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# A Robotic Platform for Targeted Studies on Biological Cells

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***Abstract*— This paper deals with the development of an open design platform for explorative cell mechanotransduction investigation. The produced setup combines SPM (Scanning Probe Microscopy) techniques and advanced robotics approaches allowing both prolonged observations and spatial measurements on biological samples. As a result, an enhanced force probing method based on scanning microscopy techniques and advanced robotics and 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. Experimentation on the mechanical cell characterization under *in vitro* conditions on human adherent cervix Epithelial Hela cells are presented to demonstrate the viability and effectiveness of the proposed setup.**

***Index Terms*— In vitro mechanotransduction; Scanning Probe Microscopy (SPM) techniques; Human adherent cervix Epithelial Hela cells mechanical characterization.**

## 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, not only in cell culture, but also extended to the behavior of the whole organism. 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 force 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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Development of effective tools for mechanotransduction studies at the molecular level is crucial for understanding the involved mechanisms. The design of such tools should address important issues in term of spatial and temporal features (e.g. measurements, positioning, monitoring). In fact, due to the complexity of the cell mechanics as well as the requirement of life science, suitable and specific solutions are needed. Robotics and microrobotics approaches can play an important role for exploring mechanotrasduction mechanisms by development toward high effective and reliable systems.

## II. Motivations of this work

A variety of approaches have been used to either mechanically stimulate cells, sense force distribution, or for cell mechanical properties determination [1]-[7]. Among these approaches, the most promising ones involve Scanning Probe Microscopy (SPM) techniques for nanoscale. These techniques have the potential to give accurate quantitative information about local forces and contact mechanics. The Atomic Force Microscope (AFM) has become a commonly used tool in the field of the biosciences [8]-[14]. A flexible cantilever with low spring constant (0.1 - 0.2 $N/m$) and an atomic sharp tip is usually 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. Some commercial solutions are available for performing experiments on life science (e.g. Veeco, Olympus, Andor) but only a few of them are effective for mechanotransduction studies. The cost as well as the flexibility are the main drawback of these devices. Since these studies need complex experiments and specific environmental conditions, an open platform design is more suitable. Furthermore, studies on mechanotransduction are usually focused on a single cell target and seldom conducted on a large cell population. Performing mechanotransduction on large samples, based on statistical approaches, can lead to a better modeling at the molecular scale.

We associate some problems with the use of a standard commercial cantilever with a sharp tip for mechanotransduction requirements. In fact, the nanometer dimensions of the tip can cause important local strains which are higher than elastic domain. Furthermore, depending on the magnitude of the force applied on the soft samples, both

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the cantilever tip and the samples can be easily damaged so that the local strain applied in the indented area becomes changed. Since mechanotransduction studies need accurate force application, a soft and non-invasive approach is more suitable. It must also be emphasized that the force measured by the cantilever is calculated by simple analytical formula (via Hooke's law) which expresses 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 greatly dependent 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 constants provided by cantilever manufacturers are incorrect [15][16]. These significant errors are mainly due to the difficulty of accurately controlling their thickness during the microfabrication process. Many efforts have been devoted to eliminating the necessity of knowing the cantilever thickness for the spring constant calibration process. As a result, various techniques have been developed and published, based on cantilever static or dynamic flexural deflection measurements [17]. 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 using sharp cantilevers. 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 shown that in the case of soft contact mechanism, models derived from linear elasticity can lead to significant errors [18][19]. 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 also been shown that depending on the applied force and the sample's thickness, large errors may result when using infinite thickness models [20][21]. 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 [22] have also shown, using finite element modeling that linear elasticity derived models lead to significant errors in case of sharp pyramidal tips.

In our opinion, mechanotransduction based on a tipless cantilever seems to be a promising solution. As studies involving such cantilevers are less prone to problems associated with a 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 into 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 mechanisms, the estimated force-deformation curves are compared with the one predicted by the Hertz theory.

## III. Experimental setup overview

The Force Bio-Microscope *FBM* device is a hybrid AFM microscope associating both scanning microscopy approach and biological environment constraints. The *FBM* 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 (Fig. 1). The overall configuration of the *FBM* and the different working components are shown in Fig. 2.

