atomic force microscopy, Young's modulus, force curve, indentation model, curve fitting

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ANALYSIS OF CELLS ELASTICITY BASED ON FORCE-DISTANCE CURVES OBTAINED FROM ATOMIC FORCE MICROSCOPY

Automated techniques for measuring elasticity parameters of cells enable development of new diagnosis methods. An important elasticity parameter is the Young's modulus (YM), which has been effectively used to characterize different cell properties, e. g., platelet activation, locomotion, differentiation, and aging. This paper deals with the problem of automated determination of cells YM based on the force-distance curves obtained from atomic force microscope. During experiments, the YM of cells was determined by using contact point detection and curve fitting algorithms. Experimental results were compared for two theoretical models of indentation: Hertz model, and Sneddon model. The results show that single indentation model allows a satisfactory accuracy to be obtained only for a subset of the force-distance curves. The most appropriate model for a given curve can be selected based on the fitting error analysis.

1. INTRODUCTION

Atomic force microscope (AFM) is a valuable tool for analysis of physical properties of cells. The key element of the AFM is a cantilever with an extremely small tip that deflects when interacting with the cell surface [2]. Deflection of the cantilever is measured in order to determine force between the tip and the surface [9]. A controller with analog-digital converters collects, processes the data, and drives the cantilever. The data collected by AFM can be represented in form of force-distance curves.

A force-distance curve is a plot of tip-sample interaction forces vs. tip-sample separation [12]. AFM is able to acquire force-distance curves on every kind of surface and in every kind of environment, with high lateral, vertical, and force resolution. In order to acquire force-distance curve, the cantilever and tip are moved towards the sample (cell surface) until the tip is in contact with it. Subsequently, the cantilever is retracted. Deflection of the cantilever is registered during both the approach and the retract stage. The tip-sample interaction force is determined from the measured cantilever deflection by using the Hooke's law, which postulates a linear relation between stress and strain [3].

Figure 1 shows a schema of the measurement procedure for the force-distance curves acquisition [10]. As the cantilever moves towards the surface of cell, the attractive forces (Van der Waals and capillary forces) are encountered that deflects the cantilever in direction of the

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surface (Fig. 1 b). When the tip is in contact with the sample, it remains on the surface as the separation between the cantilever and the sample decreases further, causing an increase of the force. The cantilever is pushed into the surface with some force to investigate physical properties of the cell (Fig. 1 c). As the cantilever is retracted from the surface, the tip remains in contact with the surface due to adhesion (Fig. 1 d). During such experiment, two force-distance curves are acquired - one for the approach stage (Fig. 1 a - c), and one for the retract stage (Fig. 1 c - e).

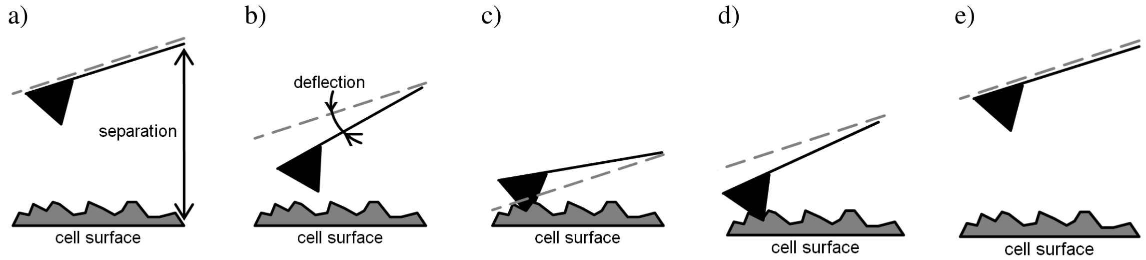

Fig. 1. Schema of the experimental procedure for force-distance curves acquisition.

Figure 2 presents an example of the force-distance curves obtained from a single approach - retract experiment on a Normal Human Dermal Fibroblasts (NHDF) cell. It should be noted here that the separation controlled during the measurement does not correspond to the actual tip-surface distance, but to the distance between cell surface and the rest position of the cantilever. These two distances usually differ owing to the cantilever deflection and the sample deformation.

