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Indentation quantification for in-liquid nanomechanical measurement of soft material using an atomic force microscope: Rate-dependent elastic modulus of live cells

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Abstract

In this paper, a control-based approach to replace the conventional method to achieve accurate indentation quantification is proposed for nanomechanical measurement of live cells using atomic force microscope. Accurate indentation quantification is central to probe-based nanomechanical property measurement. The conventional method for in-liquid nanomechanical measurement of live cells, however, fails to accurately quantify the indentation as effects of the relative probe acceleration and the hydrodynamic force are not addressed. As a result, significant errors and uncertainties are induced in the nanomechanical properties measured. In this paper, a control-based approach is proposed to account for these adverse effects by tracking the same excitation force profile on both a live cell and a hard reference sample through the use of an advanced control technique, and by quantifying the indentation from the difference of the cantilever base displacement in these two measurements. The proposed control-based approach not only eliminates the relative probe acceleration effect with no need to calibrate the parameters involved, but it also reduces the hydrodynamic force effect significantly when the force load rate becomes high. We further hypothesize that, by using the proposed control-based approach, the rate-dependent elastic modulus of live human epithelial cells under different stress conditions can be reliably quantified to predict the elasticity evolution of cell membranes, and hence can be used to predict cellular behaviors. By implementing the proposed approach, the elastic modulus of HeLa cells before and after the stress process were quantified as the force load rate was changed over three orders of magnitude from 0.1 to 100 Hz, where the amplitude of the applied force and the indentation were at 0.4–2 nN and 250–450 nm, respectively. The measured elastic modulus of HeLa cells showed a clear power-law dependence on the load rate, both before and after the stress process. Moreover, the elastic modulus of HeLa cells was substantially reduced by two to five times due to the stress process. Thus, our measurements demonstrate that the control-based protocol is effective in quantifying and characterizing the evolution of nanomechanical properties during the stress process of live cells.

I. INTRODUCTION

In this paper, a control-based approach to indentation quantification of live cells using atomic force microscope (AFM) is proposed to replace the conventional method. The indentation-based approach to measure mechanical properties of live cells using AFM has unique advantages over other techniques, as the AFM-based technique is capable of applying force stimuli and then measuring the response at the desired location in a physiologically friendly environment, with piconewton force and nanometer spatial resolutions [1–3]. Mechanical properties of a broad variety of live cells have been studied using AFM [1–4]. The force stimuli applied and the corresponding indentation generated are the input and output to the cantilever probe-sample interaction dynamics, respectively, and the nanomechanical properties (such as Young's modulus) of the cells can be quantified from the measured force-indentation data through the tip-sample interaction model (e.g., [5–7]). Therefore, error in the indentation measurement leads directly to that in the nanomechanical property quantified, and it is crucial to accurately measure the indentation in nanomechanical studies of live cells.

Despite the wide use of AFM in measuring elasticity and/or viscoelasticity, the current method for indentation quantification using an atomic force microscope is largely erroneous for live cells. Conventionally the indentation is quantified as the difference between the cantilever displacement at its fixed end (i.e., the cantilever-base displacement), and the relative displacement of the cantilever probe with respect to the cantilever base (i.e., the cantilever deflection), after the probe comes into contact with the sample surface [5,8,9]. Such a quantification, however, is only adequate when the force load rate is rather low and can be maintained at a constant—the load rate needs to be below a couple of Hz for a wide variety of live cells ranging from red blood cells (hard) to fibroblast cells (soft). As the load rate increases and/or multifrequency excitation force is applied (to measure viscoelasticity of live cells), the relative acceleration of the cantilever probe [with respect to the fixed end of the cantilever (called the *relative probe acceleration*)] becomes pronounced and substantially effects the indentation generated. For example, the indentation caused by the relative probe acceleration can account for over 45% of the total indentation at the force load rate of 50 Hz. The relative probe acceleration is induced by the viscoelastic behavior of the soft sample, and is more pronounced when the sample, like the cells, becomes softer and more viscous. The conventional indentation measurement is also plagued by uncertainty in determining the probe-sample contact point [10] and the hydrodynamic force effect [11,12]. Although the contact-point uncertainty can be alleviated through the use of a reference sample [5], and the hydrodynamic force effect might be accounted for by quantifying it via experiment [12], these efforts are still limited to ultralow force load rate, and the relative probe acceleration dominates over the hydrodynamic force (i.e., over an order of magnitude larger) when the force load rate is higher than ~ 0.1 Hz for most live cells. Therefore, to achieve accurate indentation quantification of live cells, the conventional indentation measurement needs to be replaced.

Current indentation quantification also limits the accuracy of viscoelasticity measurement (i.e., rheology measurement) of live cells in liquid. It has been proposed to measure the viscoelasticity of live cells by augmenting a sinusoidal oscillation to the load-unload force

profile of constant rate [12,13], i.e., the *force-modulation* method. This method, however, can induce large errors and uncertainties in the modulus measured due to the issues described above in indentation quantification. Particularly, the relative probe acceleration effect is pronounced and increases substantially as the increase of the measurement frequency. Secondly, the oscillation amplitude is rather small (2–5 nm), whereas the mechanical properties of live cells are (force) amplitude-dependent [14,15], and to excite a variety of biological responses of a cell, an excitation force of much larger amplitude needs to be applied. Finally, the use of this method for live cell measurement is further limited as the oscillatory force repetitively applied at the same location tends to deform and even damage the cell membrane.

In this paper, a control-based approach to achieve accurate indentation, and hence accurate nanomechanical measurement of live cells in liquid, is proposed. The proposed approach is then employed to study the rate-dependent elastic modulus of HeLa cells before and after the nutrient-deprivation process, with a comparison to those of fibroblast cells. Particularly, the cantilever dynamics during the force-indentation measurement is analyzed by taking into account both the cantilever-probe interaction and the measurement frequency range. Based on the analysis, we propose to track the *same* excitation force profile (i.e., the same cantilever deflection) on both the soft sample (e.g., the live cell) and a hard reference, and then quantify the indentation from the displacement difference of the cantilever base on the cell with respect to that on the hard reference. The main advantage of the proposed approach is that by using a hard reference, and more critically accurately tracking the same force profile on both samples, the major adverse effect—the relative probe acceleration effect—is completely removed with no need for parameter calibration. Moreover, the hydrodynamic force effect is also substantially reduced, particularly when the force load rate increases (e.g., reduced by over 50% when the force load rate is over 100 Hz as shown in the experiment results). Accurate tracking of the cantilever deflection is achieved by using advanced control techniques (e.g., [16]). The proposed method is implemented to quantify the elastic modulus of the live HeLa cells before and after the stress process, by varying the load-unload rate of the excitation force over three orders of magnitude from 0.1 to 100 Hz, with a measured indentation amplitude over two orders larger than the oscillatory amplitude in [12].

