



An active one-particle microrheometer: Incorporating magnetic tweezers to total internal reflection microscopy

Xiangjun Gong, Li Hua, Chi Wu, and To Ngai

Citation: [Review of Scientific Instruments](#) **84**, 033702 (2013); doi: 10.1063/1.4794441

View online: <http://dx.doi.org/10.1063/1.4794441>

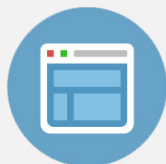
View Table of Contents: <http://scitation.aip.org/content/aip/journal/rsi/84/3?ver=pdfcov>

Published by the [AIP Publishing](#)



Re-register for Table of Content Alerts

Create a profile.



Sign up today!



An active one-particle microrheometer: Incorporating magnetic tweezers to total internal reflection microscopy

Xiangjun Gong, Li Hua, Chi Wu, and To Ngai^{a)}

Department of Chemistry, The Chinese University of Hong Kong, Shatin, N.T. Hong Kong

(Received 14 June 2012; accepted 20 February 2013; published online 11 March 2013)

We present a novel microrheometer by incorporating magnetic tweezers in the total internal reflection microscopy (TIRM) that enables measuring of viscoelastic properties of materials near solid surface. An evanescent wave generated by a solid/liquid interface in the TIRM is used as the incident light source in the microrheometer. When a probe particle (of a few micrometers diameter) moves near the interface, it can interact with the evanescent field and reflect its position with respect to the interface by the scattered light intensity. The exponential distance dependence of the evanescent field, on the one hand, makes this technique extremely sensitive to small changes from z -fluctuations of the probe (with a resolution of several nanometers), and on the other, it does not require imaging of the probe with high lateral resolution. Another distinct advantage is the high sensitivity in determining the z position of the probe in the absence of any labeling. The incorporated magnetic tweezers enable us to effectively manipulate the distance of the embedded particle from the interface either by a constant or an oscillatory force. The force ramp is easy to implement through a coil current ramp. In this way, the local viscous and elastic properties of a given system under different confinements can therefore be measured by resolving the near-surface particle motion. To test the feasibility of applying this microrheology to soft materials, we measured the viscoelastic properties of sucrose and poly(ethylene glycol) solutions and compared the results to bulk rheometry. In addition, we applied this technique in monitoring the structure and properties of deformable microgel particles near the flat surface.

© 2013 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4794441>]

I. INTRODUCTION

Complex fluids such as synthetic colloids, emulsions, suspensions, and polymer melts have wide industrial applications. When such soft materials are strained, their microstructures both store and dissipate the deformation energy in a frequently dependent manner, reflecting viscoelasticity. The rheological properties of a soft material determine its flow and processing behavior, and provide a hint into its microstructure. Traditional bulk rheometers typically measure the frequency-dependent linear viscoelastic relationship between strain and stress on milliliter-scale material samples.

Microrheology is a term for a number of techniques designed to probe small samples and to measure local viscoelastic properties of materials by tracking the motion of embedded micrometer-sized probe particles.^{1–3} Generally, microrheology is divided into two approaches that are distinguished by the driving force of the probe motion. In passive microrheology, probe particles are forced by thermal fluctuations and Brownian motion of the probe particles is commonly measured by light scattering techniques^{4–6} or video microscopy^{7–9} to extract rheological moduli using the generalized Stocks-Einstein relation.^{10,11} Particularly, passive tracer microrheology has been utilized in exploring the dynamics of multiple biologically relevant low modulus materials, such as F-actin,¹² and the cytoplasm of a live cell.¹³ This approach, however, has still remained limited, especially

at high frequencies and for stiff materials. To address this limitation, active microrheology has been developed. In active microrheology, conversely, the embedded probe particles move in response to an external force, typically generated by optical^{8–10} or magnetic tweezers.^{11–13} In magnetic tweezers, the forces are applied to superparamagnetic spherical probes using an external magnetic field. More importantly, with such probe particles, one can cover several orders of magnitude in force, ranging from femtonewtons to tens of piconewtons. In addition, due to the non-invasive feature, they hold promise for the broad application in bioanalysis. Examples include the mechanical straining and twisting of DNA,^{14–16} nanomanipulation of probes in biological systems,^{17–19} and microrheological studies of different kinds of cells,^{20–22} such as fibroblasts,²³ macrophages,²⁰ and endothelial cells.²⁴

While magnetic tweezers have been widely used, a further challenge that has to be overcome is the resolution in probe particle displacement, especially for subnanometer accuracy. To obtain this level of sensitivity, some recent approaches have combined magnetic tweezers with total internal reflection fluorescence microscopy (TIRF). In this situation, an evanescent wave is created in the vicinity of the solid-water interface in TIRF as the incident light source. In general, the penetration depth of the evanescent wave is in the range of 50–200 nm for visible light. When fluorescence probe particles move under the generated evanescent field, they produce a measureable intensity, which in turn reflects their position with respect to the interface. Since the intensity of the fluorescence probe is exponential decay with increasing distance from the surface, it makes evanescent illumination

^{a)} Author to whom correspondence should be addressed. Electronic mail: tongai@cuhk.edu.hk.

an ideal technique for measuring small probe displacement. The combination of magnetic tweezers and evanescent illumination has developed into a powerful technique for manipulating single biomolecules and studying their interactions. DNA twisting and hybridization,^{25–28} protein unfolding,^{29,30} and DNA-protein interactions³¹ have all been demonstrated at the single-molecule level. However, it is worthy to note that the practical implementation for detection using TIRF relies on the use of fluorescence probes which might lead to a complex convolution with the evanescent wave, affecting the spatial determination of the probes.²⁸ Additionally, the evanescent wave created through high-NA objective in TIRF has the problem to determine the penetration depth accuracy and the intensity of the probe image shows to decay non-exponentially with the distance from the interface.^{32,33}

Inspired by that, herein we describe a novel setup that magnetic tweezers were incorporated to a total internal reflection microscopy (TIRM), instead of the commonly used TIRF. The important advantage is that we can use a photodiode detector to measure the scattering of light by a single spherical particle when it is illuminated by an evanescent wave which is produced by reflection of a laser beam off the glass-water interface at a sufficiently glancing angle that total reflection occurs. The setup therefore uses single particle, is label-free, and, in principle, does not require a more complex sensor, such as a high resolution CCD camera. The motion of the embedded probe particle can be controlled by a magnetic driving force, produced by two sets of four electromagnetic pole pieces symmetrically arranged in the upper and lower planes of the sample stage. High precision of the magnetic force, at pN scale, can be achieved through a coil current ramp. We are able to oscillate, manipulate the distance of the embedded single particle from the surface within hundreds nm, and monitor the very small particle displacement in z-position using the evanescent wave illumination. As the motion of the single particle is affected by the surrounding medium, we demonstrated that the combination of magnetic tweezers with TIRM leads to a single-particle microrheometer for measuring local viscoelastic properties of materials by resolving the particle motion. Moreover, we showed that this setup allows us to study confinement effects on the dynamical transition and the viscoelasticity of complex fluids, which have been rarely explored.^{34–38} Understanding the rheological properties of complex fluids under the confined condition is vitally important since it has been shown that confinement – due to the presence of the walls/surfaces – might significantly alter the dynamics and structures of the systems. This situation is ubiquitously in biological processes such as cell adhesion/migration,³⁹ folding of chromatin fibers,⁴⁰ and behaviors near cell membrane,^{41,42} as well as an essential issue in many industrial processes including oil recovery, water purification, and lab on-chip designs.⁴³

II. APPARATUS

A. Single particle tracking in the TIRM

In standard TIRM, the evanescent wave is produced by reflecting a laser beam off the glass-water interface at a sufficiently glancing angle that total internal reflection occurs.

