Optical measurement of the viscoelastic and biochemical responses of living cells to mechanical perturbation

Chau-Hwang Lee, Chin-Lin Guo, and Jyhpyng Wang

Institute of Atomic and Molecular Sciences, Academia Sinica, and Department of Electrical Engineering, National Taiwan University, Taipei 106, Taiwan

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We have developed an optical method for real-time monitoring of cellular motion in a natural environment with nanometer resolution. From the motion driven by small optical forces, we measured dynamic viscoelastic responses of living cells in the linear reversible region. Cytoplasmic gel-to-sol transition that was due to the disruption of the actin-filament framework was detected, and a linear release of Ca^{2+} from intracellular storage that was related to submicrometer cell deformation was observed. The method was shown to be a powerful tool for studying the natural response of cells to mechanical perturbation. © 1998 Optical Society of America

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The viscoelasticity of a living eucaryotic cell reflects the state of the cytoskeleton, which is intimately related to intracellular biochemistry.[1] Conversely, intracellular biochemical reactions can be triggered by mechanical stress as a way by which the cell senses and responds to the environment.[2] Investigation of the viscoelastic and biochemical responses of living cells to environmental stress has been limited by the available technology. The difficulties are as follows: (1) The measurement must be done noninvasively to avoid artifacts. (2) Stress-induced motion must be kept small to ensure that the strain is in the linear reversible region. This requires nanometer instrumental resolution. (3) The speed of measurement must be fast enough to track the dynamic response of the cell. (4) The technique must be compatible with established methods of biochemical measurement.

There was no method that satisfied all these requirements. Recently we developed an optical technique called differential confocal microscopy (DCM) that is capable of high-speed surface-profile measurement with 2-nm depth resolution and 0.3-μm lateral resolution.[3] In this Letter we describe the application of DCM to study the viscoelastic response of living human fibroblasts to small optical forces. The frequency response of driven cellular motion under different biochemical conditions was measured. The sensitivity of DCM enabled us to detect the sol–gel transition of cytoplasm in living cells. Because DCM is compatible with fluorescence microscopy, we demonstrated that the two methods can be combined to measure the biochemical responses of living cells to mechanical perturbation.

The experimental setup is shown in Fig. 1. Two wavelengths were selected from an argon-ion laser as the light source. The 514-nm beam of 0.3–1.0 mW, focused to a 14-μm diameter, was used to exert an optical force on the cells. The 458-nm beam of 20 μW, focused to a 1-μm diameter, was used to measure the driven cellular motion at the center of the 514-nm beam. The location of the light spot on the cells was monitored with an inverted optical microscope. Both beams were focused by a water-immersion objective lens (Zeiss ICS Achroplan 40×; N.A., 0.75), which was mounted upon a piezoelectric-transducer-driven objective holder (Physik Instrumente PIFOC) for fine positioning of the focal plane. The setup closely resembles a conventional confocal microscope,[4] in which the 458-nm light, reflected from the cell membrane and filtered by the 5-μm-diameter pinhole at the conjugate focal point, forms the confocal signal. However, the novel characteristic that marks the essence of DCM is that the focal plane is intentionally placed slightly away from the cell membrane, so the membrane is on a steep slope of the confocal axial response curve. This makes the signal light that enters the confocal aperture depend sensitively on the position of the membrane.[3]

It can be shown by optical diffraction theory that the confocal axial response curve for a planar reflective object is $I(z) = I(0)\sin^2(az)/(az)^2$, where $I(z)$ is the optical power going through the pinhole, z is the distance between the focal point and the reflecting plane, and $a = 4\pi \sin^2(\alpha/2)/\lambda$, where $\sin(\alpha)$ is the numerical aperture. At each side of $z = 0$, the

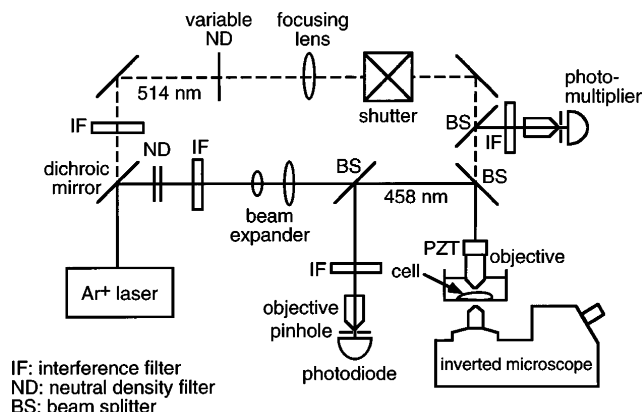

Fig. 1. Experimental setup. PZT, piezoelectric transducer.