Cell morphology serves a specialized function [17]. However, in some physiological and pathophysiological events (i.e., epithelial-mesenchymal transition), cells change their morphology due to dynamic remodeling of the internal cellular cytoskeleton in response to external physical force stimuli or internal reprogramming of gene expression profiles. More importantly, the morphological alterations are considered as important diagnostic indexes in the progression of numerous diseases such as fibrosis, cancer initiation, and metastasis. Many studies have demonstrated that the alteration of cell shapes is associated with the change of mechanical properties [18,19]; morphological alteration will affect the mechanical integrity of the cell. Conversely, the mechanical properties (i.e., stiffness) of the cell also reflect upon the dynamics of underlying molecular activities and cell behaviors [20]. Therefore, detection and quantification of cell mechanical properties will help to predict the ongoing changes of cell morphology, function, and fate.

Epithelial-mesenchymal transition (EMT) is an important biological event during embryonic development and cancer progression [21,22]. During the EMT process, epithelial cells lose adherent and tight junctions, prompting morphological alterations toward an invasive mesenchymal phenotype. However, biomarkers that quantify and predict cell predisposition to EMT are lacking. The proposed control-based technique using AFM provides the ability to quantitatively monitor the cellular viscoelastic behavior in real time [23]. In this study, we used serum starvation to treat HeLa cells, human cervical epithelial cancer cell lines, mimicking initiation of EMT and evaluating the dynamics of cell stiffness. The morphology of HeLa cells was changed toward a mesenchyme-like shape after serum deprivation for one day due to a decrease of E-Cadherin, a key component involved in the formation of an adherens junction, at the cell-cell junctions [24]. Indeed, our experimental results demonstrate that the rate-dependent elastic modulus of these stressed cells was also strikingly decreased compared to that of unstressed cells toward those of murine embryonic fibroblasts (MEF), which are mesenchymal cell lines, indicative of their acquisition of mechanical characteristics similar to that of MEFs. This quantification is consistent with the altered morphology. Thus, these findings suggest that control-based indentation and nanomechanical quantification possess a great potential to quantify and even predict cell fate during the EMT process.

II. CONTROL-BASED APPROACH TO THE ACCURATE INDENTATION MEASUREMENT OF LIVE CELLS

We start by discussing the fundamental limits of the conventional method for indentation measurement of soft materials such as live cells, particularly when the force load rate becomes high and/or the measurement frequency range is large (e.g., broadband).

A. Errors in the conventional method for indentation measurement of live cells

Without a loss of generality, we assume that, during the force-indentation measurement of live cells (or more generally, soft material with elastic or viscoelastic properties of similar range), the following assumptions hold:

Assumption 1. The vibration modes of the cantilever are not excited.

Assumption 2. A continuous and stable probe-sample contact is maintained.

Assumption 3. The amplitude of the probe vibration on the sample surface is much smaller than the length of the cantilever.

Assumption 1 holds as the measurement frequency range in nanomechanical property measurement of live cells (usually within a few hundred Hz) is at least several tens of times smaller than the first resonant frequency of the cantilever in liquid. Assumption 2 also holds as the tip-sample interaction force is dominated by the repulsive electrostatic force during the nanomechanical measurement. Thus, the repulsive tip-sample interaction is proportional to the AFM cantilever deflection [5,25], i.e.,

$$F_z(t) = k_{eqv} [z_e(t) - z_b(t)] = -k_{eqv} d(t), \quad (1)$$

where k_{eqv} is the equivalent stiffness of the cantilever in liquid (see Fig. 1), and z_e and z_b are the cantilever displacement at the free end and that at the base (i.e., the fixed end), respectively. Thus, $d(t)$, measured as the cantilever deflection, is the relative displacement of the cantilever probe with respect to the cantilever base.

Finally, Assumption 3 holds as the displacement of the cantilever free end relative to its base is usually over two orders smaller than the length of the cantilever (in the range of 100–300 μm), i.e., the bending angle (θ in Fig. 1) is rather small and $\sin \theta \approx \theta$.

By modeling the probe-sample interaction as a spring-dashpot system under Assumptions 1–3 (see Fig. 1), the dynamics model of the rotational motion of the cantilever with respect to its fixed end is

$$J\ddot{\theta}(t) = Lk_{\text{eqv}} [z_{es}(t) - \Delta_z(t)] - M_{hs}(t), \quad (2)$$

where $z_{es}(t)$, J , L , and $\Delta_z(t)$ are the displacement of the cantilever free end during the tip-sample interaction, the inertia of the cantilever relative to its fixed end, the cantilever length, and the indentation in the soft sample, respectively. Moreover, in Eq. (2), $Lk_{\text{eqv}}[z_{es}(t) - \Delta_z(t)]$ is the moment acting on the cantilever caused by the tip-sample interaction, and $M_{hs}(t)$ is the moment due to the hydrodynamic force exerting on the cantilever beam. As during the in-liquid force-indentation measurement, the Reynolds number Re is very small ($Re \ll 1$) (for the cantilever velocity is at most tens of $\mu\text{m/s}$) [5,12], the hydrodynamic force is proportional to the velocity of the cantilever, i.e.,

$$M_{hs}(t) = \int_0^L C_d w x v_s(x, t) dx, \quad (3)$$

where C_d and w are the drag coefficient of the liquid and the width of the cantilever, respectively, x and $v_s(x, t)$ denote a point on the cantilever with respect to the fixed end and the velocity of the cantilever at that point, respectively. Previous work in [5,12,26] has shown that for AFM cantilever operations on live cells, the hydrodynamic-force-generated moment $M_{hs}(t)$ can be adequately approximated by that of an equivalent force $F_{hs}(t)$ exerting at the free end of the cantilever, i.e., $M_{hs}(t) = LF_{hs}(t)$, where the equivalent-hydrodynamic force $F_{hs}(t)$ depends on the absolute velocity of the cantilever, which, in turn, can be written as the summation of the velocity of the indentation and that of the cantilever deflection, i.e.,

$$M_{hs}(t) \approx LF_{hs}(t), \quad \text{with} \quad F_{hs}(t) = \mathcal{C}_d [\dot{d}_s(t) + \dot{\Delta}_z(t)], \quad (4)$$

where $\mathcal{C}_d$ is the equivalent drag factor of the liquid—independent of the tip-sample interaction [26,27] (for cell culture media, $\mathcal{C}_d$ ranges between 0.2 and 0.5 $\mu\text{N s/m}$ [5]).