When a probe moves near the evanescent wave, it scatters a measurable intensity which in turn reflects its distance from the interface. The evanescent wave propagates parallel to the interface and its amplitude decays exponentially with the distance from the interface. As a consequence, the scattering intensity also decays exponentially as^{44–46}

$$I(h) = I_0 \exp(-\beta h), \quad (1)$$

where h is the particle-surface separation, I_0 is the scattered light intensity at $h = 0$, and β is the decay index, expressed as⁴⁵

$$\beta = \frac{4\pi}{\lambda} \sqrt{(n_1 \sin \theta)^2 - n_2^2}, \quad (2)$$

where θ is the incident angle, n_1 and n_2 are the refractive index of the incident and refractive medium respectively, and β^{-1} is the penetration depth of the evanescent wave intensity. The exponential dependence of intensity on the probe makes TIRM a highly sensitive spatial detector for tracking the vertical motion of the probe at single particle level.

In TIRM measurements, the measured instantaneous intensity, $I(t)$, generally represents the photon numbers entering the photodiode detector during time t to $t + \Delta t$. In principle, Δt should be infinitely small, however, in real experiments, Δt varies from case to case, typically ranging from 0.1 ms to 100 ms. It is noteworthy that too small value of Δt can cause an increase of the statistical noise since the Poisson statistics in photodiode detector becomes prominent. In our TIRM, a counter card PCI6602 (8-channel, 32-bit counter/timers, 80 MHz maximum source frequency, National Instruments) was connected to the photodiode detector for counting the entered photons. The accumulated counts then were sent to the PC for further analysis using Visual C program. Following Eq. (1), if the scattered intensity, I_{ref} , at a reference distance from the surface, h_{ref} , is selected, the h of the particle from the surface could be written as

$$h = \beta^{-1} \ln(I/I_{\text{ref}}) + h_{\text{ref}} \quad (3)$$

Note that in real measurements, I_{ref} is often set as the intensity I_0 at $h = 0$ (bottom surface). In this way, Eq. (3) can be expressed as

$$h(t) = \beta^{-1} \ln[I(t)/I_0], \quad (4)$$

where $h(t)$ is the absolute distance of the probe particle with respect to the surface. $I(t)$ represents the photon numbers entering the photodiode detector between time t to $t + \Delta t$. I_0 can be obtained by depositing the probe particle to the bottom surface. As a result, $h(t)$ can be calculated and used for further microrheological analysis as discussed in Secs. III A–V.

B. TIRM instrumentation

TIRM is a method to monitor the fluctuations in the instantaneous distance separating a Brownian spherical particle and a transparent plate. The details of TIRM and its applications in force measurements have been described in Refs. 45, 47, and 48. Figure 1 schematically shows our TIRM setup combined with eight magnetic poles (labelled from 0 to 7) which were arranged in the upper and lower planes of

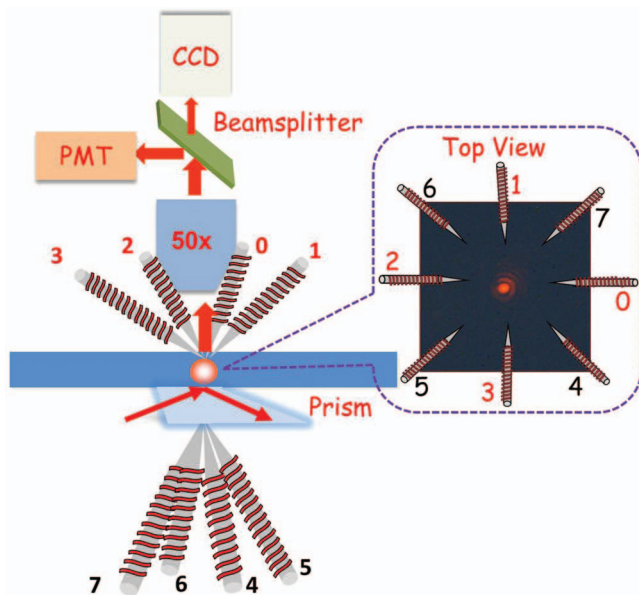


FIG. 1. The developed single-particle microrheometer that combines the magnetic tweezers with TIRM. In this setup, two sets of four electromagnetic pole pieces are symmetrically arranged in the upper and lower planes of the sample cell in standard TIRM to achieve a three-dimensional position control. A top view is inserted which shows a probe microsphere from the CCD camera under the evanescent wave illumination surrounded by the tips of the eight magnetic poles.

the sample stage, leading to a single-particle microrheometer. The laser beam (maximum = 35 mW HeNe, $\lambda = 632.8$ nm, TEM₀₀ mode, linear polarized, 1145P, JDSU) enters into a prism (three kinds of base angle 63°, 70°, and 73°; thickness: 1.5 mm; $n = 1.512$, BK-7 glass, JUKA optics) and is reflected at the interface between the sample tubing (Borosilicate glass extra wide W3520, thickness = 200 μm , VitroCom) and the sample solution. As the incident angle is larger than the critical angle, θ_c , a total internal reflection occurs and an evanescent wave is produced at the interface. The scattered intensity of the probe particle is collected by the objective (N PLAN, 50 $\times$ /0.50, WD = 7 mm, Leica) in the microscope (Leica DM LFSA with electronic focus). Typically, the scattered light passes through a beamsplitter (70% R/30% T, Edmund), in which 70% is reflected to photomultiplier (PMT, H7155 Photo Counting Head, Hamamatsu) while 30% is transmitted to charge coupled device (CCD, Qimaging MicroPublisher 3.3 MC cooled color) for imaging. A long-wave pass filter (central wavelength = 633 nm, 10LWF-600-B, Newport) was placed before the PMT to reduce the background noise.

