

## Two-Point Microrheology of Inhomogeneous Soft Materials

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We demonstrate a novel method for measuring the microrheology of soft viscoelastic media, based on cross correlating the thermal motion of pairs of embedded tracer particles. The method does not depend on the exact nature of the coupling between the tracers and the medium, and yields accurate rheological data for highly inhomogeneous materials. We demonstrate the accuracy of this method with a guar solution, for which other microscopic methods fail due to the polymer's mesoscopic inhomogeneity. Measurements in an F-actin solution suggest conventional microrheology measurements may not reflect the true bulk behavior.

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Many interesting and important materials such as polymers, gels, and biomaterials are viscoelastic; when responding to an external stress, they both store and dissipate energy. This behavior is quantified by the complex shear modulus,  $G^*(\omega)$ , which provides insight into the material's microscopic dynamics. Typically,  $G^*(\omega)$  is measured by applying oscillatory strain to a sample and measuring the resulting stress. Recently a new method, called microrheology, has been developed which determines  $G^*(\omega)$  from the thermal motion of microscopic tracer particles embedded in the material [1,2]. Microrheology offers significant potential advantages: it provides a local probe of  $G^*(\omega)$  in miniscule sample volumes and can do so at very high frequencies. While microrheology provides an accurate measure of  $G^*(\omega)$  for simple systems, its validity in common complex systems is far from certain. If the tracers locally modify the structure of the medium, or sample only pores in an inhomogeneous matrix, then bulk rheological properties will not be determined. Such subtle effects currently limit many interesting applications of microrheology.

In this Letter, we introduce a new formalism, which we term "two-point microrheology," based on measuring the cross-correlated thermal motion of pairs of tracer particles to determine  $G^*(\omega)$ . This new technique overcomes the limitations of single-particle microrheology. It does not depend on the size or shape of the tracer particle; moreover it is independent of the coupling between the tracer and the medium. We demonstrate the validity of this approach with measurements on a highly inhomogeneous material, a solution of the polysaccharide guar. Two-point microrheology correctly reproduces results obtained with a mechanical rheometer, whereas single-particle microrheology gives erroneous results. We also compare ordinary and two-point microrheology of F-actin [2–4], a semiflexible biopolymer constituent of the cytoskeleton. Different results are obtained with the two techniques, suggesting that earlier interpretations of F-actin microrheology should be reexamined.

Conventional microrheology [1,2] uses the equation:

$$\tilde{r}^2(s) = \frac{k_B T}{\pi s a \tilde{G}(s)}, \quad (1)$$

where  $\tilde{r}^2(s)$  is the Laplace transform of the tracers' mean squared displacement,  $\langle \Delta r^2(\tau) \rangle$ , as a function of Laplace frequency  $s$ , and  $a$  is their radius. Equation (1) is the Stokes-Einstein equation generalized to a frequency-dependent viscosity [ $s^{-1} \tilde{G}(s)$ ] that accounts for elasticity [1]. Equation (1) is subject to the same conditions as the Stokes calculation: overdamped spherical tracer particles in an homogeneous, incompressible continuum with no-slip boundaries. If the tracers inhabit cavities in a porous medium, or create their own cavities by steric or chemical interactions with the material itself, their mobilities may be much greater than predicted by Eq. (1) [5].

Since the effect of such inhomogeneities is difficult to quantify, we seek a means to discriminate between a tracer moving in a soft pore in an otherwise very rigid matrix from another tracer moving with the same amplitude in an homogeneous soft matrix. One difference between these cases arises from the long-range deformation or flow in the matrix due to the tracer's motion. In the homogeneous case, this strain field is proportional to the tracer motion and decays  $\sim a/r$ , where  $r$  is the distance from the tracer. For the hypothetical tracer in a soft cavity, the strain field is localized to the cavity itself.

Since one tracer's strain field will entrain a second particle, we can measure the strain field by cross correlating two tracers' motion. Recent experiments have probed such correlated motion in viscous [6] and elastic [7] materials. We use multiparticle video tracking [8] to measure the vector displacements of the tracers  $\Delta r_\alpha(t, \tau) = r_\alpha(t + \tau) - r_\alpha(t)$  where  $t$  is the absolute time and  $\tau$  is the lag time. We then calculate the ensemble averaged tensor product of the tracer displacements:

$$D_{\alpha\beta}(r, \tau) = \langle \Delta r_{\alpha}^i(t, \tau) \Delta r_{\beta}^j(t, \tau) \delta[r - R^{ij}(t)] \rangle_{i \neq j, t}, \quad (2)$$

where  $i$  and  $j$  label different particles,  $\alpha$  and  $\beta$  label different coordinates, and  $R^{ij}$  is the distance between particles  $i$  and  $j$ . The average is taken over the “distinct” terms ( $i \neq j$ ); the “self” terms yield  $\langle \Delta r^2(\tau) \rangle \times \delta(r)$ .