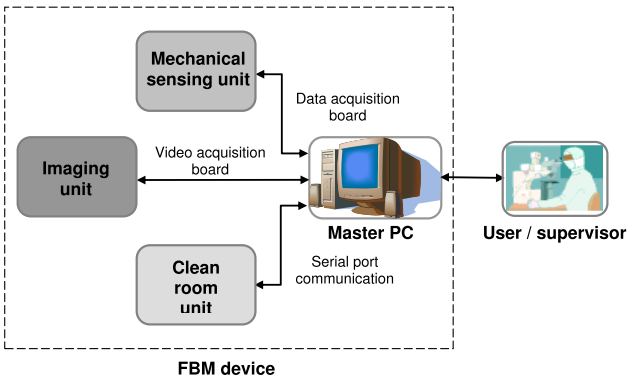


Fig. 1. Block diagram of the FBM device.

The *FBM* experimental setup provides suitable conditions for study in a controlled environment so that the biological cells can be kept several hours in a living state by using a cage incubator. Therefore, the mechanical measurement process can be done on the biological sample over an extended period of time.

A master computer is used to drive the *FBM* in an automatic operating mode based on force/vision referenced control. The data acquisition process between the master computer and the *FBM* is achieved by the use of two specialized PCI cards (Matrox and National Instrument). A user-definable graphical interface has been developed in order to make configuration of the experiments 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 Fig. 2.

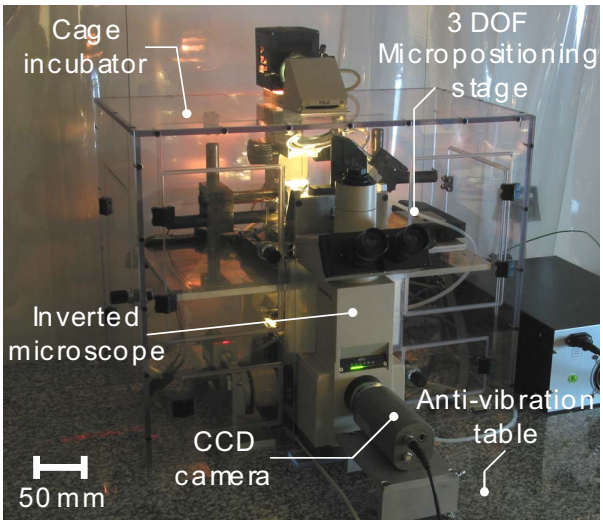


Fig. 2. The *FBM* experimental setup overview.

The mechanical sensing unit is based on the detection of the deflection of a cantilever by an optical technique. A four quadrant photodiode (CentroVision) with internal amplifiers associated to a 650 $nm$ low power collimated laser diode (Vector Technology) 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 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 our 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 (Nanosensors) is used as a probe for the cell mechanical characterization. The lever is 450 $\mu m$ long, 90 $\mu m$ wide and 2 $\mu m$ thick. The sample to be studied is accurately positioned below the cantilever by a 3 degree-of-freedom DOF (x-axial ,y-lateral and z-vertical) micropositioning encoded stages (Physik Instrumente) with a submicrometer resolution (0.1 $\mu m$). The kinematics features of the micropositioning stages allows us to achieve accurate mechanical measurements in a workspace of 25 x 25 x 25 $mm^3$ with a good repeatability.

## IV. *In vitro* MECHANICAL CHARACTERIZATION EXPERIMENTS

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 cell with a thin surrounding biomembrane which has two functions : ensuring both protection of the cytoplasm and adhesion feature on the substrate (Fig. 3). In the present study, the average dimensions of the biological sample is 10 $\mu m$ long, 9 $\mu m$ wide and 6 $\mu m$ in height.

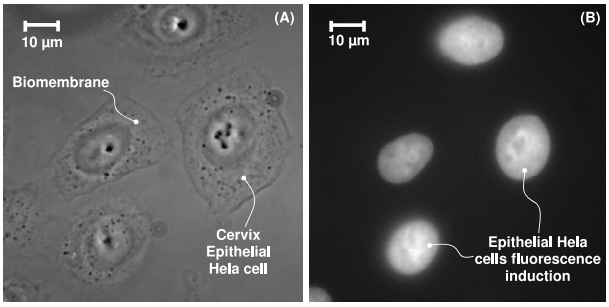


Fig. 3. (A) Magnified image of the cervix Epithelial Hela cells obtained with an 63x objective. (B) The cervix Epithelial Hela cells morphology observed by fluorescence techniques.