By the small motion assumption (Assumption 3), i.e., $\alpha \sin \theta \approx \alpha \theta \approx \frac{d}{L}$ (α is a cantilever-shape-dependent constant, e.g., $\alpha = 2/3$ for a rectangular-shaped cantilever), and invoking

the definition of cantilever deflection, $d(t) = z_b(t) - z_e(t)$ [see Eq. (2)], the above Eq. (2) can be rewritten in terms of deflection as

$$\frac{1}{\alpha L} J \ddot{d}_s(t) = L k_{\text{eqv}} [z_{bs}(t) - d_s(t) - \Delta_z(t)] - M_{hs}(t), \quad (5)$$

where $z_{bs}(t)$ is the cantilever base displacement on the soft sample. Therefore, the indentation in the soft sample, $z(t)$, can be computed as

$$\begin{aligned} \Delta_z(t) &= z_{es}(t) - \frac{J}{\alpha L^2 k_{\text{eqv}}} \ddot{d}_s(t) - \frac{1}{L k_{\text{eqv}}} M_{hs}(t) \\ &= z_{bs}(t) - d_s(t) - \frac{J}{\alpha L^2 k_{\text{eqv}}} \ddot{d}_s(t) - \frac{1}{k_{\text{eqv}}} F_{hs}(t). \end{aligned} \quad (6)$$

In the conventional method (e.g., [5,26]), the indentation is measured as the displacement difference between the base and the free end of the cantilever on the soft sample, i.e.,

$$\hat{\Delta}_z(t) = z_{bs}(t) - d_s(t), \quad (7)$$

where $\hat{z}(t)$ denotes the indentation measured by the conventional method. Thus, Eq. (6) implies that the conventional method [Eq. (7)] is valid only when the effects of both the relative probe acceleration and the hydrodynamic force are small and negligible, i.e., $\ddot{d}_s(t) = 0$ and $F_{hs}(t) = 0$. These two effects are negligible, however, only when the force load rate is low and maintained at constant during the force-indentation measurement. Thus, the conventional method becomes erroneous and should not be applied when measuring the viscoelastic behavior of soft material (including live cells) where an excitation force of multifrequencies is applied, i.e., $\ddot{d}_s(t) \neq 0$, particularly as the excitation frequency increases. Even when the base displacement of the cantilever is maintained at a constant rate, $\dot{z}_{bs}(t) = k_l$ with $k_l > 0$ a constant, the relative probe acceleration, due to the probe-sample interaction that leads to indentation in the soft material, is nonzero unless the load rate k_l is very small.

To be more concrete, the dynamics relating the cantilever-base displacement to the cantilever deflection is analyzed. First, note that by the force balance at the contact point, the indentation dynamics is given by (see Fig. 1)

$$m_s \ddot{\Delta}_z(t) + \xi_s \dot{\Delta}_z(t) + (k_s + k_{\text{eqv}}) \Delta_z(t) = k_{\text{eqv}} z_{es}(t) = k_{\text{eqv}} [z_{bs}(t) - d_s(t)], \quad (8)$$

where m_s , k_s , and ξ_s are the effective mass, the equivalent spring constant, and the damping coefficient of the sample involved in the probe-sample interaction, respectively.

$$G_{z2d}(s) = \frac{D_s(s)}{Z_{bs}(s)} = \frac{m_s s^2 + \xi_s s + k_s}{\frac{J}{\alpha L^2 k_{\text{eqv}}} m_s s^4 + \frac{J}{L^2 k_{\text{eqv}}} \xi_s s^3 + \left(\frac{J}{\alpha L^2 k_{\text{eqv}}} k_s + m_s \right) s^2 + \xi_s s + (k_{\text{eqv}} + k_s)}, \quad (9)$$

where $D_s(s)$ and $Z_{bs}(s)$ are the Laplace transforms of $d_s(t)$ and $z_{bs}(t)$, respectively. The above Eq. (9) is obtained by taking the Laplace transform of both Eqs. (6) and (8), and eliminating the indentation term.

To quantify the $G_{z2d}(s)$ dynamics in Eq. (9), we set, $\alpha = 1$, and based on the values reported in the literature for soft and hard mammalian cells [5,28], $m_s \in (34.7, 0.5)$ pg, $\xi_s \in (0.4, 0.007)$ Ns m⁻¹, and $k_s \in (0.003, 0.24)$ N/m, respectively. Using these parameter ranges and accounting for the mechanical properties of live cells (e.g., the largest mass and damping ratio are accompanied with the smallest spring constant [5]), the upper and lower bounds of the frequency response of $G_{z2d}(s)$ can be obtained, as shown in Fig. 2. It can be seen that even for hard cells (e.g., human red blood cells with Young's modulus ~ 70 kPa [29]), $G_{z2d}(s)$ is constant only for frequencies below ~ 10 Hz. This implies that for conventional indentation measurement to be valid, the load rate of the triangle force profile—used in usual force indentation measurement—needs to be below 2–3 Hz. This load rate limit becomes extremely small when the sample becomes softer—below 0.01 Hz for fibroblast cells with Young's modulus ~ 1 kPa. Thus, it is evident that the relative probe acceleration effect is pronounced in nanomechanical measurement of a broad range of soft materials, including almost all live cells—The conventional indentation measurement is largely erroneous for these soft materials.

To simplify the discussion but without loss of generality, the dynamics from the cantilever-base displacement to the cantilever deflection can be described in the frequency domain as below, by ignoring the hydrodynamic force and treating the parameters as constants (as will be discussed immediately below, the parameter variations can be accounted for by considering the variations of the frequency responses thus caused),

Next we discuss that for live cell measurement, the hydrodynamic force effect [12,27,30] on indentation quantification is much less than the relative probe acceleration effect. It can be estimated by using Eq. (4) that for force load velocity usually employed in nanomechanical property measurement of live cells ranging between 0.05 and 60 $\mu\text{m/s}$ (for a load rate between 0.1 and 100 Hz), the hydrodynamic force is within the range of 0.025–30 pN.

However, the equivalent force due to the relative probe acceleration, $\frac{J}{L^2}\ddot{d}_s(t)$, is around 10 pN–1 nN, more than 30 times larger than the hydrodynamic force. Thus, it is clear that the relative probe acceleration has a dominate impact on the indentation measurement of soft material including live cells.