C. Generation of controllable magnetic force

The basic magnetic tweezers in our setup are shown in Figure 2. We incorporated two sets of four electromagnetic pole pieces symmetrically arranged in the upper and lower planes of the sample stage in the TIRM. We opted to use the electromagnets (diameter: 6.35 mm, length: 10 cm, NI037910, Magnetic Shielding Alloy Ni77/Fe14/ Cu5/Mo4 Rod, Goodfellow Cambridge Limited), because the magnetic field can be easily changed by the core of the electromagnets,

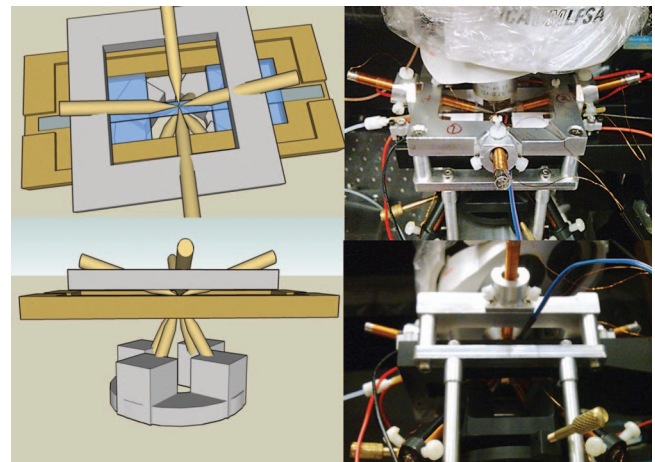


FIG. 2. Schematic drawing of the incorporated magnetic tweezers is shown on the left picture. The real instrument from the top view (upper picture) and side view (lower picture) is shown in the right picture.

electromagnet current, and coil turns. Using copper coiling wire, 200 turns were laid in each magnet pole giving a resistance of $\sim 2.2 \Omega$. Variation of the magnetic force in the sample plane can be achieved by changing the coil current. The applied electromagnet poles were mechanically sharpened with a tip diameter of $\sim 100 \mu\text{m}$ (shown in Figure 3), which could provide high magnetic fields so as the produced forces. These eight poles were fixed onto a homemade square stage and their positions were manipulated under a 10 $\times$ objective by adjusting the corresponding fixing screws (Figure 2). In our setup, the included angle between the 4 upper poles and the optical path is $\sim 70^\circ$, while it is 35° between the 4 lower poles and the optical path. This special design is due to the fact that the optical path collected by working objective (N.A. = 0.5, work distance = 7 mm) occupies a re-entrant cone, where the vertex angle is about 60° along the z direction. Besides, the lower included angle in the lower plane can avoid blocking of the total internal reflection incident light.

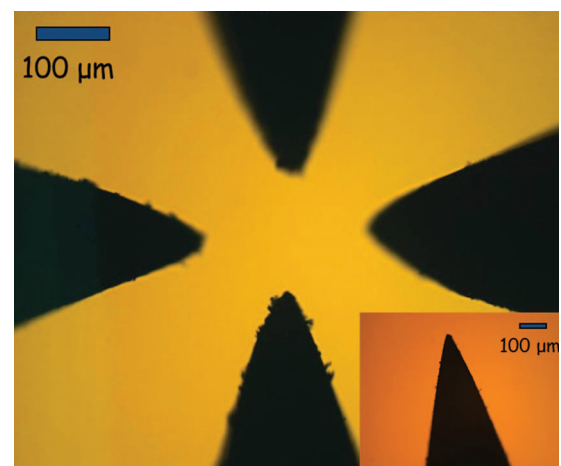


FIG. 3. The four core pieces of the upper set of magnetic poles under optical microscope. The tip of each pole is mechanically sharpened to less than $\sim 100 \mu\text{m}$ in size in order to optimize the generated magnetic driving force. The shape of the below four poles is similar, and placed with desirable distance to the sample stage.

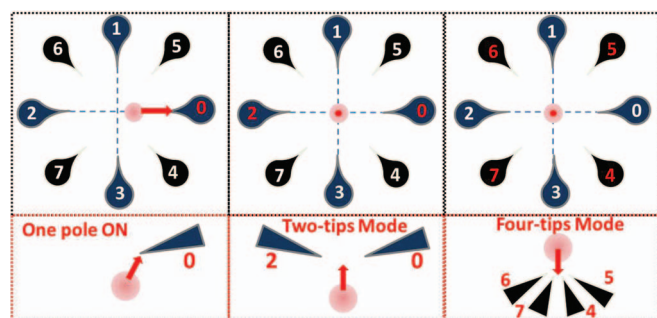


FIG. 4. Different modes are used to generate the upwards and downwards forces on the probe paramagnetic particle. The above three pictures are from the top view, and the below pictures are from the side view. From left to right: force towards the pole 0 is generated when pole 0 is turned on; a net upwards force is generated when pole 0 and 2 are turned on; and a net downwards force is generated by turning on poles 4, 5, 6, and 7 with proper voltages.

The designed magnetic tweezers can move the probe particles in three dimensions. Figure 4 shows different modes for generating either a net vertical upwards or downwards force on the probe particle. Generally, if only one pole such as pole 0 is turned on, the probe particle will be magnetized and moved towards the pole 0 tip. However, if we turn on both poles 0 and 2 at the same time with opposite currents, a net vertical upwards force is generated because the lateral forces in the plane are balanced and cancel out each other. Therefore, the upward movement of the probe particle can be achieved by such *two-tips mode*. Following the sample working principle, *four-tips mode* in which the four lower magnetic poles; 4, 5, 6, and 7 were employed to generate a net downwards force, pushing particle closer to the surface. Both *two-tips mode* and *four-tips mode* were used to generate the upwards and downwards force on the probe particle, respectively, in our later force calibration experiments.

D. Sample capillary

The sample capillary (Borosilicate glass extra wide W3520, VitroCom) used in this study has a wall thickness around $200\ \mu\text{m}$, with the hollow optical path length $\sim 200 \pm 20\ \mu\text{m}$. Atomic force microscopy showed that the inner surface is extremely smooth with RMS less than $1\ \text{nm}$. We connected the two ends of the sample capillary with two short rubber tubing by silicone adhesive (silicone window and glass sealant), which were further linked to PTFE tubing. The rubber tubings were sealed to keep the sample solution tightly inside. Tested samples with volume less than $0.1\ \text{ml}$ were required and could directly be pumped through the PTFE and rubber tubing into the capillary. The capillary was held by a copper block, fixing on the mechanical arm under the microscope objective. This design does not block the incident light, and thus allows the fixing of the magnetic poles both up and below the sample tubing. The sample tube was washed by NaOH solution before use. Four Peltier thermoelectric cooling modules (TEC1-06306 $20 \times 40\ \text{mm}^2$) were symmetrically mounted on the upper side of the copper block to control the temperature. The temperature sensor (AD590MF, two-terminal IC temperature transducer, Analog Devices) was em-

bedded into the copper block. Typically, temperature up to $45\ ^\circ\text{C}$ can be achieved with a precision of $0.1\ ^\circ\text{C}$.

E. Electronic and computer control

We control the generated current via copper coils to drive the magnetic tweezers. Since the coils are inductive and their dc resistances vary with temperature, the magnetic field cannot be accurately controlled by the coil voltage. In our setup, the eight magnetic poles were driven independently by an homemade eight-channel current amplifier, according to the design reported by Fisher and co-workers.¹⁸ The maximum current output per channel was $\pm 3\ \text{A}$. The independent 8-channel input control voltage was provided by an 8-channel voltage analog output PCI board (PCI6722 analog output 8-channel board, National Instruments.) interfaced to the computer. The direction and the generated magnetic forces were determined by a current ramp via a voltage ramp. The voltage control program was developed using Visual C language. The program was a response to generation pulse and periodic voltages combining the simultaneous TIRM signal detection. As a result, we can record the evanescent wave intensity scattered by the probe particle and generation of the control voltage at the same time. This is important to obtain a reliable result in viscoelastic measurements.