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normalized slope of $I(z)$, $I(0)^{-1}|dI(z)/dz|$, is approximately $4.5\sin^2(\alpha/2)/\lambda$. For our setup the slope is $\approx 1.0/\mu m$; hence a 10-nm change in membrane height causes a 1% change in the confocal signal. Such sensitivity enables us to measure cellular motion down to a few nanometers. The resolution is limited by the 0.2% power fluctuation of the laser. Because the technique does not depend on feedback control or phase-locking mechanisms, the measurement can be as fast as the response of the photodetector. If the signal is weak so that shot noise dominates, the speed is then limited by the time constant required for the desired signal-to-noise ratio. Before making the measurement we calibrated the slope $|dI(z)/dz|$ by measuring $I(z)$ while scanning the focal point along the z axis by the piezoelectric-transducer-driven objective holder. The calibration determines the value of $I(0)$, which is governed by sample reflectivity and the size of the pinhole.

The fibroblasts, taken from infants' foreskins, were cultured in Dulbecco's modified Eagle essential medium with 10% fetal calf serum and 1% antibiotics. The culture dish was not coated with any extracellular matrix components; the cells attached directly to the bottom surface of the polystyrene dish. In the culture medium the membrane reflectivity was $\approx 2\%$, giving rise to an optical force of 55–180 fN from the 514-nm beam. The cells' absorption to the 514-nm beam was less than 0.2%. For such small absorbed power the thermal expansion of a cell surrounded by water is smaller than 2×10^{-6}, which was negligible in our experiments. Measurement was done only on typical spindle-shaped cells that had just attached to the bottom of the culture dish before stretching out. During the measurement, we ensured the vitality of the sample cell by monitoring its morphology.

In the linear response region the displacement $d(t)$ driven by the stress $s(t')$ can be described by $d(t) = \int R(t - t')s(t')dt'$, where $R(t - t')$ is the displacement at time t that is due to the driving stress at a prior time t'. By Fourier transform one has $d(\omega) = \chi(\omega)s(\omega)$, where the susceptibility $\chi(\omega)$ is the Fourier transform of $R(\tau)$. By measuring $\chi(\omega)$ one obtains the response function $R(\tau)$, which contains a full description of the cell's linear viscoelastic properties.

In our experiment the driven motion of the cell was measured in both the time domain and the frequency domain. We applied a square-wave optical force to the membrane by chopping the 514-nm beam. The beam diameter was intentionally defocused to 14 μm to reduce the contribution of membrane bending to the restoring force. The inset of Fig. 2 shows the membrane displacement as a function of time. The underlying cytoskeleton responded to the optical force as a damped harmonic oscillator. The displacement follows $d(t) = F[1 - \exp(-\gamma t)]/k$ when force F is on, where k is the spring constant and γ is the relaxation rate. We measured the susceptibility curves $\chi(\omega)$, shown in Fig. 2, by sweeping the chopping frequency while recording the amplitude of the driven motion. The data fitted well the susceptibility curve of a damped harmonic oscillator $|\chi(\omega)| = F/|-m\omega^2 + k + i\rho\omega|$, where m is the equivalent mass of the system and ρ is the damping constant. For our system $(\rho^2 - 4mk)^{1/2} \ll \rho$; hence γ approximately equals $\rho/2m$. The spring constant and the damping constant that characterize the viscoelastic response of the cell were determined by curve fitting. The values were $(2.05 \pm 0.47) \times 10^{-7}$ N/m and $(4.4 \pm 0.23) \times 10^{-8}$ Ns/m respectively, where the uncertainties denote the standard deviations of the fittings. The quality of the fittings was carefully examined according to the criterion in Ref. 5. The signal-to-noise ratio of our experiments was large enough that the quality of all the fittings well exceeded the good-fitting criterion. The values of k remained the same for driving forces below 200 fN, indicating that our experiment was done in the linear response range.

The same measurement was repeated on the same cell after it was treated with 4 μM cytochalasin D for 90 min. Cytochalasins are known to disrupt the cytoplasmic framework of actin filaments in living cells.[6,7] Pictures of frozen dried cells under electron microscopes reveal that polymerized actin filaments cross-link each other to form an intracellular elastic framework, which provides mechanical support for the cell. In this state the cytoplasm is gel-like.[1] In contrast, the treatment of cytochalasin D dissolves the framework of actin filaments and makes the cytoplasm fluidlike, resisting the stress less strongly than in the gel state. This sol–gel transition of cytoplasm is thought to be the mechanism of cell motion.[8] The experimental result is shown as the dashed curve in Fig. 2. After the treatment, k and ρ changed to $(1.24 \pm 0.36) \times 10^{-7}$ N/m and $(3.1 \pm 0.05) \times 10^{-8}$ Ns/m, respectively. The decrease in both k and ρ clearly displayed a shift of balance toward solation of the cytoplasm, verifying that the cross linking of actin filaments is the mechanism for sol–gel transformation in living cells.