In summary, the conventional indentation measurement fails to account for both the relative probe acceleration and the hydrodynamic force effects, therefore it is largely erroneous for not only most elastic modulus measurements (with constant load rate), but also broadband nanomechanical measurements (with a multifrequency excitation force) of soft materials in liquid.

B. A Control-based method for accurate indentation measurement of live cells in liquid

We propose a control-based approach that overcomes the limits of the conventional method. Specifically, the proposed approach amounts to (i) using a hard reference sample, and (ii)

tracking the *same* excitation force profile (i.e., the same cantilever deflection) on both the live cells and the hard reference.

Note that although by Eq. (6), the relative probe acceleration effect, $\frac{J}{\alpha L^2} \ddot{d}_s(t)$, might be accounted for directly by using the measured cantilever deflection signal $d_s(t)$, significant uncertainties can be induced, as it is very challenging (if not completely impossible) to accurately calibrate and quantify the inertia of the cantilever, J , and the effective spring constant, k_{eqv} , as both parameters depend on the cantilever geometric configuration and the probe geometry. Moreover, the calibration process itself is time-consuming and prone to external disturbances. Instead, the proposed approach removes the relative probe acceleration effect with no need to calibrate or quantify these parameters. As the indentation becomes negligible (compared to that on the live cell) on the hard reference sample (e.g., silicon, with elastic modulus several orders higher than that of the live cell), the dynamics of the cantilever during in-liquid probe-sample interaction on the hard reference is reduced to (see Fig. 3)

$$\frac{J}{\alpha L} \ddot{d}_h(t) = L k_{eqv} z_{eh}(t) - M_{hh}(t), \quad (10)$$

where the subscript “ h ” denotes the variables with respect to the hard reference sample.

Combining Eq. (6) with (10) yields the indentation as

$$\begin{aligned} \Delta_z(t) &= z_{es}(t) - z_{eh}(t) + \frac{J[\ddot{d}_h(t) - \ddot{d}_s(t)]}{\alpha L^2 k_{eqv}} + \frac{M_{hh}(t) - M_{hs}(t)}{L k_{eqv}} \\ &= z_{bs}(t) - z_{bh}(t) + d_h(t) - d_s(t) + \frac{J[\ddot{d}_h(t) - \ddot{d}_s(t)]}{\alpha L^2 k_{eqv}} + \frac{[F_{hh}(t) - F_{hs}(t)]}{k_{eqv}}. \end{aligned} \quad (11)$$

Equation (11) implies that the relative probe acceleration can be completely removed by ensuring accurate tracking of the *same* desired cantilever deflection trajectory on both the live cell and the hard reference, i.e., $d_h(t) = d_s(t)$, and thereby the indentation is given by

$$\Delta_z(t) = z_{bs}(t) - z_{bh}(t) + \frac{[F_{hs}(t) - F_{hh}(t)]}{k_{eqv}}. \quad (12)$$

As discussed in Sec. II A, the contribution of the hydrodynamic force to the indentation is rather small (at most a few nm, compared to a few hundred nm of indentation in usual live cell measurement [5,31]). Moreover, Eq. (12) shows that the hydrodynamic force effect is further reduced as only the difference of the hydrodynamic force (soft versus hard sample) contributes to the indentation. To quantify the reduction, we estimated the relative hydrodynamic force difference [i.e., the ratio of $F_{hs}(t) - F_{hh}(t)$ to $F_{hs}(t)$] by using Eq. (4) and the cantilever displacement data obtained on a fibroblast cell, as shown in Fig. 4 as a function of force load rate. It is clear from Fig. 4 that the hydrodynamic force effect is reduced by over 45% when the force load rate is higher than 50 Hz (corresponding to a force load speed of 30 $\mu\text{m/s}$ with a total piezo displacement of 460 μm), and even more as the force load rate increases further, pointing to an advantage of the proposed approach for

nanomechanical measurement of live cells with relatively high load rate (e.g., a few hundred Hz).

Therefore, the proposed approach not only significantly improves the accuracy of indentation measurement on soft materials like live cells in liquid, but it is also not limited to force measurement of constant drive rate only, and equally applicable to broadband nanomechanical measurement as well, i.e., the excitation force profile can be virtually chosen freely as any signal of a bounded time derivative, e.g., a band-limited white noise for rapid broadband nanomechanical characterization [32,33].

The key to this control-based indentation quantification and nanomechanical measurement is to ensure precision tracking of the same excitation force profile, i.e., the same cantilever deflection trajectory, on both the live cell and the hard reference. We propose to implement the modeling-free inversion-based iterative learning control (MIIC) technique [34] to ensure precision tracking of the *same* desired cantilever deflection trajectory. Specifically, the control input applied to drive the AFM z -axis piezo is obtained through iteration as follows (see Fig. 14):

$$u_1(j\omega) = \alpha d_d(j\omega), \quad k=1, \\ u_{k+1}(j\omega) = \begin{cases} \frac{u_k(j\omega)}{d_k(j\omega)} d_d(j\omega) & \text{when } d_k(j\omega) \neq 0 \text{ and } d_d(j\omega) \neq 0, k \geq 1, \\ 0 & \text{otherwise,} \end{cases} \quad (13)$$

where $d_d(j\omega)$ is the desired output trajectory (i.e., the cantilever deflection measured on live cells), α is a constant, and $u_k(j\omega)$ and $d_k(j\omega)$ are the current input (e.g., the input voltage to the corresponding piezo actuator) and output signals (e.g., the cantilever deflection) in the k th iteration, respectively. The convergence of the MIIC technique in the presence of random noise is analyzed in [34], and the main results are included in the Appendix for completeness.

Care, however, shall be taken when implementing the MIIC algorithm to live cell measurement as iteration is involved to obtain the control input (to the piezo actuator) to ensure the tracking of the same excitation force on both samples—applying the force stimuli repetitively at the same location of the live cell might deform and damage the cell membrane. Thus, instead of seeking accurate tracking of a pre-specified desired excitation force on both the live cell and the hard reference, we use the cantilever deflection measured on the live cell (during the force measurement) as the desired force profile to be tracked on the hard reference, i.e., iteration is only needed for deflection tracking on the hard reference. Alternatively, we realize that the force applied on the live cell may not maintain the desired constant force load rate. This issue can be alleviated by applying the MIIC technique to the force-distance curve measurement on a hydrogel sample with elastic modulus similar to the live cell to be measured (i.e., the elastic modulus is within the same order), and obtaining the input signal to achieve accurate excitation force tracking on the hydrogel. Then, by applying the obtained input to the measurement on the live cell, the desired force profile can be tracked closely as well.

