The effects of compressibility, hydrodynamic interaction and inertia on two-point, passive microrheology of viscoelastic materials

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In two-point passive microrheology, a modification of the original one-point technique the cross-correlations of two micron-sized beads embedded in a viscoelastic material are used to estimate the dynamic modulus of a material. The two-point technique allows for sampling of larger length scales which means that it can be used in materials with a coarser microstructure. An optimal separation between the beads exists at which the desired length and time scales are sampled while keeping an acceptable signal-to-noise-ratio in the cross-correlations. A larger separation can reduce the effect of higher-order reflections, but will increase the effects of medium inertia and reduce the signal-to-noise-ratio. The modeling formalisms commonly used to relate two-bead cross-correlations to the dynamic modulus and the complex Poisson ratio neglect inertia effects and underestimate the effect of reflections. A simple dimensional analysis suggests that for a model viscoelastic solid there exists a very narrow window of bead separation and frequency range where these effects can be neglected. In a recent work [Phys. Fluids, 2012, 24, 073103] we proposed an analysis formalism that accounts for medium inertia and high-order hydrodynamic reflections and therefore significantly increases the versatility of the two-point microrheology technique. In this paper we extend our analysis to compressible viscoelastic solids. There has been a recent interest in using two-point microrheology to measure the complex Poisson ratio of biopolymers [Das and MacKintosh, Phys. Rev. Lett., 2010, 105, 138102] however a rigorous analysis of the sensitivity of the technique to the static and dynamic properties of the Poisson ratio is still lacking. There are two decoupled statistics that can be followed with such a technique: motion parallel and perpendicular to the line of centers of the probe beads. We show that the cross-correlation in the direction parallel to the line of centers is insensitive to compressibility, so may reliably be used to determine $G^*$ (dynamic modulus) alone. Although, the cross-correlation in the perpendicular direction may then be used to extract a constant Poisson ratio, it is relatively insensitive to its frequency dependence. We consider the example of a composite actin/microtubule network.

I Introduction

The two-point passive microrheology technique is based on measuring the cross-correlated thermal motion of pairs of tracer micron-sized beads ($R < 1 \, \mu m$) to determine $G^*(\omega)$.1–3 Unlike bulk rheometers, microbead rheology requires only very small samples (pico- to microliter order) and elastic modulus as small as 10–500 Pa can be measured.4 These advantages make the technique especially useful for the analysis of biological samples.5,6 The pair of particles of radius $R$ is separated by a distance $L$ larger than the microstructure of the medium. In the passive two-point microrheology experiment the beads are driven by Brownian forces. The motion of the beads creates a velocity field in the otherwise undisturbed medium. This velocity field is characterized by waves that originate at the bead–medium interface, and then reflect back and forth between the beads. The cross-correlations of the two tracer beads will therefore be determined by the nature of those waves. The waves produced at the bead–medium boundary can be characterized by a wavelength and a penetration depth. At a given frequency the wavelength indicates the velocity at which the waves propagate while the penetration length indicates a characteristic distance the wave propagates before dissipating significantly from the medium. If the medium is compressible, then there are two kinds of waves, each of which has two such length scales.

There has been a recent interest to use the two-point microrheology technique to measure the complex compressibility of biopolymers and cell components such as F-actin and
microtubules.\textsuperscript{7} This would seem at first glance an excellent benchmark application for the two-point microrheology technique. As stated above microrheology methods are specially suitable to test the often scarce and expensive biological samples. Moreover the linkage of Poisson ratio to specific microstructural characteristics of a great variety of materials has been studied extensively. For example it is well known that the Poisson ratio is intimately connected with the way structural elements are packed.\textsuperscript{8} Measurements of the Poisson ratio might be appropriate for testing or fitting microscopic models of cell mechanics. This approach has already proven successful in the understanding and molecular-based design of other type of materials.\textsuperscript{9} A rigorous theoretical study of the sensitivity of the passive two-point microrheology technique is still lacking however. Therefore the reliability of compressible data obtained using two-point microrheology has not been well established.

Data analysis for two-point microrheology usually makes two important approximations: (i) that the waves produced at the bead–boundary interface decay before producing reflections, (ii) and that the waves propagate through the medium instantaneously, in other words that the inertia of the medium is negligible. For a given material to satisfy those assumptions only data gathered with beads placed at a distance 2L at which the reflections produced by one bead will significantly decay before reaching the other bead should be used. Similarly, the methods are only applicable to data gathered at frequencies where the waves can be assumed to be propagating much faster than the relaxation times being measured. However finding the experimental conditions where all these assumptions are met might not be possible for a viscoelastic material, especially if a wide spectrum of relaxation times is to be measured.

The compressibility of the medium adds another factor to consider in the analysis of the two-point microrheology data. Levine and Lubensky\textsuperscript{10} have previously pointed out there are two basic reasons to question the validity of the traditional microrheology data analysis (generalized Stokes–Einstein relation) in compressible viscoelastic solids. A probe particle moving at a given frequency will excite modes other than simple shear modes, and its response to external forces will, in general, depend on all of these modes in a way not simply described by the shear modulus. To model the effect of medium compressibility in the two-point microrheology experiment they considered a model viscoelastic medium consisting of a viscoelastic network that is viscously coupled to an incompressible, Newtonian fluid. They calculated an approximate mutual response function for their two-fluid medium and showed that in the limit that the bead–bead separation (L) is large compared to the radius of the beads (R), this response function measures the bulk rheological properties of the medium independently of the rheological properties of the regions immediately surrounding the two beads.\textsuperscript{2} This conclusion was reached when the response functions were derived with lowest-order reflections only. The corrections due to higher-order reflections were estimated and shown to be small for a large bead–bead separation (L). Their analysis however was based on the steady Stokes equation and therefore the effects of medium inertia in the mutual response functions were not considered.