### A. Cell's mechanical response characterization

Fig. 4(A) shows the experimental curves of the photodiode output as a function of the sample vertical displacement ($\Delta z$) performed on both single *EpH* cell and a hard surface. The single step of the sample displacement is 200 $nm$ and the total displacement is 8 $\mu m$. Deformation $\delta$ of the *EpH* cell is monitored by calculating the difference between the sample displacement $\Delta z$ and the cantilever deflection $\Delta d$. The non-linear elastic behaviour of the *EpH* can be seen in the Fig. 4(B) which presents the sample deformation $\delta$ as function of the load force applied by the cantilever.

The viscoelastic behaviour of the *EpH* cells are also investigated by the *FBM* device. Cyclical automatic approach and retract experimentations were conducted on the same biological sample over 2 hours at 3 minute intervals. For this given study, the motion amplitude and the single step of the vertical microstage are fixed to 8 $\mu m$ and 200 $nm$,respectively. In order to reduce the cantilever damping oscillations during the mechanical characterization process, velocity of the sample positioning stage is chosen small (0.5 $\mu m/s$). Fig. 5(A) shows 3 approach and retract curves monitored at different time intervals (t=0 $minutes$, 40 $minutes$ and 80 $minutes$) of the cyclical experiments. A single referenced approach and retract curves performed on hard surface are given in Fig. 5(B). According to the collected data, the *EpH* sample exhibit the same viscoelastic behaviour during all the experimentation.

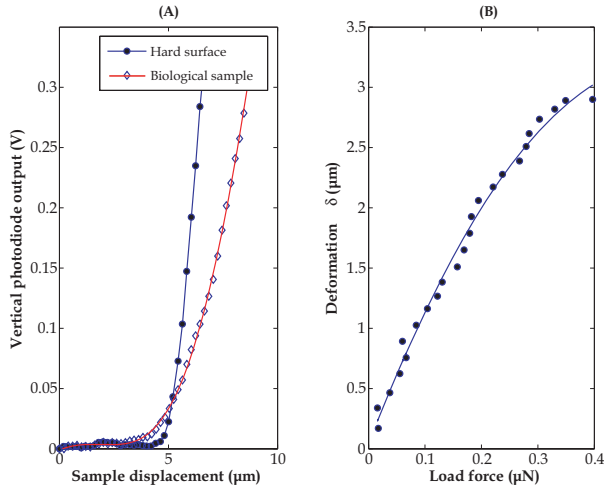


Fig. 4. (A) Experimental data of the photodiode output as function of the sample displacement performed on both single *EpH* cell and hard surface. (B) Experimental curve of the sample deformation $\delta$ as a function of the applied load by the cantilever.

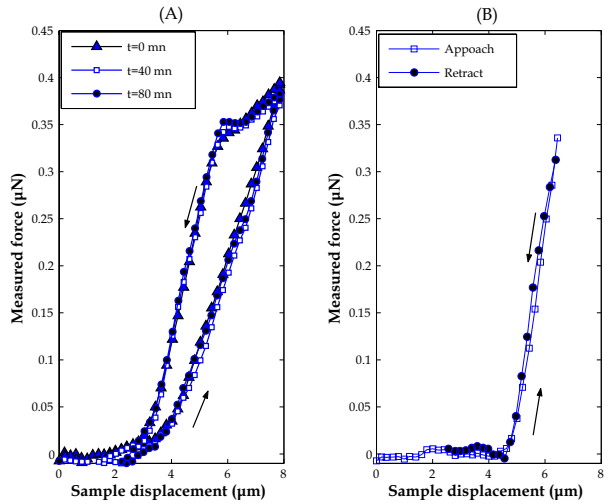


Fig. 5. (A) Experimental spectroscopy curves (approach and retract) performed on a single *EpH* cell at different time intervals (t=0 $minutes$, 40 $minutes$ and 80 $minutes$). (B) Single referenced approach and retract curves performed on hard surface.