III. EXPERIMENTAL MEASUREMENT OF THE RATE-DEPENDENT ELASTIC MODULUS OF LIVE CELLS

In this section, we present the implementation of the above-proposed control-based technique to investigate the effect of the nutrient-deprivation process on the mechanical property of live mammalian cells. Specifically, the proposed technique is employed to measure the indentation (and thereby the elastic modulus) of live HeLa cells, nutrient-deprived HeLa cells, and fibroblast cells when the force load rate is changed by three orders of magnitude. The outcome of the following experimental results shed light on the application of the proposed technique to study mechanical evolution of dynamic cellular processes such as the EMT process. We realize that HeLa cells are pathological in their properties. However, they served well as an example in this experiment to illustrate the proposed approach. Moreover, the experiment provided pilot data to explore future implementation of the proposed approach to cancer-related studies.

A. Cell preparation

MEF cells and HeLa cells were maintained in DMEM (Mediatech Cat. 10017CV) supplemented with 10% fetal bovine serum (FBS, Sigma, Cat. F6178) and 1% penicillin/streptomycin (Gibco, Cat. 15070063). For AFM detection, 5×10^5 HeLa cells were seeded onto Collagen I-coated glasscover (BDbiosciences, Cat. 354089) in a six-well plate and grew to complete confluence overnight. The next morning, HeLa cells were washed with sterile 1xPBS three times and then cultured in DMEM with or without 10% FBS for one more day before detection.

B. Experimental setup

A triangle excitation force profile with constant load and unload rate (as employed in usual force-distance curve measurement) was applied as the desired force profile, and the load rate was varied over four orders of amplitude from 0.1 to 100 Hz (see the Results part below for the ten different load rates tested), corresponding to the force velocity of 0.01–59 $\mu\text{m/s}$.

For ease of implementation, the amplitude of the input voltage (to the piezo actuator) was kept the same during the force-displacement measurement on the HeLa cells while the load-unload rate was changed. Then the applied force and the indentation generated were measured and used in the Hertz contact model [1,35] to compute the elastic modulus at that load rate:

$$F_z = \frac{4}{3} \frac{E \sqrt{R \Delta_z^3}}{1 - \nu^2}, \quad (14)$$

where R is the tip radius, and E and ν are Young's modulus and Poisson ratio of the live cell, respectively.

An AFM system (Dimension Icon, Bruker AXS Inc.) that allowed direct access to the drive of the piezo actuator and both the cantilever deflection and the z -axis sensor signal was employed in the experiments. All of the control and sensor signals were acquired through a data acquisition system (NI PCI-6259) under the Matlab xPC-target environment. A

cantilever of a normal spring constant of 0.01 N/m was used in the experiments. As listed in Table I, the specification of the cantilever ensures that Assumptions 1–3 were satisfied in the experiments (where the resonant frequency is experimentally calibrated for in-liquid cantilever oscillation).

Before the indentation measurement, an AFM image of a HeLa cell topography was acquired under contact mode imaging (scan rate: 0.2 Hz, scan size: $20\text{ }\mu\text{m} \times 20\text{ }\mu\text{m}$), as shown in Fig. 5. Then the elastic modulus measurements were conducted near the center of the nuclei of the cell (as marked by the red cross in Fig. 5). Note that the center of the cell was chosen—as commonly done in other nanomechanical measurements of live cells using AFM (e.g., [2,9,26])—to (i) eliminate the substrate effect, and (ii) make the comparison between different cells easy and less prone to measurement uncertainties.

To quantify the elastic modulus of the HeLa cell and the stressed HeLa cell, a triangle voltage signal was sent to drive the z -axis piezo actuator of the AFM system during the force curve measurement on the cell, and the load-unload force rate (i.e., the frequency of one entire push and retract operation) was varied between 0.1 and 100 Hz for the following 10 different values (while the amplitude of the signal is maintained the same): $\{0.1, 0.2, 0.5\} \times k$ Hz ($k = 1, 10, 100$) and 100 Hz. To minimize the distortion to the cell membrane, the triangle drive was applied for only one or two periods when the force load rate was lower or higher than 50 Hz, respectively. The drive inputs were applied successively separately with a separation time of ~ 3 min between each to allow the cell to recover from the previous force stimuli. For each load rate, the excitation force exerted (i.e., the cantilever deflection) on the live cell was measured and regarded as the desired excitation force profile to be tracked on the hard reference sample (a silicon sample). Then the MIIC technique was utilized to achieve accurate tracking of the given desired force profile for each load-unload rate. The iteration was terminated when the relative-RMS-tracking error of the cantilever deflection is smaller than 3%. Finally, the indentation was quantified as the difference of the cantilever-base displacement on the silicon sample from that obtained on the HeLa cell. For the force load rate employed in this study (< 100 Hz), the dynamics of the z -axis piezo actuator and that of the cantilever fixture (connecting the cantilever to the z -axis piezo actuator) were not excited, and hence the cantilever-base displacement can be directly measured from the z -axis sensor (that measures the z -axis piezo actuator displacement).

To study the effect of the stress process on the elastic modulus of HeLa cells, the above protocol for indentation and nanomechanical measurement was applied to the stressed HeLa cells as well. Furthermore, to evaluate the accuracy and consistency of the method, the above indentation and nanomechanical measurement protocol was repeated on 11 different HeLa cells and 16 stressed HeLa cells.

