A Dynamic Feedback Algorithm of AFM Based on Cell Morphology Changes

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Abstract—The atomic force microscope (AFM) used in biological research as a powerful tool has been for many years. However, the imaging of living cells is still a problem as the sample is too high and too soft to obtain their accurate morphologies. Especially in the high-speed scanning mode, AFM does not have enough judgments to make accurate measurements at the down part of the sample. In this study, we propose an improved control method to improve the image quality of living human colon cancer cells (SW480) especially in the down part during the scanning.

Keywords—AFM, Living cell imaging, Control method, Cell morphology

I. INTRODUCTION

The atomic force microscope (AFM) has gained lots of important applications in the biological field [1-3] because it is a suitable character for providing the topography of biological samples at nanometer resolution in their natural aqueous environments. With the great potential of the AFM, worldwide scholars have used it to study the living cells extensively, and obtained a great amount of knowledge. Researchers are increasingly aware of the essence of life activities and AFM provides a new method for diagnosing diseases and drug discoveries. Thus, AFM has become an important tool in the biological field since it appeared more than 30 years ago. AFM not only can obtain the high-resolution image of the sample but also can measure many different physiological parameters, such as the mechanical properties, topographic parameters of cells and the forces between receptors and ligands [3-8]. Obtaining the topography of biological samples is still the general application in the biological filed by AFM. However, for the complicated biological features of living cells and the time-delay processes of AFM, it is still a difficult problem to get a good map of a living cell. In this work, we present a method to control the AFM developed by ourselves in our lab in order to obtain a clear topography map of SW480 cells (established from a primary adenocarcinoma of the colon).

II. SAMPLE AND METHODS

A. Sample Preparation

Human colon cancer cells (SW480) were used in this study. SW480 cells were seeded on sterilized 20 mm coverslips and kept in an 8-well microplate which was filled with the culture medium. For the purpose of nature aqueous environment, SW480 cells were maintained at 37°C in a 5% CO₂ incubator. In the incubator, SW480 cells would stay for 24-48 hours prior to measurements. During the experiment, we flushed out the dead cells using the pure culture medium, and mounted the coverslips on a cell filled with the culture medium. Then put the cells on the AFM stage to perform the measurements.

B. Methods

It is known that AFM can obtain topographic images using a tip attached to the end of a cantilever to scan the surface of sample, based on the beams that reflected from the cantilever and form a feedback loop (PI control) vertically moving an actuator to keep the interaction forces between the tip and the sample in a constant deflection [9-10]. The most important force in the interactions between the tip and the sample is Van der Waals force, and its empirical equation of the energy is [11]

\[ U = \frac{B}{r^{12}} - \frac{A}{r^6} \]  

(1)
Fig. 1 Principle of measuring the cell using AFM. (A) measuring the cell in the force curve mode; (B) the curve of the cantilever’s deformation by distance.

where A and B are interaction parameters. The first term describes the repulsive exchange interaction, and the second term the attractive van der Waals force. r is distance between the atoms of interests. From Equation (1), we can get the Van der Waals’ force [11-13]. Then the change of long-range force and short-range force can be obtained. The long-range force \( F_{\text{att}} \) is [11]

\[
F_{\text{att}} = -\frac{AR}{6d^2}(1-3\frac{d^2}{H^2})
\]  

where \( R \) is the curvature radius of the tip, \( d \) is the distance between the tip and the sample, and \( H \) is the height of the tip. The short-range force \( F_{\text{rep}} \) is [11]

\[
F_{\text{rep}} = 4\pi^2Bn_1n_2r_0^4R(1+\frac{\Delta d}{r_0})\exp\left(-\frac{d+\Delta d}{r_0}\right)
\]  

where \( d \) is the distance between the sample and the cantilever which is not curved, \( \Delta d \) is the deformation quantity of the tip, \( r_0 \) is the radius of interaction, \( n_1 \) is the atomic density of the sample, and \( n_2 \) is the atomic density of the tip. Thus, the total force between the tip and the sample is [11]

\[
F_{\text{tot}} = 12\pi^2\frac{BN}{d^3} - \frac{BR}{6d^2}(1-3\frac{d^2}{H^2})
\]  

where \( N \) is the effective atomic number.

The deformation of cantilever with the distance change can be imaged from the curve (Fig.1). Through analyzing the interaction forces mentioned above, we render topographic images by directly scanning the tip over the sample, but there are some factors affecting the precision of measurement. As in AFM a feedback loop usually is a PI measure, it is difficult to have accurate measurements for cells which have complex surfaces, especially in the down part during the scanning process [14]. When scanning the falling edge of cells, the deflection of the cantilever may at the point of A, B or C from the curve. If the distance is b or c which in the linear portion of the curve, the feedback loop can give a precise error compensation \( \Delta U(t) \) by the constant deflection:

\[
\Delta U(t) = K_p(1+\frac{T_i}{T_0})e(k)-K_p e(k-1)
\]  

where \( K_p \) is the scale factor, \( T_i \) is the integral time, and \( e(k) \) and \( e(k-1) \) are the error signals of this moment and the last one, respectively.

