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Modeling and Analysis of Mechanical Properties of Single Cells

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***Abstract*—Cells can be regarded as a complex network, which contains thousands of criss-cross signal pathways. The mechanical properties of a cell reflect the structure and composition of its cytoskeleton and are closely related to the cellular biological functions and physiological activities. In this study, we develop a dynamical model with cellular viscoelasticity properties as the system parameters to describe the stress-relaxation phenomenon of a single cell under the indentation of atomic force microscope (AFM). The system order and parameters were identified and analyzed. The parameters identified with this model represent the cellular mechanical elasticity and viscosity respectively and can be used to classify cell types and discriminate cellular state.**

Keywords—single cell; dynamic model; mechanical properties; principal component analysis; atomic force microscopy; viscoelasticity

I. INTRODUCTION

Cells are the basic component units of organisms and contain the important and abundant biological information. The complete genetic information of humans can be obtained from a single cell [1]. On the other hand, a cell is a complex network, which contains thousands of criss-cross signal pathways. Traditional Method to describe this dynamics of the network is extremely complicated. Zhang et al. describe the local dynamic behaviors of a cellular signal network by using 23 equations and 82 parameters [2]. Otte et al. use a 118-equations-model with 177 parameters to describe the dynamics of ion channels [3]. Moreover, it is difficult to measure the variation of the chemical components on the pathways of the network on a living cell. Therefore, it is hard to analyze the global properties of cells via the underlying mechanism, let alone how to control.

Mechanical properties of a cell are closely related to a cell's life activities such as cell growth, cell division, cell movement and cell adhesion, and reflect the structure and composition of cytoskeleton. Recently, some researches show that the variation of mechanical properties of cells is associated with the emergence and development of human disease. Many diseases, taking cancer as an example, can drastically affect cell's mechanical properties at cellular level [4]. The development of nanotechnologies, including atomic force microscope (AFM) [5], magnetic and optical tweezers [6], and so on, enables the measurement of mechanical properties on single living cells. So the cellular mechanical information can be utilized as a label-free biomarker for cell recognition [7], early diagnosis of disease and drug efficacy evaluation [8], and the study on the mechanical properties of single cells may provide a potential method for detecting abnormal cells, early diagnosis of serious disease and drug screening. Therefore, it is important to measure and quantitatively describe the mechanical properties of a single cell using a mathematical model.

In order to acquire the mechanical properties with the AFM technology, kinds of theories and models are used to calculate the mechanical performance indexes, commonly Young's modulus. In the indentation process, the tip of a AFM probe will detect the specimen and will measure the relationship between the force and the depth of indentation. Hertz model [9] is a widely used model to describe the mechanical properties of a single cell. Based on Hertz model, the theoretical relationship between the force and the depth of indentation depends on the probe shapes, including pyramid [10], cone [11] and sphere [12]. However, many issues of Hertz model still exist and need to be addressed. For example, Hertz model, with the assumption of linear elastic for cells, cannot explain the dynamic behavior commonly observed on living cells [13, 14]. A.H.W. Ngan et al. developed the so-called rate-jump approach to successfully evaluate the mechanical parameters of viscoelastic materials analytically [15]. The rate-jump method has been extensively and effectively used to estimate viscoelasticity of living cells [16, 17]. And, considering the high complexity of the viscoelastic parameters evaluation, analytical solution is not yet accurate enough for the characterization of living cells' mechanics. Recently, Wei et al. developed a rectification approach based on the finite element simulation with cells material assumed to be viscoelastic and acquired the viscoelastic parameters that reflect the actual dynamical mechanics of cells [18]. This finite element simulation-based approach can obtain the parameters of viscoelastic model accurately, but cannot obtain an analytical solution so that the characteristic of the system cannot be reflected roundly.

In general, the more complicated a system, the harder to model by using mechanical approaches. Even though with the mechanical model, it is difficult to analysis the system because of the complexity due to the high order and nonlinearity. However, an input-output-based view will make the modeling and analysis convenient, and more properties of the system will be easy to obtain from the input-output view. In present work, we develop a modeling method based on viscoelastic model to describe the mechanical properties of a single cell. The order and parameters of the dynamic system are determined by system identification methods. We also use the principal component

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analysis to reduce the dimension of parameter space, and then we use the principal components classify different kinds of cells. Furthermore, this work provides a method for detecting abnormal cells and early diagnosis of serious disease.

II. METHOD

In this section, the experiment process of how to obtain the input and output curves is to be given firstly. Then, the process of modelling the mechanical properties of a single cell are described, and we perform mechanical parameters extraction under the viscoelastic assumption.