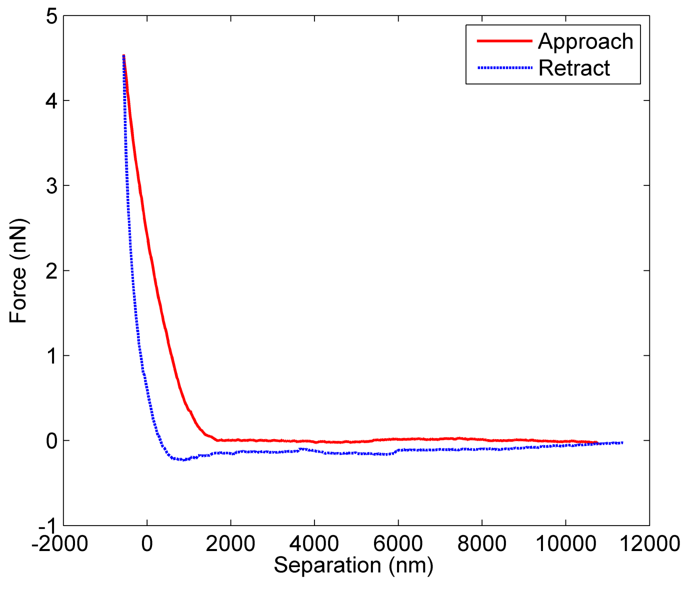

Fig. 2. Approach and retract force-distance curves.

So far, a number of studies have demonstrated dependencies between the elasticity of cells and various diseases, such as cancer, arthritis, malaria, and ischemia [3], [6]. Most types of cells, like muscle, epithelial, blood cells, neurons, etc., stay under a permanently changing force environment. The changes in cell mechanics may change the mechanical response of tissue or organs. Therefore, the alteration of cell mechanics may lead to various pathologies or diseases [4]. In this context, the AFM is a unique tool to enable the development of new methods of diagnosis. It is important to provide robust automated methods that allow measuring the elastic parameters of cells. One of such parameters is the Young's modulus (YM), which has been effectively used to characterize different cell properties, e.g., platelet activation, locomotion, differentiation, and aging [5], [6].

This paper deals with the problem of automated determination of cells YM based on the approach force-distance curves obtained from AFM. The YM of NHDF cells was determined by using contact point detection and curve fitting algorithms. A comparison is presented, which involves the experimental results obtained for two indentation models: Hertz model, and Sneddon model. Error of indentation model fitting is discussed as an indicator of the YM evaluation accuracy for cells. The paper is organized as follows. In Section 2 details of the utilized methods and algorithms are described. Section 3 discusses results of the experiments on the AFM-based YM evaluation for cells. Finally, summary and conclusions are given in Section 4.

2. METHODS

According to the approach, which is used in this study, the YM of a cell is determined by fitting an indentation model to the approach force-distance curve obtained from AFM. Prior to model fitting, it is necessary to determine a contact point in the examined force-distance curve. The applied algorithm for contact point detection and the considered indentation models are described in the following subsections.

2.1. CONTACT POINT DETECTION

The contact point is the value of separation (z_{CP}) at which the tip reaches the surface of the cell. For separation values below the contact point ($z < z_{CP}$), the force increases and therefore the slope of the force curve is higher.

A simple contact point detection algorithm was used in this study, which is based on the method implemented in NanoScope Analysis software [1]. This algorithm finds a line between the first and last points of the experimental force curve ($F(z)$). The force values that correspond to the considered line are subtracted from each value in the force curve, effectively rotating it. The separation, at which the rotated curve has a minimum, is selected as the contact point. Formally, the position of contact point is calculated as follows:

$$z_{CP} = \arg\min \left(F(z) - z\frac{F(z_{MAX}) - F(z_{MIN})}{z_{MAX} - z_{MIN}} \right), \tag{1}$$

where z_{MIN} and z_{MAX} denote respectively the minimum and the maximum separation value for the considered approach force-distance curve.

This method emphasizes the minimum force at the contact point while de-emphasizing forces due to noise or interference in the non-contact region, reducing the likelihood that the wrong point is selected.

2.2. INDENTATION MODEL FITTING

In order to evaluate the YM, an indentation model has to be fitted to the part of experimental force curve below the contact point, i. e., $F(z), z < z_{CP}$. Two indentation models are considered in this study: the Hertz model, and the Sneddon model.

The Hertz model assumes a contact between a sphere and an elastic half-space [7]. When using this model, the theoretical force values are calculated according to the formula:

$$\hat{F}(z) = \frac{4}{3} \cdot \frac{E}{1-\nu^2} \cdot \sqrt{R} \cdot (z_{CP} - z)^{3/2} + F(z_{CP}), \tag{2}$$

where: $F(z_{CP})$ is the experimental force value measured at the contact point, E is the YM (fit parameter), ν denotes Poisson's ratio (sample dependent), and R is radius of the tip.

