.-J. Beveridge, "Atomic force microscopy and theoretical considerations of surface properties and turgor pressures of bacteria", Colloids and Surfaces B: Biointerfaces, vol. 23, pp. 213-230, May 2002.

[12] R. Mahaffy, C. -K Shih, F. -C. Mackintosh and J. Kas, "Scanning probe-based frequency-dependent microrheology of polymer gels and biological cell", Phys. Rev. Lett., vol. 85, pp. 880-883, 2000.

[13] R. Levy and M. Maaloum, "Measuring the spring constant of atomic force microscope cantilevers : thermal fluctuations and other methods", Nanotechnology, vol. 13, pp. 33-37, 2002.

[14] J. Domke and M. Radmacher, "Measuring the elastic properties of thin polymer films with the atomic force microscope". Langmuir, vol. 14, pp. 3320-3325, 1998.

[15] B. Akhremitchev and G. Walker, "Finite sample thickness effects on elasticity determination using atomic force microscopy". Langmuir, vol. 15, pp. 5630-5634, 1999.

[16] K. Costa and F, -C. Yin, "Analysis of indentation: implications for measuring mechanical properties with atomic force microscope", J. Biomeh. Engr. Trans. ASME, vol. 121, pp. 462-471, 1999.

[17] D.Maugis, "Contact, adhesion and rupture of elastic solids", Springer-Verlag, 2000.