A. *Indentation Process*

Main idea of the input-output-based modelling is to describe the system with appropriate external signals instead of interior structure of the system. We use the distance AFM tip pushing down as the system's input, and the output is the force that AFM measures. Schematic of stress relaxation experiment is shown in Fig. 1. In stress relaxation experiment, probe, which is located just above cell, travels towards the cell at a speed of 4 μm/s, and then keeps still on the cell after the predefined peak force is reached.

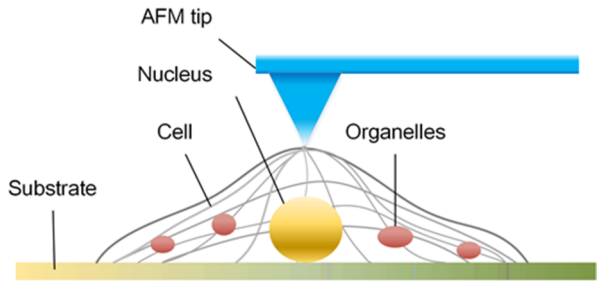

Fig. 1. Schematic diagram of using an AFM tip to push a single cell.

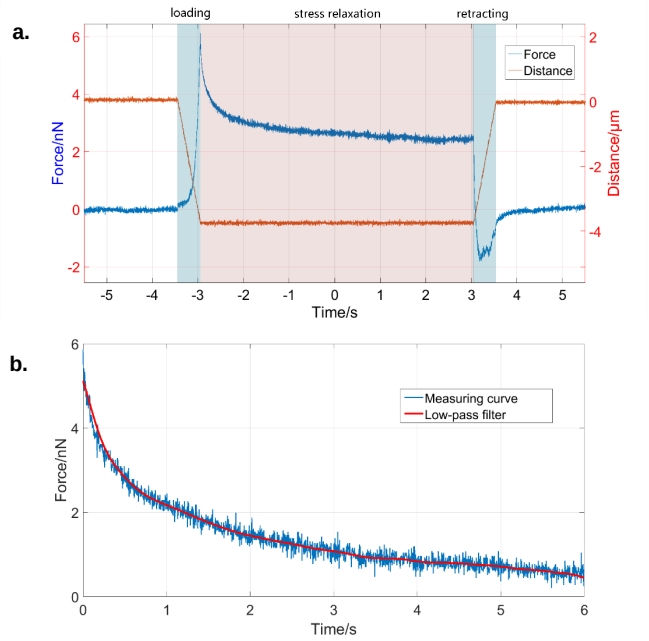

Fig. 2. Illustration of the stress experiment and one whole F–t curve obtained. (a) shows the stress relaxation experiment curves (blue) and AFM probe press curve (red). (b) The stress relaxation part and low-pass filter of the stress relaxation curve.

Fig. 2 (a) shows the as obtained force-z position-time curve. It obviously includes three stages of indentation process, i.e., loading, stress relaxation and retracting, respectively. Before the loading stage, both the distance of AFM tip pushing down and the force are zero. During the loading stage, the distance of z position decrease with a constant speed and the force AFM measured increase rapidly, and in the stress relaxation part, the distance is staying and the force decrease slowly. In the retracting stage, the distance of z position increase with a constant speed and the force decrease rapidly. As shown in Fig. 2 (b), the force-time curve of relaxation stage that AFM measures can be considered approximatively as the step response curve of the system. To avoid the measurement noise from AFM, a low-pass filter with 10 Hz cut-off frequency is used (red curve in Fig.2 (b)).

B. *Viscoelastic model of a single cell*

As we mentioned above, the distance that AFM tip push the cell down is system's input $u(t)$, and the system's output $y(t)$ is the force on the cellular surface detected by AFM. The cellular mechanical properties can be modeled by Maxwell model for viscoelastic material in the indentation progress shown in Fig. 3 [18]. A spring k_0 and n spring-damping paths are in parallel, and each path is constituted by a spring and a damping in cascade. Then we can write the state-space equation of Maxwell model in the following.

$$\begin{cases} \dot{x}_i = -\frac{k_i}{b_i}x_i + \frac{k_i}{b_i}u\,, \; i = 1,2,\dots,n \\ y = -\sum_{i=1}^{n} k_i\, x_i + (k_0 + \sum_{i=1}^{n} k_i)u \end{cases} \tag{1}$$

where x_i is the moving distance of the point between spring and damping in the ith path. The state-space equation (1) completely describe the mechanical properties of the cell under assumptions of Maxwell model for viscoelastic material.