For an incompressible continuum, the expected two-point correlation is computed by multiplying the displacement predicted in Eq. (1) by the strain field of a point stress [9]. The result, in the limit  $r \gg a$ , is

$$\tilde{D}_{rr}(r, s) = \frac{k_B T}{2\pi r s \tilde{G}(s)}, \quad D_{\theta\theta} = D_{\phi\phi} = \frac{1}{2} D_{rr}, \quad (3)$$

where  $\tilde{D}_{rr}(r, s)$  is the Laplace transform of  $D_{rr}(r, \tau)$  and the off-diagonal tensor elements vanish. Significantly, Eq. (3) has no dependence on  $a$ , suggesting that  $D_{\alpha\beta}(r, \tau)$  is independent of the tracer’s size, shape and boundary conditions with the medium in the limit  $r \gg a$ .

While we will treat our samples as incompressible, Eq. (3) can be generalized to compressible media by using a different Stokes-Einstein relation and strain field [9]. The compressibility changes the anisotropy of the strain field, and modifies the relative amplitude of the tensor elements. Thus, measuring the different tensor elements should enable measurements of compressibility [7].

We use Eq. (3) as the basis for the microrheology of inhomogeneous media which cannot be modeled by Eq. (1). We demonstrate the effectiveness of this approach empirically, and present a simple argument in its favor. The thermal motion of a soft viscoelastic medium can be described as a stochastic, time-fluctuating strain field characterized by a spectral density that depends on frequency and spatial wavelength [10]. The Brownian motion of a single tracer is the superposition of such modes with wavelengths greater than the particle radius,  $a$ . The correlated motion of two tracers is driven by those modes with wavelengths greater than their separation  $r$  rather than  $a$ , since shorter wavelength modes do not move the tracers in phase. Thus, the correlated motion of two tracers separated by more than the coarse-graining length scale in an inhomogeneous medium will depend only on the coarse-grained  $G^*(\omega)$ .

Comparing Eq. (3) and Eq. (1) suggests that we define a distinct mean squared displacement,  $\langle \Delta r^2(\tau) \rangle_D$  as

$$\langle \Delta r^2(\tau) \rangle_D = \frac{2r}{a} D_{rr}(r, \tau). \quad (4)$$

This quantity is just the thermal motion obtained by extrapolating the long-wavelength thermal undulations of the medium down to the bead size. If and only if the assumptions implicit in Eq. (1) are valid will  $\langle \Delta r^2(\tau) \rangle_D = \langle \Delta r^2(\tau) \rangle$ . Any difference in the displacements provides insight into the local microenvironment experienced by the tracers. We can then understand  $\langle \Delta r^2(\tau) \rangle$  as a superposition of a long-wavelength motion described by  $\langle \Delta r^2(\tau) \rangle_D$  plus a local motion in a cavity.

In practice, we first confirm that  $D_{rr} \sim 1/r$ , which indicates that the medium can be treated as an (coarse-grained) homogeneous continuum. This was the case for all our samples over the length scales we studied,  $3 < r < 30 \mu\text{m}$ . We then use the average value of  $rD_{rr}$  over that range to calculate  $\langle \Delta r^2(\tau) \rangle_D$  from Eq. (4). Finally, we calculate the two-point microrheology result by substituting  $\langle \Delta r^2(\tau) \rangle_D$  into Eq. (1) in place of  $\langle \Delta r^2(\tau) \rangle$ .

Several numerical procedures for calculating the shear modulus from  $\langle \Delta r^2(\tau) \rangle$  have appeared in the literature [1,2,4]. We first approximate  $\langle \Delta r^2(\tau) \rangle$  locally by a second-order polynomial (spline) in the logarithmic plane to obtain a smoothed estimate of the function and its first logarithmic derivative. We then use approximate, local algebraic expressions to calculate the storage,  $G'(\omega)$ , and loss,  $G''(\omega)$ , moduli (defined by  $G^* = G' + iG''$ ), from the smoothed value and derivative [1]. This method approaches the exact result as  $\langle \Delta r^2(\tau) \rangle$  approaches a power law, and has the advantage that it does not require the experimental data to be fit to an analytic model, nor does it suffer from the truncation errors of numerical integral transforms. On the basis of numerical tests, we expect all the moduli presented here to have systematic errors smaller than 5% of  $|G^*|$ , although larger errors are possible near the frequency extrema.