The board PCI6722 was able to generate pulse wave with variable lasting time (usually $\geq 1\ \text{ms}$) and time interval for force calibrations. The pulse wave was generated by adding constant or opposite voltages on the upwards *two-tips mode* where the component force in the x-y plane was balanced. Moreover, the board allows a maximum sample rate of $800\ \text{ks/s}$ for single channel, which corresponds to a frequency up to $8\ \text{kHz}$ and $1\ \text{kHz}$ for 8-channels. The control software therefore is capable for precise generation of sine waves at controlled frequency up to $1\ \text{kHz}$, and with independent controls of the 8-channels at one time. It is noteworthy that the frequencies ranging from 0.1 to $10\ \text{Hz}$ are commonly used in our microrheology measurements to ensure that there are enough photons entering into the PMT detector during one sampling period. Considering that the probe was positioned in the middle plane of the eight incorporated magnetic poles, the sine oscillatory movement of the probe was achieved by generation of a half sine wave variation of the voltages on the upwards *two-tips mode*, and the other half on the downwards *four-tips mode*. The applied voltage on each tip was determined by the calibration to ensure that the force amplitude of the up and down half period was the same. To minimize the heating effects, fans with strong wind were turned on to cool the coils. The temperature of the environment was strictly monitored and controlled.

III. DATA ANALYSIS

A. Force calibration and uniformity confirmation

We used viscous drag method to calibrate the generated magnetic force acting on a probe microparticle when it moved very near to a glass surface. Typically, we used evanescent illumination to track the probe particle displacement in the

z-position from the glass surface. Note that the particle-surface distance, h , is usually much smaller than the size of the probe, r , i.e., the ratio $h/r \ll 1$. In this situation, the hydrodynamic effect cannot be ignored because the motion of the particle may be hindered when the particle is nearby the wall. This so-called confinement effect could lead to a large deviation of the commonly applied Stoke's equation in the force calibration. Prieve and co-workers⁴⁹ have addressed this problem in TIRM measurements and demonstrated that the hydrodynamic force $F_{hydro}(h)$ at specific distance h can be represented as

$$f(h) = \frac{6h^2 + 4rh}{6h^2 + 18rh + 8r^2} \quad (5)$$

$$F_{hydro}(h) = 6\pi r \eta v(h)/f(h),$$

where $f(h)$ is a reducing factor, modifying the bulk diffusion coefficient with hydrodynamic effects. The hydrodynamic force $F_{hydro}(h)$ can be further simplified as^{35,50}

$$F_{hydro}(h) = 6\pi r^2 \eta v(h)/h. \quad (6)$$

Equation (6) will show a very small deviation to replace Eq. (5) in a proper h ranged between ~ 200 to 800 nm. Herein, Eq. (6) essentially represents the force exerting on the probe particle after coupling with the hydrodynamic effect. Note that the velocity, v , changes as h because the magnetic force $F_{magnetic}$ is kept as a constant in the range of probe motion which will be confirmed in this section. Accordingly, the absolute height, $h(t)$, of the probe from the surface under a constant force can be obtained using Eq. (6). Moreover, the maximum scattered light intensity, I_0 , at $h = 0$ could be determined by dragging the probe particle down to the surface using the below *four-tips mode*. As a consequence, $v(h) = dh/dt$ can be calculated from the first derivative of h - t curve.

In the calibration experiment, a selected particle dispersed in a standard sucrose aqueous solution with known η is moved upwards by the upwards *two-tips mode*. By varying the corresponding voltages, the resultant driving force generated on the probe particle can be varied which not only balances the forces in the parallel plane but also counterbalances the downwards gravity. The trajectory of the particle is tracked, and as a result the velocity can be obtained as mentioned above. Figure 5 shows a typical particle displacement from the glass surface, together with the primitive intensity $I(t)$ under an alternative upwards *two-tips mode* and downwards *four-tips mode*. From Eq. (3), a decrease of I corresponds to an increase of h . It can be clearly seen that the probe particle can be fluctuated from the bottom surface ($h \sim 0$ nm) to a distance as far as 1200 nm from the surface. Note that the particle displacement with distance higher than 1200 nm was not included since the evanescent wave signal was too small to be detected. In general, the data between 250 to 750 nm were selected and used to calculate $v(h)$. Based on the above discussion, we are now ready to construct the calibration curve relating the voltage V to the magnetic force, $F_{magnetic}$. For convenience, we replace $F_{magnetic}$ with F_{net} ($= F_{magnetic} \pm F_{gravity}$) since F_{net} equals to F_{hydro} which can be directly obtained via Eq. (6) and $F_{gravity}$ can be calculated from paramagnetic probe particles with known size and density. Due to the specific design of our sample cell, the calibration process should be done

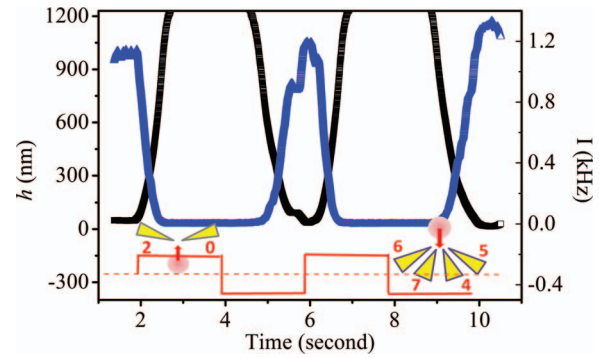


FIG. 5. The typical displacement, h ($\square$ -, left Y axis) and the original intensity I (Δ -, right Y axis) of the probe particle under the alternative forces in upwards and downwards directions during the calibration. The experiments were performed in 40 wt. % sucrose solution containing 4.5 μm paramagnetic particles coated with PEG polymers. The red square wave indicates the direction of the generated magnetic force, with the starting upwards force by two-tips mode, following by downwards force by four-tips mode.

every time. This is because fixing sample cell to the stage needs to remove and reinstall the two sets of the magnetic tweezers.