The model derived by Sneddon assumes a rigid cone indenting a soft flat surface [11]. The theoretical force value for this model is calculated as follows:

$$\hat{F}(z) = \frac{2}{\pi} \cdot \frac{E}{1-\nu^2} \cdot \tan(\alpha) \cdot (z_{CP} - z)^2 + F(z_{CP}), \tag{3}$$

where α denotes half-angle of the tip, and the remaining symbols have the same meaning as in Eq. (2).

In this study, for the experiments performed on NHDF cells, the parameters of indentation models had the following values: $\nu = 0.5$, $R = 20$ nm, $\alpha = 18°$.

The theoretical force-distance curves were fitted to the experimental ones by using the trust-region-reflective least squares algorithm [8]. The model fitting operation was executed for different contact point positions in range between $z_{CP} - 0.05 \cdot z_{CP}$ and $z_{CP} + 0.05 \cdot z_{CP}$. The value of YM was determined based on the solution for which the residual sum of squares (RSS) is minimal.

3. RESULTS

The YM of NHDF cells was evaluated on the basis of 20 force-distance curves by using Hertz and Sneddon indentation models. This section includes presentation and discussion of the obtained model fitting errors and the resulting values of YM.

Accuracy of the indentation model fitting was evaluated by means of two measures: the residual sum of squares (RSS) and the root-mean-square error (RMSE). The fitting errors obtained for both compared models are presented by the scatter plots in Fig. 3. In these plots, the data points above the diagonal line correspond to the force-distance curves that were fit more accurately by the Hertz model. The data points below diagonal represent the test force-distance curves for which lower error was encountered while fitting the Sneddon model. It can be observed that the higher fitting accuracy was obtained for 13 curves by using the Sneddon model and for the remaining 7 curves by applying the Hertz model. The average RSS value equals 0.024 for Hertz model and 0.023 for Sneddon model. In case of RMSE, the average error value amounts to 0.039 for Hertz model and 0.031 for Sneddon model.

Figure 4 shows two examples of experimental force-distance curves acquired for cells and the corresponding fitted theoretical indentation models. Maximal indentation was assumed to be equal to 1200 nm. In Fig. 4 a) the detected contact point corresponds to the separation of 602 nm. The better fit was achieved for the Hertz model (RSS = 0.006 nN^2 for Hertz model, and RSS = 0.036 nN^2 for Sneddon model). An opposite situation is shown in Fig. 4 b, where the lower fitting error was obtained by using Sneddon model (RSS = 0.035 nN^2 for Hertz model, and RSS = 0.001 nN^2 for Sneddon model). In this example, the contact point was detected at separation of 1477 nm.

The resulting values of YM for the 20 analyzed force-distance curves are presented in Fig. 5. The results are compared for both considered indentation models. A general observation is that the values of YM obtained for Hertz model are higher than those extracted from Sneddon model. The average YM equals 0.0057 MPa for Hertz model and 0.0041 MPa for Sneddon model.

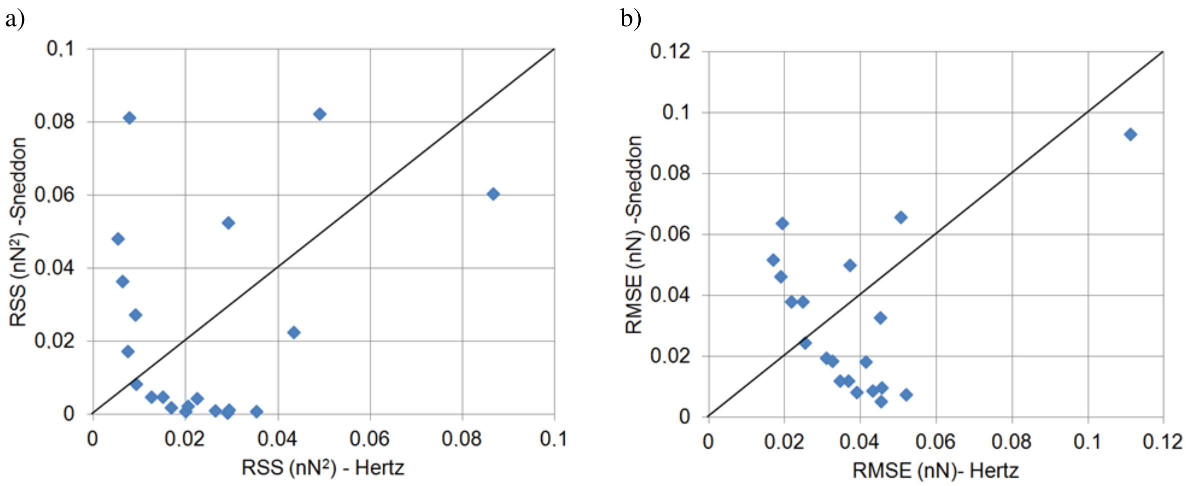

Fig. 3. Comparison of curve fitting errors for Hertz and Sneddon models: a) RSS, b) RMSE.