IV. RESULTS AND DISCUSSION

A. Indentation measurement using the control-based protocol

We first show the indentation of the live HeLa cell measured by using the proposed control-based protocol. Central to this protocol is to ensure accurate tracking of the *same* excitation force profile on both the cell and the silicon sample. Such an accurate tracking has been

maintained (via the use of the MIIC technique [34]) across all the load-unload force rates. As an example, the tracking of the excitation force profile (measured on the HeLa cell) on the silicon sample for the load rate of 50 Hz is shown in Fig. 6. The need for control and the efficacy of the MIIC technique to compensate for the relative probe acceleration and other adverse effects are illustrated in Fig. 6: Initially, the cantilever deflection measured on the silicon sample was largely different from that measured on the HeLa cell—the relative RMS tracking error was at 23.4%. With the use of the MIIC technique, such a large tracking error was dramatically reduced to 2.83% after only three iterations. The same level of tracking precision ($<3\%$) was maintained across all the other nine different load rates and during the measurements of all cells. We note that the remaining error was mainly a static offset [see Fig. 6(c)], which was due to the small drift of the cantilever probe in liquid. Thus, using the proposed protocol, the indentation in the HeLa cell was obtained directly from the difference of the cantilever-base displacement trajectory on the HeLa cell from that on the silicon sample. As examples, the cantilever-base displacement trajectory on both samples and the corresponding indentation measured were plotted in Fig. 7 for the load rates of 0.5, 20, and 100 Hz, respectively. Note that as we discussed before, as even the highest load rate (100 Hz) was still far below the resonance of the z -axis piezo actuator and the cantilever fixture (for the AFM system used in this work, around 5.6 and 4.3 kHz, respectively), the effects of these two dynamics were negligible. As shown in Fig. 7 (top row), the cantilever base displacement measured on the HeLa cell followed the triangle profile of the input voltage very closely, whereas on the contrary, the cantilever-base displacement measured on the silicon largely deviated from the input triangle profile, reflecting the compensation for the relative probe acceleration and the cell viscoelasticity effect (on the cantilever deflection).

B. Indentation measurement errors in the conventional method

Next, we present experimental results that demonstrated the limits of conventional indentation measurement. As an example, the cantilever-base deflection curve on the cell is plotted in Fig. 8 for a load rate of 0.5, 20, and 50 Hz (top row), respectively, along with the corresponding indentation-force plot obtained by using the proposed control-based protocol compared to those using the conventional protocol at these three rates (bottom row).

The measurement errors of the conventional method are evident, particularly when the force load rate increased and became relatively high. When using the conventional method, the cantilever-base displacement needs to be greater than the cantilever deflection during the probe-sample interaction for the indentation obtained to be positive [5,26] [see Eq. (7)], i.e., the base-deflection plot should stay above the dashed-line of unit slope in Figs. 8(a1) and 8(a2). However, the base-deflection curve crossed below the unit-slope line when the load rate was increased to 20 Hz and then further to 50 Hz, respectively. As a result, the indentation measured by the conventional method became largely erroneous as the force load rate increased. As shown in Figs. 8(b1)–8(b3), the indentation measured using the conventional method was close to that measured using the proposed method at a low load rate of 0.5 Hz, but then it decreased with the force load rate increase and became negative during the initial portion of the measurement as the load rate increased to 20 Hz, and even more so at 50 Hz—the decrease of indentation with the force increase and negative indentation contradicted the physical reality. Therefore, the experimental results clearly

demonstrated the limit of the conventional method for force indentation (nanomechanical) measurement of live cells.

The measurement error in the conventional method was further investigated along with the analysis of Sec. II A. First, the indentation caused by the relative probe acceleration,

$\frac{J}{\alpha L^2 k_{eqv}} \ddot{d}_s(t)$ (choose $\alpha = 1$), was quantified by using the measured cantilever deflection and nominal cantilever geometric values (see Table I), and then added to the indentation measured by the conventional method. As shown in Figs. 8(b1)–8(b3), with such a modification, the difference between the indentation measured using the conventional method and that using the proposed method was largely reduced. The difference was almost completely removed at a load rate of 0.5 Hz, and the modified indentation was almost completely positive even at a load rate of 50 Hz. Thus, the experimental results showed that the relative probe acceleration effect played a significant role in the conventional indentation measurement. We noticed that a residual difference existed between the modified indentation and that measured using the proposed method. The residual difference most likely was due to the uncertainty of the cantilever parameter values (J and L) used in computing the relative probe acceleration effect. Moreover, the relative probe acceleration effect was also compared to the hydrodynamics force effect for all 10 measured frequencies in Fig. 9, where $b(0) = 0.4 \mu\text{N s/m}$ was used in Eq. (4). It is clear that the indentation caused by the relative probe acceleration was much higher than that caused by the hydrodynamics force across all tested load rates (about 30–50 times higher). Hence, our experiment demonstrated the incapability of the conventional indentation measurement raised from the ignorance of both the relative probe acceleration as the load rate increased and the hydrodynamic force effect.

It is evident from the experimental results that the conventional protocol cannot accurately measure the indentation of live cells in liquid with high force load rates (i.e., when the relative probe acceleration becomes pronounced), which is needed to study the rate-dependent viscoelasticity of live cells.

C. Rate-dependent elastic modulus of HeLa cells

With the proposed control-based protocol for indentation measurement established by the experiment results, we present next the rate-dependent elastic modulus measurement results. First, the indentation-force curves for the nine load rates tested in the experiment are shown in Fig. 10. The rate dependence of the viscoelasticity of HeLa cells is evident—the slope of the indentation-force curve consistently decreases with the increase of the load-unload rate, reflecting that the HeLa cell “behaves” stiffer when the excitation force is exerted on the cell membrane at a higher rate—agreeing with both the previous observations (e.g., [1]) and theoretical modeling study (e.g., [31]). The increasing viscoelastic behavior of the HeLa cell upon an increasing force load-unload rate can also be seen from the indentation-force relation being less linear with a higher load-unload rate. Moreover, we realize that as the amplitude of the input voltage (to the z -axis piezo actuator) is kept the same for each force load rate, thereby the cantilever base displacement range (at 500 nm) is kept the same in all the force-distance measurements on the HeLa cell (see Fig. 7, top row). The maximum indentation in the HeLa cell should decrease while the maximum reaction force from the cell

membrane (equal to the applied force as the load-unload rate is very close to constant) should decrease—a direct result of the HeLa cell's appearing stiffer upon force stimuli of a faster load-unload rate—exactly presented in Fig. 8. Note that the load-unload speed corresponding to the ten load-unload rates are varying between $0.01 \mu\text{m/s}$ (for 0.1 Hz) and $59 \mu\text{m/s}$ (for 100 Hz). Thus, the experimental results show that the control-based protocol clearly captures the rate dependence of the viscoelastic behavior of the HeLa cell.