### *B.* In vitro *efficiency approach for cell mechanical characterization*

In order to address either the efficiency of the *in vitro* clean room unit or how mechanical cell properties can be affected by the environmental culture conditions, we have experimented with automatic and cyclical spectroscopy operation on a single *EpH* cell over several minutes without the use of the incubating system. As the precedent study, the sample displacement and the single step of the vertical micropositioning stage are fixed to 8 $\mu m$ and 200 $nm$ respectively. Since the purpose of this study is to observe the difference which can occur on mechanical behaviour of the studied biological sample, experimentation is initially conducted using the incubating system. Fig. 6 shows evolution of

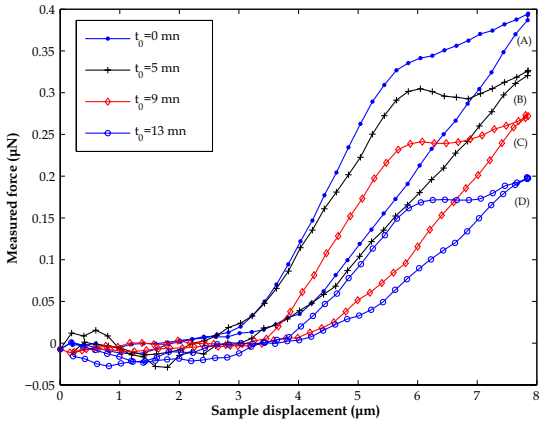


Fig. 6. Evolution of the measured force as a function of the sample displacement for different elapsed times $t_0 = 0, 5, 9$ and $13$ $min$.

the *EpH* cell mechanical behaviour of cyclical spectroscopy operation with and without the use of the incubating system. More specifically, curve (A) shows the approach and retract curves using the cage incubator. Curves (B), (C) and (D) show the mechanical behaviour of the studied *EpH* cell for different elapsed times $t_0$ once the cage incubator is turned off.

These mechanical characterization experiments obviously reveal that mechanical properties of the studied sample are affected by the temperature environmental culture conditions. This difference suggests that the intra or extra-cellular matrix react to the variation of temperature.

### *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 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 Fig. 6 the quasi linear elastic behaviour is satisfied for load $P$ less then 0.15 $\mu N$. 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. 7 presents the mechanical interaction between the silicon tipless cantilever and the biological sample. Noting $R$ the radius of the biological sample ($R$=5 $\mu m$), $w$ the adhesion work and $P$ the load force applied by the cantilever, the contact area $a$ can be expressed respectively according

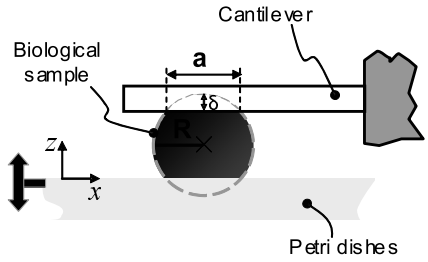


Fig. 7. Mechanical interaction scheme between the silicon tipless cantilever and the biological sample.

to the Hertz, the JKR and the DMT theories [23]:

$$a^3 = \frac{RP}{K} \quad (1)$$

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

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

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:

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

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

The JKR and the DMT theories suggest that adhesion work $w$ can be expressed in two ways according to the pull-off force $P_{off}$ needed to overcome adhesion forces as [23]:

$$P_{off} = \frac{3}{2}\pi Rw \quad (JKR) \quad (5)$$

$$P_{off} = 2\pi Rw \quad (DMT) \quad (6)$$

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 3 and 3 to estimate the contact area $a$.

The deformation $\delta$ of the elastic body is expressed respectively using the Hertz, the JKR and the DMT analytical models as [23]:

$$\delta_{Hertz} = \delta_{DMT} = \frac{a^2}{R} \quad (7)$$

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

Fig. 8 (A) shows the estimation of the biological sample deformation $\delta$ as a 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 section IV-A. The