It can be seen from this model that the elasticity of cells can be represented by the parameters k_i, and the viscosity can be represented by the parameters b_i. It is worth pointing that the nonlinear of cellular elasticity and viscosity can be produced by different combinations of different parameters. Then, the order of the system n and the parameters will be determined by a system identification approach later.

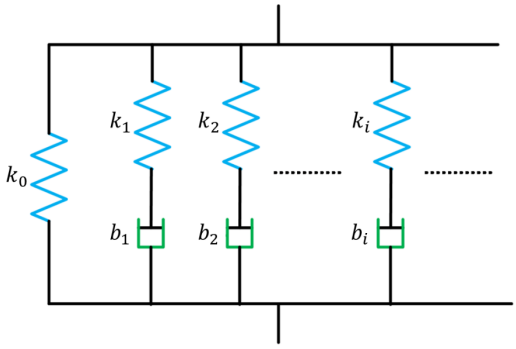

Fig. 3. An n-order equivalent mechanical models for viscoelastic material of a single cell.

C. Order and Parameters Identification

We have determined the structure of the system, and we are going to determine the order and parameters of systems by a system identification approach. System identification determines the mathematical model which describes the behavior of the system by using the input and output functions [19, 20, 21]. Here, we use a classic system identification approach named Hankel matrix method to determine the system's order. Traditionally, one identifies from input-output data the Markov parameters from which the Hankel matrix is built. We have obtained the step response sequence as we mentioned above, and the impulse response sequence of the system can be obtained by calculating the difference of step response sequence. The impulse response sequence is denoted as $\{g(i)|i = 1, 2, \dots, L\}$. Construct Hankel matrix as follows

$$H(l,k) = \begin{bmatrix} g(k) & g(k+1) & \cdots & g(k+l-1) \\ g(k+1) & g(k+2) & & g(k+l) \\ \vdots & & \ddots & \vdots \\ g(k+l-1) & g(k+l) & \cdots & g(k+2l-1) \end{bmatrix}$$

Where l determines the dimension of Hankel matrix, and k is any integer and $k \in [1, L-2l+2]$. If $l > n_0$ (the real order of the system), the rank of the Hankel matrix equals n_0, i.e.

$$rank[H(l,k)] = n_0, l \geq n_0, \forall k \tag{2}$$

Because of the impulse response sequence includes noises, so the average ratio of the determinant of Hankel matrix D_l is used to judge that the Hankel matrix is singular or not. Where

$$D_l = \frac{\frac{1}{L-2l+2}\sum_{k=1}^{L-2l+2} \det[H(l,k)]}{\frac{1}{L-2l}\sum_{k=1}^{L-2l} \det[H(l+1,k)]} \tag{3}$$

Let l gradually increase from 1, and when D_l reaches a maximum, the l can be considered as the order of the model. Once we obtain the order of dynamic system of a single cell, the parameters of state-space equations can be easily determined by least square method. The system identification toolbox of Matlab can easily use to determine the parameters by least square method.

Up to now, we have introduced a method on how to obtain the structure and parameters of a single cell by the input-output response curves. These dynamic equations can describe the mechanical properties of a single cell completely in the viscoelastic assumptions.

III. Results

In this section, an example and an application of the method mentioned above will be given. The experimental material we used are four different kinds of cells, namely MCF-7, Hek293, L929 and Neuro 2A. The system order is identified using the Hankel matrix method and order 2 is selected for these four kinds cells, and then 5 parameters in the second-order system are identified by the least square method. The solution of the system can be easily calculated as follows.

$$y(t) = k_0 u(t) + u(t) k_1 e^{-\frac{k_1}{b_1}t} + u(t) k_2 e^{-\frac{k_2}{b_2}t} \tag{4}$$

The system parameters of the average of example cells are shown in Table 1. Twenty cells for each kind of cell are selected and two indentation process are made for each cell. The vector of these 5 parameters represents the elasticity (k_i) and viscosity (b_i) properties of a single cell respectively and therefore can be used to classify cell types and discriminate cellular state.