Next, the force-indentation curve is utilized to compute the elastic modulus of the HeLa cell at ten different load rates via the Hertz model, where the maximum force-indentation point is used for each load rate. The obtained elastic modulus is plotted in Fig. 11 with respect to the load rate for the *twelve* different HeLa cells. Three observations are readily in-line: (i) The measured values of the elastic modulus of the HeLa cell for low force load rates of 0.1–5 Hz at 6.71–20.76 kPa compared well to the that reported in [36,37] for HeLa cells at ~4–30 kPa for a force load rate lower than 10 Hz. However, the measurements of the elastic modulus of live cells using the force-displacement curve as reported in the literature are all limited to a low force load rate (< 10 Hz)—10 times lower than the load rate reported in this work. (ii) The elastic-modulus-load rate plot (in logarithmic scale) in Fig. 11 clearly follows a power-law relation—the simple linear fitting results in $E = 13.39 \omega^{0.40 \pm 0.03}$ (kPa), as shown in Fig. 11. Such a power-law modulus-rate relation agrees with those results obtained on a wide variety of live mammalian cells and via various micro- and nanorheology methods (see, e.g., review [38] and references therein), including force modulation using AFM [26,31]. However, unlike those results using AFM [26,31] that are all based on the concept of force modulation, we report in this work the measurement of such a power-law dependence via the quasistatic force-displacement curve with the force load rate spanning over four orders of magnitude and up to 100 Hz. (iii) Figure 11 also demonstrated that the measurement results were highly consistent—the standard deviation was between 4.6% (at 50 Hz) and 11.2% (at 100 Hz). The effect of the static preload force that was applied in the experiment and substantially reduced the uncertainty of the initial contact point on the cell was small: the preload applied was kept small (< 50 pN), and the comparison of the indentation measured under three different preload levels (50, 80, and 100 pN) across the 10 different load rates showed that the variations of the indentation measured across these three different preloads were small across all the load rates at less than 8%.

D. Effect of the serum-starvation process on the elastic modulus of HeLa cells

Finally, the rate-dependent elastic moduli of the HeLa cell before and after the stress process are compared. The elastic modulus for the ten different load rates measured on 16 different HeLa cells after the stress process (called “stressed HeLa cells” in the figures) is plotted in Fig. 12, and is compared with that before the stress process in Fig. 13. Three observations can also be drawn: (i) A similar power-law dependence of the elastic modulus on the load rate (frequency)—as observed for HeLa cells before the stress process (Fig. 11)—also holds for the HeLa cells after the stress process [$E = 5.48 \omega^{0.26 \pm 0.01}$ (kPa)]. Thus, this result also supports the statement that “the power law behavior and scale-free rheology are a common feature of cell mechanics” [38]. (ii) As for the HeLa cells before the stress process, the measured modulus results are highly consistent. The standard deviation of the measured modulus among the 16 stressed HeLa cells is between 4.29% (at the load rate of 10 Hz) and

12.19% (at the load rate of 20 Hz). (iii) The dramatic effect of the stress process on the mechanical properties of the HeLa cell is evident. As shown in Fig. 13, the reduction of the elastic modulus induced by the stress process is substantially larger than the different-cell-caused variations of the modulus, and there is no overlap between the elastic modulus of the HeLa cell before the stress process and those after the stress process across all 10 load rates. Moreover, the stress-process-caused reduction of the elastic modulus becomes even more significant as the load rate increases. For example, the mean value of the elastic modulus at the load rate of 100 Hz reduced by 5.07 times after the stress process. Note that experiments have also been conducted to excluded other potential causes to such a significant change of elastic modulus, including aging of the HeLa cell. The experiments were repeated on HeLa cells that were kept in the same incubator environment along with those HeLa cells undergoing the stress process. The measurement elastic modulus values were similar to those measured on the unstressed HeLa cell without one more day of incubation (the difference is within the standard deviation at each measured frequency). Thus, this result measures the stress-process effect on the elastic modulus of HeLa cells for a load rate of four orders of magnitude range and up to 100 Hz.

The cellular cytoskeleton determines cell morphology and mechanical properties [39,40]. The highly organized cytoskeleton is dynamically regulated by internal genetic and external physical or biochemical cues. Therefore, the dynamics of the cytoskeleton will affect cell morphology and mechanical properties. E-Cadherin complexes form adherens junctions and transduce mechanical forces via association with actin cytoskeletal networks [41]. Once the number of adherens junctions is reduced (i.e., in serum starvation), the integrity of the original cytoskeleton will be compromised and will consequently influence local mechanical properties. Here, our result verifies that the elasticity of HeLa cells was decreased when they acquired a mesenchyme-like phenotype due to a loss of cell-cell junctions. In fact, many studies have elucidated that the elastic modulus of cells can be shaped due to cellular cytoskeleton reorganization during the process of cell proliferation, differentiation, and transformation [18–20,42]. For example, invasive tumor cells become mechanically soft when they lose connection to their neighbors [43]. Highly metastatic cells have more reduced stiffness due to actin cytoskeleton remodeling compared to less invasive parental cells [44]. Thus, accurate quantification of cell mechanical properties provides a novel window to evaluate the cell predisposition and fate. Taken together, cell mechanical properties are one of the important epigenetic parameters and also important indices which can be utilized to quantitatively assess cell function, plasticity, and fate.

V. CONCLUSION

In this paper, we have developed a control-based approach to rate-dependent elastic modulus quantification of live cells using AFM. The limits of the conventional indentation measurement in liquid have been discussed, and the probe-sample interaction dynamics in liquid AFM nanomechanical measurement has been analyzed. It was then proposed to measure the indentation as the difference of the cantilever-based displacement on the live cells with respect to that on the hard reference sample when the same excitation force profile was tracked on both samples during the force-distance measurement. The MIIC technique has been implemented to achieve accurate tracking of the excitation force profile (measured

on a live cell) on the hard reference sample. The proposed approach overcame the limits of the conventional indentation measurement and accounted for the effect of the cantilever deflection acceleration induced by the tip-sample interaction. The proposed method was applied to measure the rate-dependent elastic modulus of both a live HeLa cell before and after nutrient deprivation and a fibroblast cell for comparison, all in cell culture media when the force load rate was changed four orders of magnitude and up to the 100 Hz range. The experimental results demonstrate that the control-based indentation and nanomechanical quantification technique provides a powerful means to quantify and even predict cell fate during and after evolutions such as EMT process.

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APPENDIX

1. Modeling-free inversion-based iterative learning control

The modeling-free inversion-based iterative learning control (MIIC) method [34] is implemented to ensure precision tracking of the deflection profile (measured on the live cell) on the hard reference sample.

Specifically, the control input applied to drive the AFM z -axis piezo is obtained through iteration as follows (see Fig. 14):

$$\begin{aligned} U_1(j\omega) &= \alpha D_d(j\omega), \quad k=1, \\ U_{k+1}(j\omega) &= \frac{U_k(j\omega)}{D_k(j\omega)} D_d(j\omega), \end{aligned} \quad (A1)$$

where $D_d(j\omega)$ is the desired output trajectory (i.e., the cantilever deflection measured on live cells), α is a constant, and $U_k(j\omega)$ and $D_k(j\omega)$ are the current input (e.g., the input voltage to the corresponding piezo actuator) and output signals (e.g., the cantilever deflection) in the k th iteration, respectively. The convergence of the MIIC technique in the presence of random noise is presented below [34].