If the distance is a, while the deformation almost is equal to C, we cannot determine the exact position of the tip. From Fig.2 we can see when the distance of the tip is between a and c, the error compensation line is curved, so the error compensation cannot move an actuator to the right position. Especially, when the falling edge is steep, as each point of the processing time is fixed if the parameters of the feedback are not suitable, the compensation may not be enough and it can make the tip to contact the cells before the tip go to the next point. As mentioned above, these factors influence the imaging quality seriously.

To solve the above-mentioned problems, we present a new method to improve the control
mode. In the design, as the path of the tip has the rising stage and the falling stage with the morphology changes of the sample in the scanning path, we divide the scanning process into two parts: the up part and the down part (Fig.3). We use the error signal $e(t)$ to detect whether the tip is in the up or down part:

$$e(t) = \text{setpoint} - y(t)$$  \hspace{1cm} (6)

$y(t)$ is the signal of the deflection of the cantilever collected by the photodetector. If the $e(t)$ is higher than the setpoint minus $y_c$, where $y_c$ is the signal of the cantilever’s deflection in the position $c$ in Fig.1, we identify the tip is in the down part. In other case, we identify the tip is in the up part. While in the flat part of the sample, as the deflection of cantilever is small it is often identified in the up part.

In the up part, the tip always contacts with the sample where in the area between $c$ and $d$ in Fig.1, so the PI controller can satisfy the request. While in the down part, where the tip is in the area between $a$ and $c$ in Fig.1, we add some judgments before the tip goes to the next point and this will ensure that in every point the tip and the cells are contacted, so we can get more accurate topographic data of the sample. In other words, the shadow in the topographic image is disappeared after the new design implemented, instead of the shadow is the topographic image of the falling edge and the area we cannot measured before (Fig.4).

As more judgments are needed in the down part, the longer scan is also needed. To reduce the scanning time, the dynamic parameters of PI control are used in the system. The scale coefficient is proportional to the error signal $e(t)$. Once there is an error, the PI controller will reduce the deviation immediately. The integrator is mainly used to reduce the static error to improve the system accuracy. The longer of the integration period, the better of the control accuracy. When the tip is scanning in the down part, as the tremendous changes of the error signal, we can appropriately increase the proportion coefficient and the integration period [15-17]. In the design (Fig.3), the parameters of $P_a$ and $I_a$ are larger than the parameters of $P_{ap}$ and $I_{ap}$. The error signal dynamically changes the parameters of $P$ and $I$ and the method can better adapt a change of the topography and reduce the scanning time.

III. RESULTS AND DISCUSSIONS

By the method mentioned above, at first, we measured the SW480 cells which had been cured in the air environment. The scanning range is 50 μm×50 μm and the resolutions of these images are 256×256 pixels. From Fig.4 we can see that there are some shadows in the area where the tip is down from the cell in the surface image (a) by the normal PI control. Compared with the height map (d) which is calculated based on the feedback voltage by the improved PI control, the height map (c) shows that the shadow area in the surface image (a) cannot reflect the sample’s height accurately. From the curve (e) and the curve (f) can intuitively reflect the improved control method which is better.

Fig.3 The control principal diagram.

![Fig.3 The control principal diagram.](image)

Fig.4 AFM images of the SW480 cells which have been cured. (a) and (b) are the topography images of the cell, from the red circles we can see the shadows in (a) by the PI control while in (b) shadows are disappeared by the improved PI control; (c) and (d) are the height maps of the cell, from the blue circles we can see the influence of the shadows to the measurement of the height of the cell; (e) and (f) are the curves of the height of the cell at the 75th line (the red lines) in the maps of (c) and (d).

Then we used the living SW480 cells which were cultured for 24-48 hours as the sample. During the scanning we kept the sample in a cell filled with the culture medium to remain the activity. The scanning range was 80 μm×80 μm...
and the resolution of the image was 400×400 pixels. By the improved PI control, we can get the topographic image (a) and the height map (c) in Fig.5. Compared with the optical image (b) we can see that the topographic image (a) and the height maps (c) and (d) provide good representations of the topographic characteristics of the cell.

But there are some problems that affected the topographic image in the experiment. As the membranes of living cells have the fluidity and adhesion characteristics, when the tip scanning, the membranes will be deformed according the scanning path. As shown in the height maps in Fig.5, the round images in the trace map (c) and retrace map (d) illustrate the cell’s deformation. Along the scanning path the tip glued the cell membranes together when in the down area of the cell it will affect the topographic image. These problems will be further studied in future.

IV. CONCLUSION

In this paper, an improved control method is used in the AFM system developed by our lab. The main idea is adding some judgments at the down part during the scanning and dynamical changing the parameters of P and I to adapt the surface change. The results show that this method can measure cells precisely even at the down part in the scanning process.

ACKNOWLEDGMENT

This work was supported by National Key R&D Program of China (No. 2017YFE0112100), EU H2020 Program (MNR4SCell No. 734174), National Natural Science Foundation of China (No. 62175020), Jilin Provincial Science and Technology Program (Nos. 2020C022-1, 20190201287JC, 20190702002GH and 20200901011SF), Jilin Province Education Department Program (Nos. JJKH20210833KJ), and “111” Project of China (No. D17017).

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