There have been a few mechanical methods for measuring cell elasticity, such as computing the viscosity of cytoplasm from the motion of intracellular test objects driven externally,[9,10] pulling the cell with micropipettes,[11] and poking the cell with a stylus while measuring the bending of the stylus's supporting

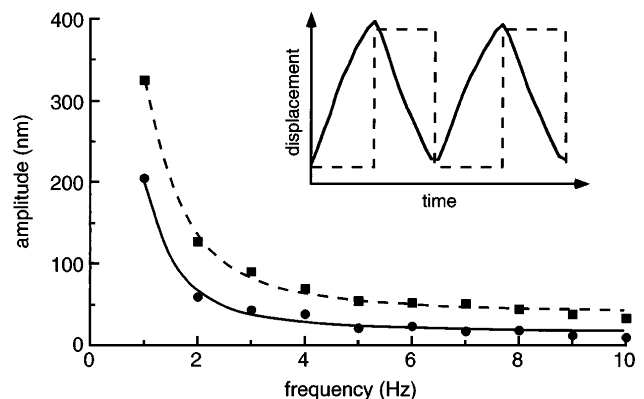

Fig. 2. Viscoelastic susceptibility curves. Solid curve and filled circles, normal fibroblast. Dashed curve and filled squares, the same cell treated with cytochalasin D. Inset: membrane displacement (solid curve) induced by a square-wave optical force (dashed curve).

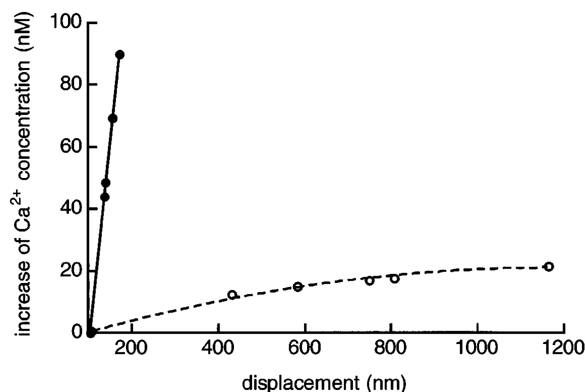

Fig. 3. Ca^{2+} response to deformation. Filled circles, normal fibroblast; open circles, the same cell treated with cytochalasin D.

beam.[12] In comparison with these techniques, the one reported in this Letter offers advantages not only in being noninvasive and dynamic but also in having the resolution to keep the deformation small, which is essential for studying the cell's response in a natural environment. In our measurement the deformation was kept well below 1 μm, ensuring that the cell remained in its natural state and that the experimental data could be characterized by linear coefficients. Recently developed confocal interference microscopy is the only noninvasive alternative that offers comparable resolution.[13,14] A detailed comparison of confocal interference microscopy and DCM was discussed in Ref. 3.

We can study the biochemical response to cell deformation in a similar way by combining DCM with fluorescence microscopy. As a demonstration, we measured the intracellular concentration change of Ca^{2+}, an important regulator of actin-binding proteins,[15] with respect to cell deformation. Owing to DCM's great axial resolution, we were able to monitor the intracellular Ca^{2+} response to minute mechanical perturbation. The Ca^{2+} was labeled by Calcium Orange, whose fluorescence rate changes after it combines with Ca^{2+}. The cells were transferred to a calcium-free medium for the experiments, and 10 mM of Ca^{2+} chelator EDTA was added to ensure that no Ca^{2+} signal came from the medium. The experimental results are shown in Fig. 3. The filled circles show that Ca^{2+} was released from intracellular storage when the cell was deformed and the response was linear for small deformation. To probe the mechanism of this stress-induced Ca^{2+} mobilization we dissolved the cytoplasmic actin-filament framework by treating the cell with 50 μM cytochalasin D for 20 min and repeated the experiment. The result is shown as the open circles. The Ca^{2+} response was almost completely suppressed, even with much larger deformation. The data suggest that the mechanical structure of actin filaments plays an important role in stress-signal transduction, in conjunction with the biochemical signal paths.

In summary, we have demonstrated that DCM is a powerful method for studying motions and elastic responses of living cells. Compared with other nanometer techniques, such as atomic force microscopy and scanning electron microscopy, DCM offers great advantages in simplicity, noninvasiveness, and measurement speed. By measuring membrane displacement synchronously with fluorescence from suitable indicators, one can also study various biochemical responses of living cells to deformation. Such experiments can provide much insight into the ways in which a cell reacts to mechanical perturbation from the environment.

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Address correspondence to Jyhpyng Wang, P.O. Box 23-166, Taipei, Taiwan.

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