We used submicron fluorescent beads as tracers (molecular probes, Rhodamine Red-X labeled carboxylate-modified latex/polystyrene). We sealed the samples between a No. 1.5 glass cover slip and microscope slide with UV-curing epoxy (Norland No. 81). The tracers were imaged with epifluorescence (Leica, inverted microscope, DM-IRB/E) using a 100 $\times$ , NA = 1.4, oil-immersion objective at a magnification of 129 nm/CCD pixel and a video shutter time of 2 msec. To minimize wall effects, we focused at least 25  $\mu\text{m}$  into the 150  $\mu\text{m}$  thick sample chambers. A few hundred particles were located within the field of view and 2  $\mu\text{m}$  depth of focus. For each sample, 10 min of video were recorded, which yields a few million two-dimensional positions with 60 Hz temporal and 20 nm spatial resolution [8].

As a control, we measured the two-point correlation,  $D_{\alpha\beta}$ , of 0.47  $\mu\text{m}$  diameter tracers in a glycerol/water mixture. The results confirm our expectations for a simple fluid, as shown in Fig. 1(a). The functional form is  $D_{rr}$ ,  $D_{\phi\phi} \propto \tau/r$  to within statistical error, with  $D_{\phi\phi}/D_{rr} = 0.43 \pm 0.06$ , in agreement with Eq. (3) for an incompressible medium. As shown in Fig. 1(b), we find that  $\langle \Delta r^2(\tau) \rangle_D$  is equal to  $\langle \Delta r^2(\tau) \rangle$  and is linear to within statistical errors, at least when a small constant is subtracted from the latter. This small constant added onto  $\langle \Delta r^2(\tau) \rangle$  is simply the squared measurement error of the tracer positions. Since the errors for two tracers are uncorrelated,  $\langle \Delta r^2(\tau) \rangle_D$  is unaffected.

To demonstrate the effectiveness of two-point microrheology in inhomogeneous media, we compared measurements of a guar solution with those from a mechanical rheometer. Guar is a naturally occurring neutral polysaccharide ( $M_W \approx 10^6$ ) extracted from the guar gum bean. A

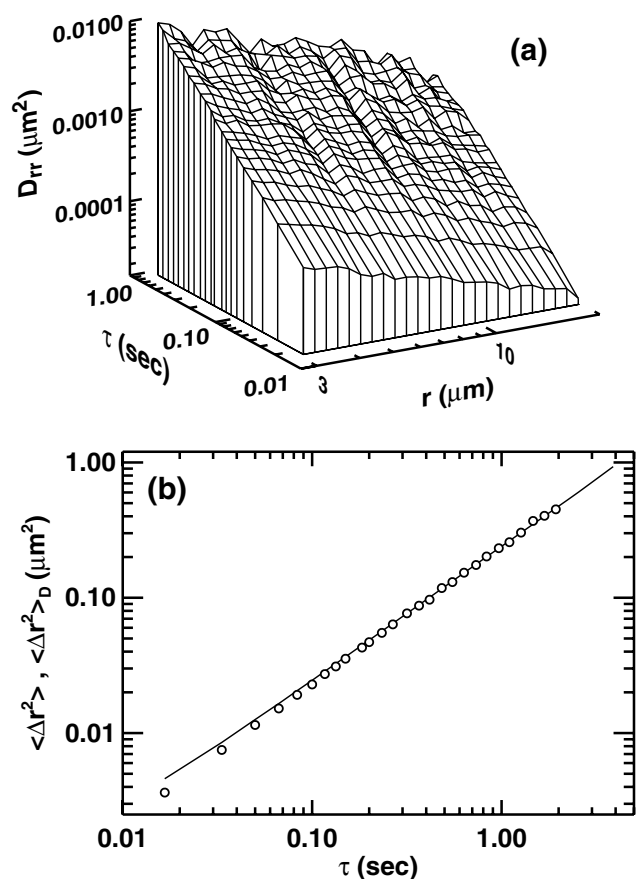


FIG. 1. (a) Two-point correlation function,  $D_{rr}$ , for  $0.47 \mu\text{m}$  diameter beads dispersed in a glycerol/water solution, as a function of  $r$  and  $\tau$ . In a triple-log plot, the surface is a plane and is  $\propto \tau/r$ . (b)  $\langle \Delta r^2(\tau) \rangle_D$  calculated from  $D_{rr}$  (circles) overlaid on  $\langle \Delta r^2(\tau) \rangle$  (line). The agreement between the two indicates that the fluid satisfies Eq. (1).

small concentration of guar in water dramatically changes the viscoelastic properties, because of the formation of high-molecular weight, mesoscopic aggregates [11] resulting from random associations of the guar molecules. This presents a highly inhomogeneous medium that is ideally suited to testing our technique.