Figures 6 and 7 show the dependence of the applied voltage on the F_{net} during the movement of the paramagnetic particles M450 (Invitrogen, diameter = $4.5 \mu\text{m}$, density = 1.60 g/cm^3) upwards and downwards, respectively. The particles were coated with poly(ethylene glycol) (PEG) ($M_w \sim 1000 \text{ g/mol}$) to prevent the sticking of particle to the surface in salty solution through the steric repulsion. The inserted figures in Figures 6 and 7 were examples of a series of resultant F_{net} in dependence of h between 250 to 750 nm in both upwards and downwards directions, respectively, which were deduced from Eq. (6) using the data of h - t in Figure 5. It was clear that small deviation of F_{net} was found in the given h , indicating that a constant magnetic force was exerting in this region. As h increases, intensity decreases and noise increases, leading to a larger fluctuation of F_{net} . Following Eq. (6), averaged F_{net} with corresponding standard deviation under different voltages were presented in Figures 6 and 7. As can be seen

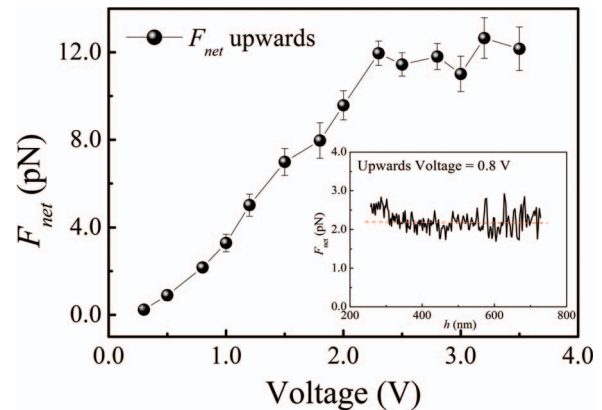


FIG. 6. The applied voltage dependence of the upwards effective magnetic force, F_{net} , generated on the $4.5 \mu\text{m}$ paramagnetic particle coating with poly(ethylene glycol) (PEG) ($M_w = 1000 \text{ g/mol}$) polymers. The insets show F_{net} in dependence of h under upwards drag at voltage = 0.8 V . The dashed line gives the averaged value. As h increases, noises increase, leading to a larger fluctuation of F_{net} .

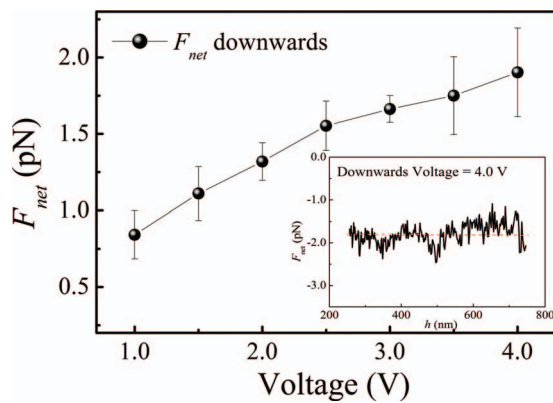


FIG. 7. The applied voltage dependence of the downwards effective magnetic force, F_{net} , generated on the $4.5 \mu\text{m}$ paramagnetic particle coating with PEG ($M_w = 1000 \text{ g/mol}$) polymers. The inset shows F_{net} in dependence of h under downwards drag at voltage = 4.0 V . The dashed line gives the averaged value.

in Figure 6, when the applied voltage V is less than 0.5 V , the magnetic force and V shows a nonlinear relationship. However, it becomes linear when V is further increased up to 2.5 V . After that the induced magnetic moment (M) is saturated. The slight fluctuation of force at higher voltages may be related to the maximum permeability of the electromagnetic core. In this way, the magnetic induction intensity B reaches a critical value so that it will not further increase even increasing the current of the electromagnetic coil. In Figure 6, it can be seen that the maximum upwards F_{net} generated in our setup is more than 12 pN . If the distance between the particle and the magnet tips is around 1 mm , our magnetic tweezers enable generating the force up to 20 pN on a $4.5 \mu\text{m}$ paramagnetic particle. Unlike the upwards force, the maximum generated downwards F_{net} in our magnetic tweezers is around 3 pN (Figure 7). This is because the prism under the sample capillary does not allow the magnetic poles positioning closely to the sample capillary. Nevertheless, we can manipulate the upwards and downwards forces by producing oscillatory force on the probe particle with the same amplitude in the pN scales.

On the other hand, to confirm the uniformity of the generated magnetic force in our magnetic tweezers, we further checked the relationship between the magnetic force and the z -position of the probe particle using the results obtained under the upwards *two-tips mode*. By employing the simple model proposed by de Vries *et al.*,⁵¹ the magnetic field gradient, ∇B , was derived in an analytical expression as

$$\nabla B(r) = \frac{4\mu_0 M_m \alpha}{(4\alpha r + 1)^2}, \quad (7)$$

where r is the distance of the particle from the pole tip, μ_0 is the vacuum permeability, and M_m is the magnetization of the pole. Note that the pole tip was considered as a parabolic shape with a sharpness α and was equaled to the height of the parabolic shape divided by the square of the radius of the corresponding intersection. Replacing r with the vertical component z in Eq. (7), we can obtain dB/dz as

$$dB/dz = \frac{4\mu_0 M_m \alpha x}{(4\alpha \sqrt{z^2 + x^2} + 1)^2 \cdot \sqrt{z^2 + x^2}}, \quad (8)$$

where x is defined as the parallel distance between the pole and the particle. As x is often between 50 and $100 \mu\text{m}$, less than $1/10$ of z , $z^2 + x^2$ can be approximated as z^2 . In this way, dB/dz is proportional to $1/[z(4\alpha z + 1)^2]$. The value of α in our poles was around 0.111 (with intersection radius = 3 mm and length = 1 cm). In our measurements, the probe paramagnetic particle moves within $1 \mu\text{m}$ (0.001 mm) from the internal surface of the tubing. The vertical distance between the surface and the pole tips is between 1 mm and 2 mm , including the optical path of the tubing (0.2 mm), the top wall thickness (0.2 mm), and the air gap between the top of the tubing and the pole tips. As a result, dB/dz is expected to change less than 0.1% as z changes from 1 mm to 1.001 mm , showing that the magnetic driving force exerted by single magnetic pole has little influence on the range of the probe particle. Therefore, the magnetic driving force under the *two-tips* and *four-tips* modes can be considered as constant as the particle moving within μm range near the sample surface.

B. Oscillatory measurement

After the calibration, we performed the additional measurements on some selected complex fluids. In rheology measurements, the rheological properties are usually described by a frequency dependent parameter; complex modulus $G^*(\omega)$ which reflects the extent of a material to be solid or liquid like. To yield the $G^*(\omega)$ [$= G'(\omega) + iG''(\omega)$], where $G'(\omega)$ and $G''(\omega)$ are elastic and loss modulus, respectively, oscillatory measurements are often employed. In the oscillatory measurements, a sine function, $F(t) = F_0 \sin(\omega t)$, was generated on the probe paramagnetic particle and the displacement of the particle was monitored. Our magnetic tweezers are able to generate stable periodic forces at frequency up to 10^3 Hz , which is high enough for studying most of the biomaterials and macromolecules. Figure 8 represents the displacement of a probe particle in gelatin aqueous solution measured under the same force and varied frequencies (from $1, 2$, to 5 Hz). It clearly shows that our setup has the feasibility to conduct the oscillatory measurements in complex solutions. Note that the displacement at each frequency was shifted up 200 nm for the observation purpose. It is clear that at the same force

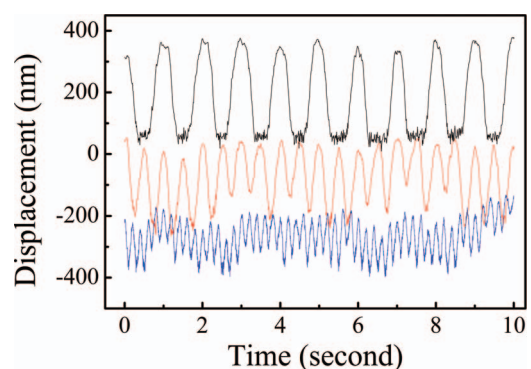


FIG. 8. Probe particle displacements, $h(t)$, measured under oscillatory forces at 1 Hz (black), 2 Hz (red), and 5 Hz (blue) with the same force amplitude in gelatin aqueous solution.

amplitude, a higher frequency leads to the smaller displacement amplitude.