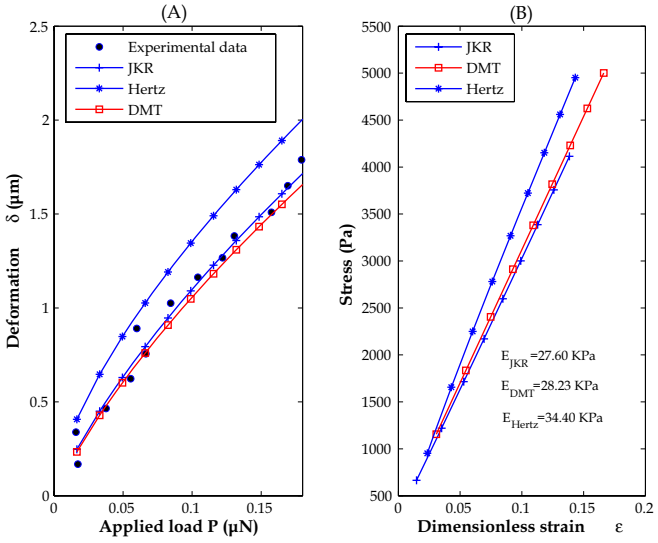


Fig. 8. (A) Estimation of the biological sample deformation $\delta$ as function of the simulated load force $P$ using the Hertz, JKR and DMT theories compared to the experimental data.(B) Estimated stress $\sigma = \frac{P}{a}$ as function of the estimated strain $\varepsilon = \frac{\delta}{2R}$ using the Hertz, JKR and DMT theories.

*EpH* cells Young's modulus $E$ is estimated using the biological sample deformation $\delta$ and the contact area $a$ obtained by the different modeling approaches. Fig. 8 (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 is satisfied, the Young's modulus $E$ of the studied biological sample can be determined by calculating the slope of the obtained curves ($\sigma$=$E\varepsilon$). 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 (on the order of 0.2 $\mu m$ of magnitude). We have observed small deviation between the JKR and the DMT models for estimating the force-deformation curve. According to literature [23], the DMT theory is applied in the case of hard solids, with small radius of curvature and low energy of adhesion. The JKR theory is more often applied for soft solids, with large radius and large energy of adhesion. Based on these considerations, we chose the JKR model as the model reference in our case. This model used in conjunction with the experimental data lead to an accurate detection of cell mechanical property modification needed in mechanotransduction studies. In the same way, we notice that the DMT model strongly diverge for small strains. The assessment of Young's modulus for $\epsilon < 0.2$ should lead to

### *D. Parametric models accuracy evaluation*

Errors resulting on the determination of the Young's modulus as well as the contact area are mainly due on the one hand to the accuracy/precision of the measured forces distribution and on the other hand on the estimation of

the contact between the probe (cantilever) and the cell.In the first case, the measurements accuracy of the forces distribution has been discussed on the previous sections. By combining dynamic and static approaches for the spring cantilever calibration, the overall sensitivity of the optical detection device has been enhanced. In the second case, the morphology of cells (which depends on the cell life evolution) induces local modification on the contact mechanics between the probe and the cell. Furthermore, common models predicting the elastic deformation of soft material is restricted to spherical shape materials. As the size of the cell is involved in the Hertz, DMT, and JKR analytical models, the change on cell morphology can affect the mechanical cell properties determination.

In the following, we propose to conduct analytical study for evaluating the uncertainties depending of this two cases. First, uncertainties dues to measurements can easily be estimated. Regarding the deformation, which is dependent of the applied load, errors can be attributed to the acquisition system starting with the measurement of the engines displacement. Uncertainties on these displacements are already estimated at $3\%$. Both the calibration of the stiffness of the cantilever, seen below, and the measurement of the spot deviation are used to obtain load data. The estimated error for the measurement of the spot deviation is $0.5\%$. The last uncertainty regards the location of the cell on the cantilever and the equivalent contact point. They were evaluated [24] and the global uncertainties on the measurement of load and strain are about of $8\%$. This value calculated in the worst case, can be reduced to $5\%$ for a person expert on the *FBM*.

The second error source in the assessment of Young's modulus come from the contact between the cell and the cantilever. The modeling of this contact needs the knowledge of the cell's radius. Its determination, without prejudicial contact, is based on visual estimation of the cell surface under the microscope. The ratio between the surface and the radius were preliminary determined on several tests on cells. Moreover, for adhesion models, adhesion energy estimation is realized by the measurement of pull-off forces. The great variability of theses forces implies the assessment of its influence on Young's modulus.