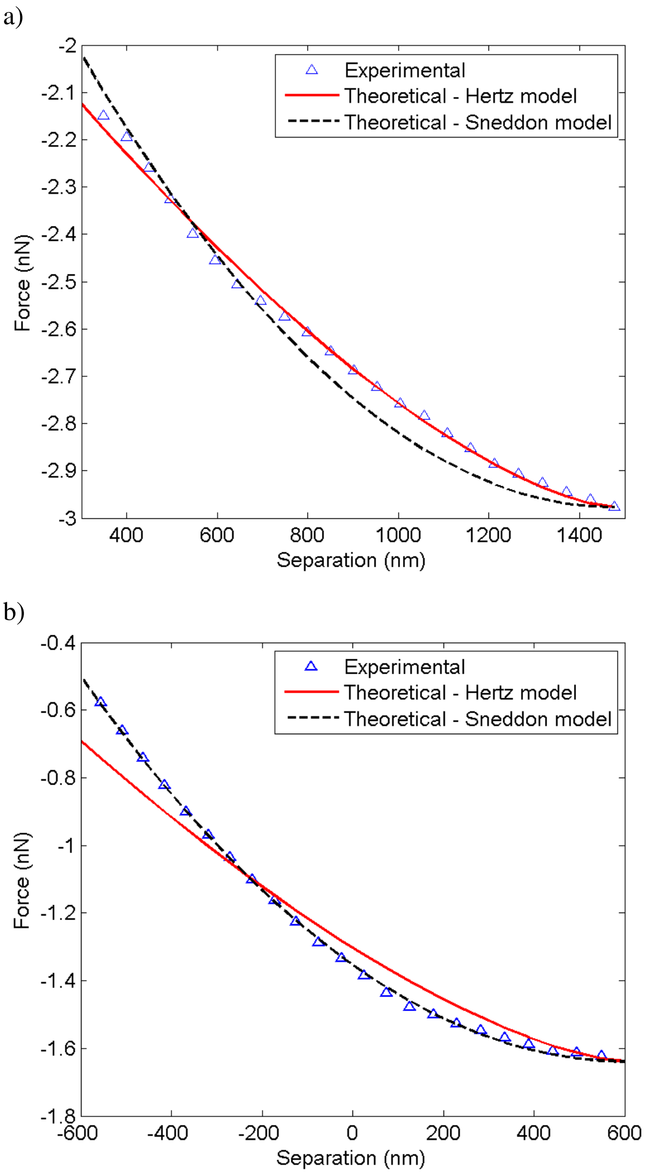

Fig. 4. Fitting theoretical indentation models to experimental force-distance curves.

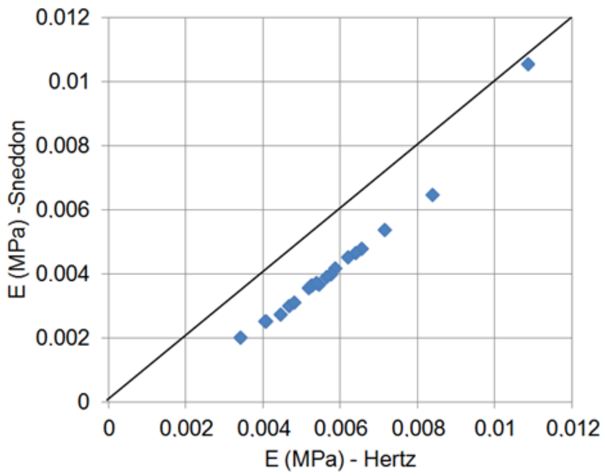

Fig. 5. Comparison of Young's modulus evaluated for Hertz and Sneddon models.

4. CONCLUSIONS

The results presented in this paper show that the AFM force-distance curves acquired during experiments conducted on NHDF cells vary significantly. Thus, when using single indentation model, a satisfactory fitting accuracy can be obtained only for a subset of the curves. In order to ensure accurate evaluation of the YM for cells, multiple indentation models can be used. Different indentation models can be fitted to one curve and then the most appropriate model can be selected based on the fitting error measures.

In this study the two most popular indentation models were taken into account. Application of the other available models will be considered in future research with a larger dataset. Moreover, further experiments will be necessary to test more sophisticated algorithms for automatic detection of contact point in force-distance curves.

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