However, the 5 parameters k_0, k_1, b_1, k_2, b_2 in the second order system are not completely independent due to the fact that the physical elasticity and viscosity properties of a single cell usually co-exist and are highly correlated. Therefore, in this study, the principal component analysis (PCA) method is used to reduce the dimension of parameter vector. The main idea of PCA is to calculate the eigenvalue of covariance matrix of sample matrix. The first principle component owns the biggest eigenvalue so it has the biggest distinction degree. We calculate the sample matrix, that we mentioned above, with the dimension 160×5, and find that the eigenvalues of the first two principle components are bigger than 1 and the total contribution rate of the first three principle components equals to 96.35%. It means that the first three principle components include about 96.35% information of all the five parameters (Table 2). The first three principle components of the four different kinds of cells are shown in Fig. 4. It can be obviously seen from Fig. 4 that different kinds of cells present different clustering patterns and this method provides a label-free biomarker for cell recognition. MCF-7 and Neuro 2A have a more intensive cluster patterns than the other two kinds of cells. The causes of this phenomenon maybe the different shape of cells. As shown in Fig.5, different MCF-7 and Neuro 2A cells have similar shapes respectively, and the Hek 293 and L929 cells do not have fixed shapes. We can use this method to classify different kind cells, also, cells with different states, for example, normal cells and pathological cells. Moreover, effects of drugs on cells can be evaluated with this method.

TABLE I. FOUR DIFFERENT KINDS OF CELLULAR PARAMETERS

Cellular Types	Elastic Parameters			Viscosity Parameters	
	k_0	k_1	k_2	b_1	b_2
MCF-7	2.984	1.110	1.385	0.118	3.108
Neuro 2a	2.425	1.519	1.259	0.242	1.934
Hek 293	1.099	0.472	0.609	0.158	1.956
L929	0.534	0.195	0.235	0.0124	0.374

TABLE II. THE RESULTS OF PCA OF CELLULAR PARAMETERS

Principle component	Eigenvalue	Difference value	Contribution rate	Total
1st	3.3158	2.2298	66.3153	66.3153
2nd	1.0860	0.6685	21.7197	88.0351
3rd	0.4175	0.2631	8.3498	96.3849
4th	0.1544	0.1281	3.0884	99.4733
5th	0.0263	-	0.5267	100

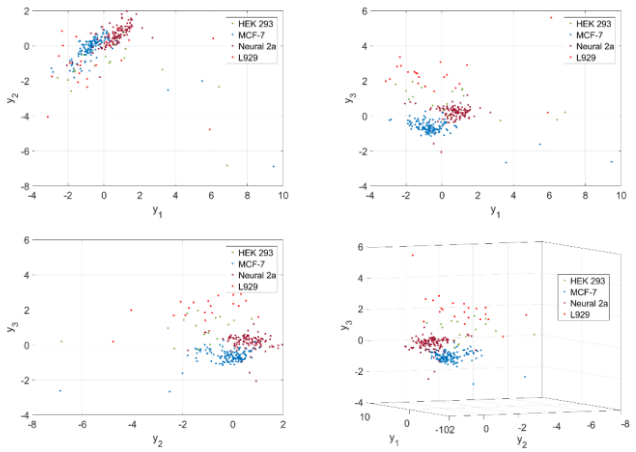

Fig. 4. Principle component analysis of four kinds of cells. These four pictures are the first component to the second component, the first component to the third component, the second component to the third component, and distribution of different cells in these three components space.

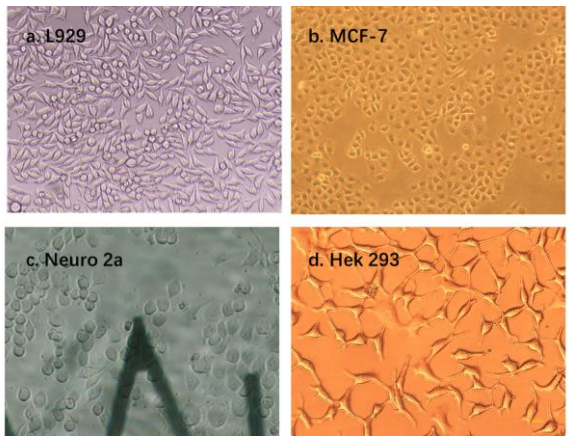

Fig. 5. Different geometry of four different kinds of cells.

IV. Conclusion

To be conclude, we presented a modelling method for the mechanical properties of a single cell and an application of this method, which classify different kinds of cells based on a principle component analysis of the parameters of cellular dynamic model. The cell's viscoelastic parameters model can be obtained from experimental stress relaxation curves. The 5 parameters k_0, k_1, b_1, k_2, b_2 in the second order system can describe the mechanical properties in terms of elasticity and viscosity. Then, by means of PCA, a classification of different kinds of cells was given. Finally, we demonstrated that the parameters identified with this model represent the cellular mechanical properties and can be used to classify cell types and discriminate cellular state. The future work on the effects of different drugs on cells are under the authors' investigation.

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