In order to identify the comportment of our model regarding theses two parameters, the crushing simulation of a perfect and homogeneous sphere is used. This first study investigate the influence of the error made on Young's modulus related to the cell's radius uncertainty. The plot of the stress as a function of the strain of the spheres with rising radius (2, 4, 6, 8 and 10 $\mu m$) submitted to a known load $[0 - 0.5\mu N]$ is realized for the three models (Fig. 9). Several conclusions regarding the Young's modulus (represented by the slope of theses curves) can be drawn. The steeply slopes with small strain, for the DMT model, represent the stiffness effect by adhesion and do not correspond to the cell stiffness. The JKR model is the one where slopes are the most steady during all the load test. Moreover, the influence of the radius is really soft for this model and negligible for strains

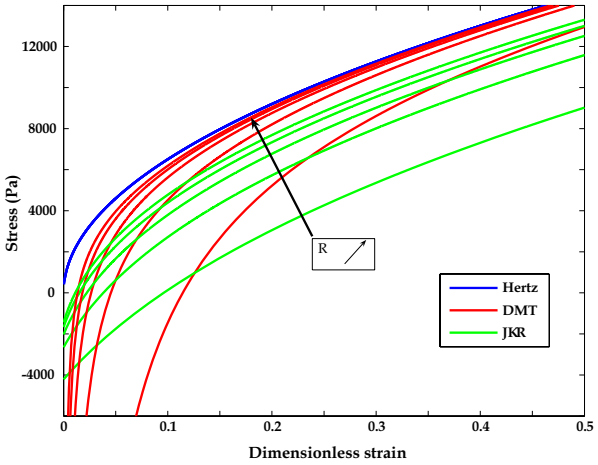


Fig. 9. Stress vs strain for indentation of a perfect sphere with different radius.

such as $\epsilon > 0.05$. The JKR model, which is, by definition, appropriate for cellular studies, is also the less sensitive to errors in the determination of the cell's radius.

The indentation of a sphere (radius $5\mu m$) is then realized for variable pull-off forces $[20,\ 40,\ 60,\ 80,\ 100\ nN]$. For each model, the stress versus strain curves are plotted on Fig. 10. In the same way, we notice that the DMT

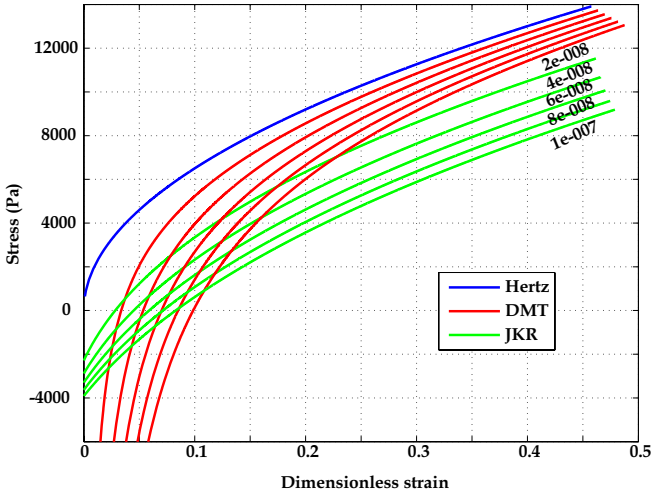


Fig. 10. Stress vs strain for indentation of a perfect sphere with different pull-off forces.

model strongly diverge for small strains. The assessment of Young's modulus for $\epsilon < 0.2$ should lead to great errors. On the contrary, the JKR model is steady for all the elongations investigated and show a small deviation for $\epsilon > 0.05$.

The JKR model for soft solids with high adhesion energy (as cell does), is slightly sensitive to both radius variations and pull off forces which are the two uncertain parameters for this modeling. Young's modulus are estimated for $\epsilon > 0.05$, with a cumulative error of $5\%$ on the model. Nevertheless,

given the large diversity and the great complexity of cells, studies will lead on as many cells as possible.

## V. Conclusion

This paper has presented the development of a micro-force sensing system for *in vitro* mechanotransduction investigation. The experimental setup combines Scanning Probe Microscopy (SPM) techniques with advanced robotics approaches. As the developed system operates in a fully automatic mode based on visual and force tracking control, effective mechanical characterization and reliable data acquisition are achieved. The Force Bio-Microscope device *(FBM)* consists of three modules with autonomous force sensing and measurements capabilities. Each module is designed, calibrated or configured toward an effective and reliable device.

Experiments have been conducted using the *FBM* on human adherent cervix Epithelial Hela cells. The experiments demonstrate the efficiency of the experimental setup developed to explore the mechanical response in *in vitro* conditions of adherent biological samples. The contact mechanisms resulting from the cell mechanical characterization process are predicted using appropriate models taking into account both adhesion forces and finite sample thickness.

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