In near-wall case, the viscoelasticity of the complex fluids not only depends on the applied frequency but also depends on the distance with respect to the surface. Thus, at one specific angular frequency ω and distance h_c , the oscillatory experiments yield the complex modulus $G^*(\omega, h_c) = G'(\omega, h_c) + iG''(\omega, h_c)$ which can be deduced from⁵²

$$\frac{F_0 h_c}{6\pi r^2} = h^*(\omega) G^*(\omega, h_c), \quad (9)$$

where $h^*(\omega)$ is the resultant displacement, $h(t) = A \sin(\omega t - \varphi)$ is in frequency domain. Note that Eq. (9) is valid as long as the amplitude A of $h(t)$ is small and the hydrodynamic force is constant at the oscillatory height ranging from $h_c - A$ to $h_c + A$. The value of A depends on the specific case and it is usually set within around 20 nm in our experiments. As a result, the viscoelastic modulus of the sample at different ω and h_c can be obtained, respectively, from the oscillatory experiments. Based on Eq. (9), the modulus G' and G'' can be obtained from the phase shift of $h(t)$ compared with the oscillatory force as

$$\begin{aligned} G'(\omega, h) &= \frac{F_0 h_c}{6\pi r^2 A} \cos \varphi \\ G''(\omega, h) &= \frac{F_0 h_c}{6\pi r^2 A} \sin \varphi. \end{aligned} \quad (10)$$

Therefore, the microrheological properties at different distances from the surface can be measured under this mode. This unique feature would help us to further explore the interesting structures of soft matter near a flat surface, such as the physical packing of soft microgel nanoparticles, the structure of the polymer brushes grafting on the surface, and the adsorption and desorption of polymer layers.

Oscillatory experiments involve the extraction of the phase shift and amplitude of the signal under arbitrary frequency. In our case, the initial data spectrum was filtered and the response under the arbitrary frequency was extracted. Consequently, the phase shift and amplitude were obtained by fitting the resultant responses with a waveform function. We will later establish the procedure of exacting the phase shift from $h(t)$ utilizing lock-in-amplifier to improve the performance.

IV. TESTING ON STANDARD POLYMER SOLUTIONS

In order to gauge our setup, we applied this microrheology to measure the viscoelastic properties of two standard samples using oscillatory mode and compared the results to bulk rheometer. We selected sucrose (BDH) and PEG ($M_w = 900\,000$ g/mol, Sigma-Aldrich) as the model samples. For concentrated sucrose solutions, the viscoelasticity at different heights from the glass surface may vary slightly compared to the bulk solution since it is a Newtonian liquid and such small molecules do not form any heterogeneous structures. On the other hand, for the PEG solutions, the viscoelastic behavior in bulk or near the surface may be similar because the size of the probe particle is much larger than the correlation length ξ of these polymers in concentrated solution (ξ is in the or-

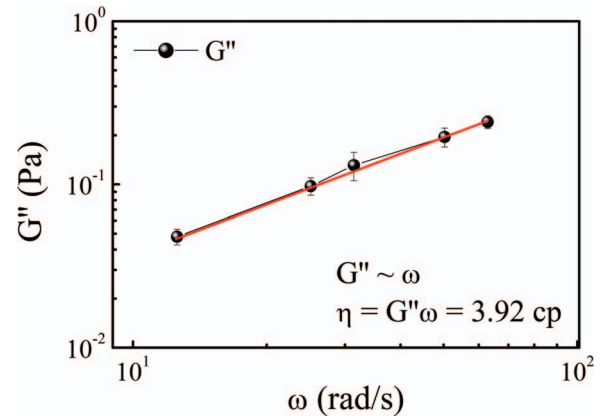


FIG. 9. The modulus G'' of sucrose solution measured by our magnetic tweezers (MT) microrheometer under force amplitude = 1.14 pN (black solid circle). The solid line shows a linear fit, leading to the viscosity $\eta = G''/\omega$ which is a constant in the measurable range.

der of several tens nm).¹⁰ These two samples were proved to have little adsorption on the probe particles and the use of low M_w polymers can suppress the depletion attraction. Bulk rheological measurements were performed on a strain-controlled rheometer (Anton Paar RHEOPLUS MCR301) with parallel plate geometry from 0.5 to 200 rad/s for PEG sample. The strain was fixed at 5% for the linear viscoelastic region.

A complex fluid containing the sucrose solution at a concentration of 40.0 wt. % or PEG aqueous solution at 2.0 wt. % with the dispersed M450 particles (number density $\sim 10^6$ ml⁻¹) was prepared and pumped into the sample tube, respectively. Note that 1 mM sodium chloride (NaCl) solution was added to screen the electrostatic interaction between the polymers. As shown in Eq. (10), G' and G'' at frequencies varying from 0.1 to 0.8 Hz can produce the forces ranging from 1.14 and 1.75 pN. We manipulate the probe particle oscillating at distance around 200 nm from the glass surface so as to avoid the complicated interactions at the short-ranges. The experiments were repeated several times at room temperature and at different heights from the glass surface in order to show that no heterogeneous structures are formed near the surface.

As a standard Newtonian fluid, sucrose solution displays a fully liquid behavior, which is reflected in a constant viscosity at arbitrary frequency and having little elastic behavior. The G' of the sucrose solution is small and the microrheological results show some irregular fluctuations. However, Figure 9 shows that the measured G'' validates this principle that the viscosity η calculated from the modulus yields a constant value of 3.92 cp, which is close to the literature value 5.32 cp at ambient conditions.⁵³ On the other hand, for PEG solution at concentration of 2.0 wt. %, similar to the sucrose solution, a power scaling of $G'' \sim \omega$ was found, indicating that at this frequency range, the solution mostly behaves like a Newtonian liquid since the static viscosity η determined by G''/ω is a real constant. This scaling law might be invalid as the frequency increases. The reason is that at high frequency, the internal structure of the entangled polymer chains might not respond to the frequency change and act as solid-like behavior. Moreover, Figure 10 clearly shows that for both

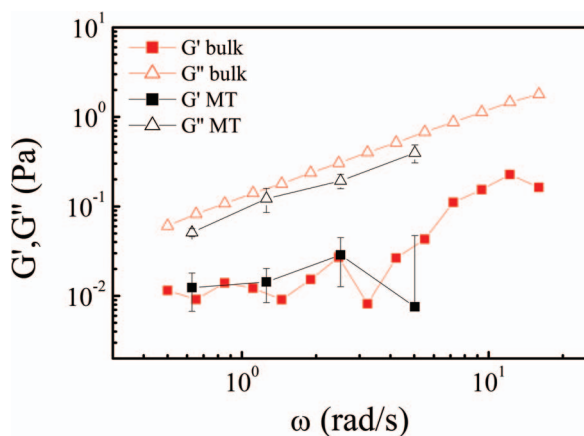


FIG. 10. The modulus G' and G'' of 2 wt. % PEG solution measured by our MT microrheometer under force amplitude = 1.14/1.75 pN (G' : $\blacksquare$ -, G'' : $\blacktriangle$ -) and the bulk rheometer (G' : $\blacksquare$ -, G'' : $\blacktriangle$ -) with strain = 5%.

moduli, the measured results had a good agreement with the bulk measurements, indicating that the local physical properties of the polymer solutions are not interrupted by the driving forces at pN scales as well as the surface confinement.