Our results for  $0.20 \mu\text{m}$  diameter tracers in a 0.25%-by-weight guar solution are shown in Fig. 2. Unlike the simple fluid case, the two mean squared displacements do not correspond, but disagree by roughly a factor of 2 and have a somewhat different functional form, as shown in Fig. 2(a). We converted both to their corresponding  $G'(\omega)$  and  $G''(\omega)$ , and compared the results to directly measured moduli from a controlled-strain rheometer (Rheometric Scientific, Ares F). As shown in Fig. 2(b) the moduli calculated from the two-point correlation function are in good agreement with the results obtained with the rheometer, correctly capturing the crossover of  $G'(\omega)$  and  $G''(\omega)$  at 40 rad/s. Single-particle microrheology provides qualitatively different moduli and completely fails to detect the crossover. This confirms the underlying concepts of the two-point method, and verifies its accuracy in

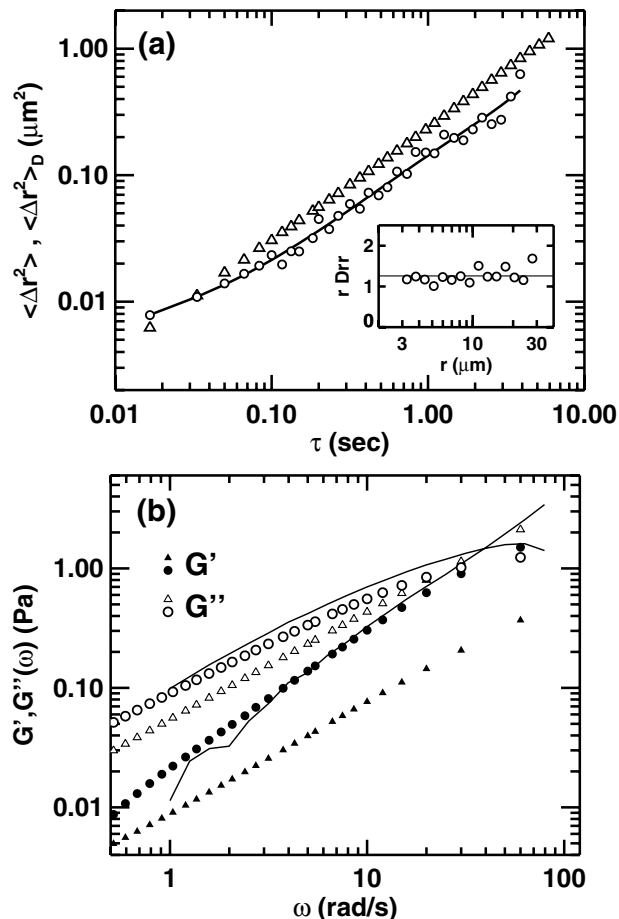


FIG. 2. (a) Comparison of the self (triangles) and distinct (circles) displacements of  $0.20 \mu\text{m}$  diameter beads in 0.25% weight guar solution. The solid line is a smooth fit to the data, used for calculating the rheology. The inset shows the  $r$  dependence of  $rD_{rr}$  for  $\tau = 100$  msec, in units of  $10^{-3} \mu\text{m}^3$ . (b) The storage (filled circles) and loss (open circles) moduli calculated using  $\langle \Delta r^2(\tau) \rangle_D$ , showing a crossover to elastic behavior at high frequencies, are in good agreement with rheometer measurements (solid curves). The moduli calculated using  $\langle \Delta r^2(\tau) \rangle$  (triangles) do not agree.

determining the bulk rheological behavior of an inhomogeneous medium.

As an application of the two-point method, we measured polymerized F-actin, obtained from purified rabbit muscle [4,12]. The sample was prepared at 1 mg/ml in G-buffer (2 mM tris-Cl, 0.2 mM ATP (Adenosine triphosphate), 0.5 mM DTT (Dithiothreitol), and 0.1 mM  $\text{CaCl}_2$ ), mixed with  $0.47 \mu\text{m}$  diameter tracers and polymerized in the sample chamber by the addition of  $\text{MgCl}_2$  to 75 mM. Phalloidin was added in a 1.2:1 molar ratio to G-actin to stabilize the actin filaments.