Let $G(j\omega)$ be a stable single-input–single-output (SISO), linear time invariant (LTI) system, and at each frequency ω , consider the system output $D(t)$ to be affected by the disturbance and/or the measurement noise $D_n(t)$ as

$$D(j\omega) = D_l(j\omega) + D_n(j\omega), \quad (\text{A2})$$

where $D_l(j\omega)$ denotes the linear part of the system response to the input $U(j\omega)$, i.e., $D_l(j\omega) = G(j\omega)U(j\omega)$, and $D_n(j\omega)$ denotes the output component caused by the disturbances and/or the measurement noise. Then, the following holds true:

- i. The ratio of the iterative input $U_k(j\omega)$ to the desired input $U_d(j\omega)$ [the input which corresponds to the desired output $D_d(j\omega)$] is bounded in magnitude and phase, respectively, as

$$1 - \varepsilon(\omega) \leq \lim_{k \rightarrow \infty} \left| \frac{U_k(j\omega)}{U_d(j\omega)} \right| \leq \frac{1 - \varepsilon(\omega)}{1 - 2\varepsilon(\omega)}, \quad (\text{A3})$$

$$\lim_{k \rightarrow \infty} \left| \angle \left(\frac{U_k(j\omega)}{U_d(j\omega)} \right) \right| \leq \arcsin \left(\frac{\varepsilon(\omega)}{1 - \varepsilon(\omega)} \right), \quad (\text{A4})$$

provided that the noise to signal ratio (NSR), as defined below, is upper-bounded by a less-than-half constant, $\varepsilon(\omega)$,

$$\left| \frac{D_{k,n}(j\omega)}{D_d(j\omega)} \right| \leq \varepsilon(\omega) \leq \frac{1}{2}, \quad (\text{A5})$$

where the desired input $U_d(j\omega)$ enables the linear part of the system output to exactly track the desired output, i.e., $D_d(j\omega) = G(j\omega)U_d(j\omega)$, and $U_{k,n}(j\omega)$ denotes the part of the output caused by disturbances and/or measurement noise in the k th iteration. Moreover, the relative tracking error is bounded as

$$\lim_{k \rightarrow \infty} \left| \frac{D_k(j\omega) - D_d(j\omega)}{D_d(j\omega)} \right| \leq \frac{2\varepsilon(\omega)[1 - \varepsilon(\omega)]}{1 - 2\varepsilon(\omega)}. \quad (\text{A6})$$

- ii. The use of the MIIC algorithm will improve the output tracking at frequency ω , i.e.,

$$\lim_{k \rightarrow \infty} \left| \frac{D_k(j\omega) - D_d(j\omega)}{D_d(j\omega)} \right| < 1, \quad (\text{A7})$$

provided that the upper bound of the NSR is less than $1 - \frac{\sqrt{2}}{2} \approx 0.3$, i.e.,

$$\left| \frac{D_{k,n}(j\omega)}{D_d(j\omega)} \right| \leq \varepsilon(\omega) < 1 - \frac{\sqrt{2}}{2}. \quad (\text{A8})$$

FIG. 1.

(Color online) Simplified model of the tip-sample dynamics during the in-liquid nanomechanical measurement of live cells.

FIG. 2.

(Color online) The (red) upper bound and (blue-dashed) lower bound of the frequency response of the dynamics from the cantilever-base displacement to the cantilever deflection, corresponding to “hard” cells (red blood cells) and “soft” cells (fibroblast cells), respectively.

FIG. 3.
 (Color online) The free body diagram for the simplified cantilever-probe-sample interaction dynamics during the nanomechanical measurement on the hard reference sample.

FIG. 4.
(Color online) Relative reduction of the hydrodynamic force.

FIG. 5.
(Color online) AFM topography image of the HeLa cell: (a) height and (b) deflection error, where the cross marks the location at which the force-curve measurements were executed.

FIG. 6.

(Color online) (a) The cantilever deflection tracking results (first and third iterations) on the silicon sample; (b) the zoomed-in view of the tracking results for 2 ms; and (c) the tracking error.

FIG. 7.

(Color online) (a) The cantilever-base displacement on the HeLa cell and the silicon sample at 0.5 Hz; (b) the cantilever-base displacement on the HeLa cell and the silicon sample at 20 Hz; (c) the cantilever-base displacement on the HeLa cell and the silicon sample at 100 Hz; and indentation quantified (d) at 0.5 Hz, (e) at 20 Hz, and (f) at 100 Hz.

FIG. 8.

(Color online) Indentation measured using the conventional method: (a1)–(a3) cantilever deflection–base displacement curve on the HeLa cell and the silicon sample at 0.5, 20, and 50 Hz using the conventional approach, respectively; (b1)–(b3) comparison of the indentation measured using the conventional method, the proposed approach, and the corrected conventional results, taking the relative probe acceleration into account at 0.5, 20, and 50 Hz, respectively.

FIG. 9.

(Color online) Comparison of the relative probe acceleration effect and the hydrodynamics force on the indentation.

FIG. 10.
(Color online) The indentation-force curves measured by using the proposed control-based protocol for nine different load rates between 0.1 and 100 Hz.

FIG. 11.

(Color online) The elastic modulus vs force load rate of the HeLa cells measured on the 12 different HeLa cells using the proposed control-based protocol.

FIG. 12.
(Color online) The elastic modulus vs force load rate of the nutrient-deprived HeLa cells measured on the 16 different HeLa cells using the proposed control-based protocol.

FIG. 13.

(Color online) Comparison of the elastic modulus vs the load rate for the control HeLa cells, nutrient-deprived HeLa cells that took on a mesenchymal phenotype, and the fibroblast cells. The straight lines are the power-law curve fit.

FIG. 14.
(Color online) Block diagram of the MIIC technique to achieve precision tracking of the desired force profile in the proposed method to indentation measurement in liquid.

TABLE I

Specifications of the AFM probe used in the experiments.

Geometry	Triangular	Tip radius	20 nm
Spring Constant	0.01 N/m	Tip height	5 μm
Length	310 μm	Thickness	0.55 μm
Width	20 μm	Resonant frequency	2.17 kHz