V. DEMONSTRATION OF THE NEAR-WALL RHEOLOGICAL MEASUREMENTS

Our developed microrheological method has further been used to measure the rheological properties of concentrated poly(*N*-isopropylacrylamide) (PNIPAM) microgel aqueous solution near the surface. Because of being soft and deformable, the physiochemical properties of PNIPAM microgel particles are entirely different from the conventional rigid-solid particles.⁵⁴ The synthesis and purification of the PNIPAM microgel particles have been reported before.^{38,55} The resultant microgel particles have a diameter around 125 nm at room temperature as determined by dynamic laser light scattering (ALV-5000). The probe M450 particles coated with PEG were dispersed in the microgel solution and NaCl (1 mM) was added to screen the electrostatic interaction between the particles. The displacement of probe particle around two distances; $h_c = 135$ and 17 nm, from the surface was tracked to determine the rheological properties of the microgel solutions. We employed the oscillatory mode at frequency of 0.1 and 0.2 Hz and force amplitude was 8.60 pN. We opted to oscillate the probe particle at these two special heights because they cover the distance from very near the surface to a height larger than the size of a single microgel. Figure 11 shows that very different viscoelastic properties were obtained when the probe particles moved at different heights from the glass surface. The G'' obtained at $h_c = 17$ nm is smaller than at $h_c = 135$ nm. In contrast, G' is larger at $h_c = 17$ nm. Besides, G''/ω at $h_c = 135$ nm equals to 1.59 and 1.61 Pa s at 0.1 and 0.2 Hz, respectively. The close values at these two frequencies likely indicate that when microgel particles are far away from the surface, the dispersion still behaves as a liquid and does not affect the dynamic of the probe particle. On the other hand, as the probe particle is forced closer to the surface such as at $h_c = 17$ nm, the elastic property of the dispersion becomes dominant, resulting in $G' > G''$ at both frequencies.

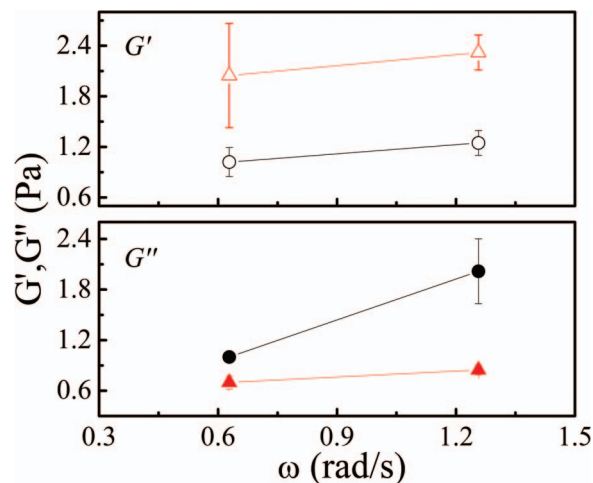


FIG. 11. The elastic modulus G' at $h_c = 135$ ($\circ$ -) and 17 nm ($\triangle$ -) (upper figure); and the loss modulus G'' at $h_c = 135$ ($\bullet$ -) and 17 nm ($\blacktriangle$ -) (lower figure) measured at frequency = 0.1 and 0.2 Hz. The measurements were conducted in 4.5 wt. % PNIPAM microgel solution under force amplitude = 8.60 pN.

This can be attributed to the existing near-wall boundary, as well as the adsorption of microgel particles to the glass surface, resulting in a soft layer sandwiched between the probe and the surface. This simple demonstration shows the feasibility of using our setup to study the confinement effect on the complex fluids and their related structures near the wall.

VI. CONCLUSION

We have demonstrated a practical implementation of magnetic tweezers with evanescent illumination for quantitative detection of viscoelastic properties of complex fluids, especially near solid surface. The exponential distance dependence of the evanescent field makes our technique extremely sensitive to detect particle displacement in *z*-position at the subnanometer level. Using TIRM, we do not require a laterally resolved image, only the scattered intensity, making it possible to view a single, label-free particle in parallel with a low-resolution objective. Moreover, the integration of a magnetic driving force into TIRM enables us to effectively manipulate the embedded particle in three dimensions. We have shown that local viscoelastic properties of two standard solutions can be measured over proper time ranges and showed good agreements with the results from a bulk rheometer. Additionally, the effect of confinement on the structure property of deformable nanoparticles has been briefly demonstrated. Thereby, the combination of the magnetic tweezers and evanescent wave-scattered particle tracking has great potential for noninvasive and spatially resolved characterization of rheological properties in soft materials including biological samples.

It is noteworthy that when measuring the viscoelastic properties of materials at distance several hundreds nanometers from the surface, some kinds of interaction forces, such as double layer repulsion, depletion interaction, and the surface entanglements might contribute to the motion of probe particle. These effects could be minimized by adding salt, surface

modification of the probe particles, and avoiding the probes too close to the interface. Further improvements of our setup will include the enhancement of both upwards and downwards poles to extend the force and frequency ranges. Although the magnetic forces generated by our instrument are not strong compared to some reported literatures,⁵⁶ they are high enough to exceed most of weak interactions in solutions and the accurate displacement of the probe be manipulated. Moreover, with the improved performance in both creep and oscillatory experiments, we expect that this active microrheology technique can offer a totally different way to study the heterogeneous structures of fragile and time-dependent systems under spatial limitation.

ACKNOWLEDGMENTS

The financial support of this work by the Hong Kong Special Administration Region (HKSAR) General Research Fund (CUHK402809, 2160387) is gratefully acknowledged.