The measured  $\langle \Delta r^2(\tau) \rangle$  and  $\langle \Delta r^2(\tau) \rangle_D$  are shown in Fig. 3(a);  $\langle \Delta r^2(\tau) \rangle$  resembles that reported in other microrheology experiments [2,4], increasing subdiffusively with a weak turnover at 50 ms.  $\langle \Delta r^2(\tau) \rangle_D$  shows no turnover, scaling as  $\tau^{1/2}$ . Significantly,  $\langle \Delta r^2(\tau) \rangle$  is up to 5 times larger than  $\langle \Delta r^2(\tau) \rangle_D$ , suggesting that most of the motion

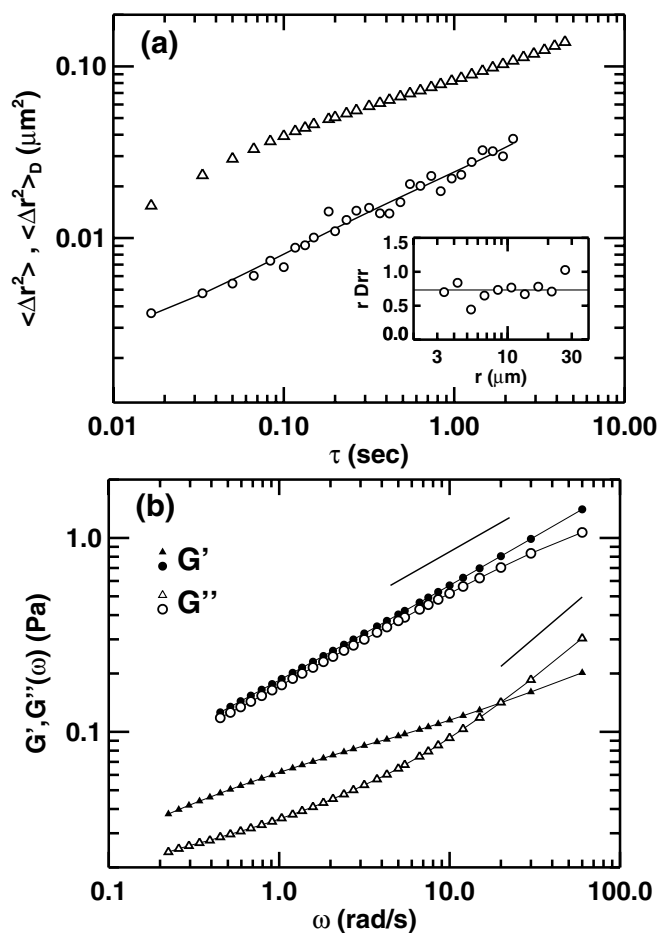


FIG. 3. Comparison of the self (triangles) and distinct (circles) displacements of  $0.47 \mu\text{m}$  diameter beads in a  $1 \text{ mg/ml}$  F-actin solution. The inset shows the  $r$  dependence of  $rD_{rr}$  for  $\tau = 67 \text{ msec}$ , in units of  $10^{-3} \mu\text{m}^3$ . (b) The storage (filled circles) and loss (open circles) moduli calculated using  $\langle \Delta r^2(\tau) \rangle_D$  vary as  $\omega^{1/2}$ . The moduli calculated with  $\langle \Delta r^2(\tau) \rangle$  (triangles) show a different form approaching  $\omega^{3/4}$ . The short lines indicate slopes of  $1/2$  and  $3/4$ .

of the beads is inside a “cage” formed by the actin filaments [5]. Since it is not due to the fluctuation spectrum of the actin solution, the turnover in  $\langle \Delta r^2(\tau) \rangle$  may result from collisions of particles with the cage. This highlights the important distinction between effects due to continuum mechanics and those due to the tracers’ microenvironments. Some of the discrepancy may also be due to compressibility of the network at the small spatial wavelengths probed in the single-particle data.

The corresponding moduli for F-actin are shown in Fig. 3(b). While  $\langle \Delta r^2(\tau) \rangle$  gives moduli similar to other microrheology results [2,4],  $\langle \Delta r^2(\tau) \rangle_D$  yields a very simple rheological spectrum varying as  $\omega^{1/2}$ , which, interestingly, corresponds with the exponent seen at lower frequencies in macroscopic measurements [3]. The significant difference between the two-point and single-particle measurements suggests that previous microrheology results must be reexamined. More work in both

theory and experiment is required to connect the internal dynamics of the actin filaments, shown recently to scale as  $\tau^{3/4}$  [5,13,14], to the  $G^*$  determined by microrheology.

Two-point microrheology has several advantages, in addition to its ability to probe inhomogeneous media. Its robustness should enable accurate microrheology with polydisperse, nonspherical, or unknown size tracers, as in studies of the cytoskeleton using organelles. Its ability to simultaneously probe both bulk rheology and the tracers’ microenvironments will likely be key to understanding such complex media as biopolymers and cells.

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