- ¹T. A. Waigh, *Rep. Prog. Phys.* **68**, 685 (2005).
- ²P. Cicuta and A. M. Donald, *Soft Matter* **3**, 1449 (2007).
- ³T. M. Squires and T. G. Mason, *Annu. Rev. Fluid Mech.* **42**, 413 (2010).
- ⁴T. G. Mason, H. Gang, and D. A. Weitz, *J. Opt. Soc. Am. A* **14**, 139 (1997).
- ⁵B. Dasgupta, S.-Y. Tee, J. Crocker, B. Frisken, and D. Weitz, *Phys. Rev. E* **65**, 051505 (2002).
- ⁶T. G. Mason and D. A. Weitz, *Phys. Rev. Lett.* **74**, 1250 (1995).
- ⁷J. Wu and L. L. Dai, *Langmuir* **23**, 4324 (2007).
- ⁸D. Chen, E. Weeks, J. Crocker, M. Islam, R. Verma, J. Gruber, A. Levine, T. Lubensky, and A. Yodh, *Phys. Rev. Lett.* **90**, 108301 (2003).
- ⁹I. Wong, M. Gardel, D. Reichman, E. Weeks, M. Valentine, A. Bausch, and D. Weitz, *Phys. Rev. Lett.* **92**, 178101 (2004).
- ¹⁰T. G. Mason, K. Ganesan, J. H. vanZanten, D. Wirtz, and S. C. Kuo, *Phys. Rev. Lett.* **79**, 3282 (1997).
- ¹¹J. C. Crocker, M. T. Valentine, E. R. Weeks, T. Gisler, P. D. Kaplan, A. G. Yodh, and D. A. Weitz, *Phys. Rev. Lett.* **85**, 1770 (2000).
- ¹²M. Gardel, M. Valentine, J. Crocker, A. Bausch, and D. Weitz, *Phys. Rev. Lett.* **91**, 198101 (2003).
- ¹³Y. Tseng, T. P. Kole, and D. Wirtz, *Biophys. J.* **83**, 3162 (2002).
- ¹⁴S. B. Smith, *Science* **258**, 1122 (1992).
- ¹⁵T. Strick, J. F. Allemand, V. Croquette, and D. Bensimon, *Prog. Biophys. Mol. Biol.* **74**, 115 (2000).
- ¹⁶F. Mosconi, J. Allemand, D. Bensimon, and V. Croquette, *Phys. Rev. Lett.* **102**, 078301 (2009).
- ¹⁷C. Gosse and V. Croquette, *Biophys. J.* **82**, 3314 (2002).
- ¹⁸J. K. Fisher *et al.*, *Rev. Sci. Instrum.* **76**, 053711 (2005).
- ¹⁹K. Kim and O. A. Saleh, *Nucleic Acids Res.* **37**, e136 (2009).
- ²⁰A. R. Bausch, W. Moller, and E. Sackmann, *Biophys. J.* **76**, 573 (1999).
- ²¹F. G. Schmidt, B. Hinner, E. Sackmann, and J. X. Tang, *Phys. Rev. E* **62**, 5509 (2000).
- ²²P. Kollmannsberger, C. T. Mierke, and B. Fabry, *Soft Matter* **7**, 3127 (2011).
- ²³A. R. Bausch, F. Ziemann, A. A. Boulbitch, K. Jacobson, and E. Sackmann, *Biophys. J.* **75**, 2038 (1998).
- ²⁴A. R. Bausch, U. Hellerer, M. Essler, M. Aepfelbacher, and E. Sackmann, *Biophys. J.* **80**, 2649 (2001).
- ²⁵M. Singh-Zocchi, S. Dixit, V. Ivanov, and G. Zocchi, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 7605 (2003).
- ²⁶H. Brutzer, F. W. Schwarz, and R. Seidel, *Biophys. J.* **100**, 10 (2011).
- ²⁷P. M. Oliver, J. S. Park, and D. Vezennov, *Nanoscale* **3**, 581 (2011).
- ²⁸H. Brutzer, F. W. Schwarz, and R. Seidel, *Nano Lett.* **12**, 473 (2012).
- ²⁹A. Sarkar, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 12882 (2004).
- ³⁰R. Liu, S. Garcia-Manyes, A. Sarkar, C. L. Badilla, and J. M. Fernandez, *Biophys. J.* **96**, 3810 (2009).
- ³¹S. Dixit, M. Singh-Zocchi, J. Hanne, and G. Zocchi, *Phys. Rev. Lett.* **94**, 118101 (2005).
- ³²A. L. Mattheyses and D. Axelrod, *J. Biomed. Opt.* **11**, 014006 (2006).
- ³³C. Gell, M. Berndt, J. Enderlein, and S. Diez, *J. Microsc. (Oxford)* **234**, 38 (2009).
- ³⁴P. Sharma, S. Ghosh, and S. Bhattacharya, *Nat. Phys.* **4**, 960 (2008).
- ³⁵C. Derail, F. Lapique, and J.-P. Montfort, *Macromolecules* **44**, 7438 (2011).
- ³⁶A. K. Kandar, R. Bhattacharya, and J. K. Basu, *Phys. Rev. E* **81**, 041504 (2010).
- ³⁷M. H. Lee, D. H. Reich, K. J. Stebe, and R. L. Leheny, *Langmuir* **26**, 2650 (2010).
- ³⁸Y. Song and L. L. Dai, *Langmuir* **26**, 13044 (2010).
- ³⁹E. M. Balzer, Z. Tong, C. D. Paul, W. C. Hung, K. M. Stroka, A. E. Boggs, S. S. Martin, and K. Konstantopoulos, *FASEB J.* **26**, 4045 (2012).
- ⁴⁰J. Mateos-Langerak *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 3812 (2009).
- ⁴¹A. Kusumi, Y. Sako, and M. Yamamoto, *Biophys. J.* **65**, 2021 (1993).
- ⁴²A. Kusumi, *Biophys. J.* **66**, A18 (1994).
- ⁴³P. Peyla, *EPL* **80**, 34001 (2007).
- ⁴⁴D. C. Prieve, F. Luo, and F. Lanni, *Faraday Discuss.* **83**, 297 (1987).
- ⁴⁵D. C. Prieve, *Adv. Colloid Interface Sci.* **82**, 93 (1999).
- ⁴⁶H. Chew, D. S. Wang, and M. Kerker, *Appl. Opt.* **18**, 2679 (1979).
- ⁴⁷F. Jin, X. Gong, J. Ye, and T. Ngai, *Soft Matter* **4**, 968 (2008).
- ⁴⁸T. Ngai, X. Xing, and F. Jin, *Langmuir* **24**, 13912 (2008).
- ⁴⁹D. C. P. Michael and A. Bevana, *J. Chem. Phys.* **113**, 1228 (2000).
- ⁵⁰D. Y. C. Chan and R. G. Horn, *J. Chem. Phys.* **83**, 5311 (1985).
- ⁵¹A. H. B. de Vries, B. E. Krenn, R. van Driel, and J. S. Kanger, *Biophys. J.* **88**, 2137 (2005).
- ⁵²M. L. Gardel, M. T. Valentine, and D. A. Weitz, *Microscale Diagnostic Techniques*, edited by K. Breuer (Springer Verlag, 2005).
- ⁵³P. Honig, *Principles of Sugar Technology* (Elsevier, 1953).
- ⁵⁴J. Mattsson, H. M. Wyss, A. Fernandez-Nieves, K. Miyazaki, Z. Hu, D. R. Reichman, and D. A. Weitz, *Nature (London)* **462**, 83 (2009).
- ⁵⁵S. Q. Zhou and B. Chu, *J. Phys. Chem. B* **102**, 1364 (1998).
- ⁵⁶P. Kollmannsberger and B. Fabry, *Rev. Sci. Instrum.* **78**, 114301 (2007).