In an earlier paper\textsuperscript{10} we presented a data analysis algorithm for the two-point microrheology technique that accounts for medium inertia and high-order hydrodynamic reflections and therefore expands the versatility of the technique. We have shown that the waves produced by two micron-sized beads trapped close together inside a viscoelastic fluid travel through a media at a frequency-dependent speed. This is unlike purely viscous or elastic media. In the purely viscous media the penetration length is identical to the wavelength, therefore there is only one relevant length scale. On the other hand in a purely elastic solid the waves travel without ever dissipating. Therefore the only relevant length scale is the wavelength of this oscillatory wave. In a viscoelastic material the waves created at the bead–medium interfaces will display two different length scales, the penetration length and the wavelength. Moreover these two length scales will have a frequency dependence determined by the dynamic modulus of the material. In the two-point microrheology technique rheological properties are inferred from the particular characteristics of these waves. However it is usually assumed that the waves travel infinitely fast between the two beads and that they decay significantly before wave reflections can reach the beads. The first of these assumptions is usually valid at low frequencies, but can break down at high frequencies where the time scales being measured become comparable to the time it takes the waves to travel between the beads. The second of these assumptions is expected to be valid when the beads are separated by a distance much larger than the penetration length of the wave. We have shown that if these assumptions are used outside this range then observable errors can be introduced in the inferred rheological properties. In this paper we extend our data analysis formalism to viscoelastic compressible solids. In contrast to incompressible materials, two types of waves can propagate through a compressible medium, namely shear waves and longitudinal waves. Characterizing the velocity at which these two types of waves travel and the distance they travel before significantly dissipating from the medium is important for the correct analysis of two-bead microrheology data of compressible materials. We address these issues here.

In Section II we derive generalized Stokes tensors with medium inertia and an infinite number of reflections for compressible viscoelastic solids. We make use of well-known analytic solutions for an isolated sphere in a compressible elastic medium,\textsuperscript{11–12} the method of reflections,\textsuperscript{13–14} and exploit the correspondence principle. Simple dimensional arguments are then used to construct a phase diagram for the shear and longitudinal waves propagating through a viscoelastic solid, which can serve as a guide to indicate experimental conditions under which inertia and reflections may become important for compressible materials. In Section III a generalized Langevin equation is given for two hydrodynamically interacting beads embedded in a viscoelastic solid. The equation is solved in the frequency domain to obtain relations between the beads auto- and cross-correlations and the components of the generalized Stokes tensor derived in Section II. In Section IV we present a detailed analysis of the sensitivity of the two-point
microrheology technique to changes in the complex compressibility of viscoelastic solids. We consider the sensitivity of two-bead cross-correlations to dynamic and static properties of the Poisson ratio. Finally in Section V we make use of generalized Brownian dynamics (GBD)\textsuperscript{14} and a microscopic model which relates the Poisson ratio to the microstructure of the medium\textsuperscript{15} to simulate the two-bead passive microrheology experiment in F-actin and microtubule composites. We use the simulated two-point cross-correlations to test the traditional data analysis formalism which neglects inertia and high-order hydrodynamic interactions, and show the errors that can be generated in the inferred Poisson ratio. As comparison, we use the data analysis formalism which is obtained from the equations derived in Section III and show that a better estimation of the Poisson ratio, and therefore of microstructural characteristics of the actin–microtubules composites, can be obtained.

II High-frequency generalized compressible Stokes tensors

We begin this section by deriving a generalized Stokes tensor for the two-point microrheology of compressible materials that accounts for inertial effects and an infinite number of reflected waves. To construct the Stokes tensors for compressible media we make use of two components: (i) the well known response function for a sphere in a compressible viscoelastic fluid\textsuperscript{11,12} and (ii) the method of reflections for unsteady Stokes flows introduced by Ardekani and Rangel,\textsuperscript{13} and previously generalized by us to viscoelastic incompressible fluids.\textsuperscript{18} Previous analyses of two-point microbead rheology are based on solutions obtained from the steady Stokes equation,\textsuperscript{3,5,6,16} which neglects the effects of medium inertia and higher-order hydrodynamic reflections.

Zwanzig and Bixon\textsuperscript{11} were the first to derive the unsteady response function for an isolated sphere in a compressible 1-mode viscoelastic medium. However as Metiu, Oxtoby, and Freed\textsuperscript{12} later pointed out their derivation contains a minor error which causes an additional erroneous term to appear in their expression for the response function. The correct unsteady response function for the isolated sphere was first derived by Metiu, Oxtoby, and Freed.\textsuperscript{12}

Ardekani and Rangel\textsuperscript{13} used the method of reflections,\textsuperscript{17} which is an approximate method for calculating the force exerted on two small spheres moving in Stokes flow to solve the unsteady problem. The particles are assumed to be sufficiently close to each other to interact hydrodynamically but sufficiently distant from boundary walls so that the surrounding medium is regarded as an infinite sea. Solutions for a single time-dependent point-force or for an isolated sphere are used in combination with the method of reflections.

The velocity field that satisfies boundary conditions on two spheres is constructed by a linear superposition of an infinite number of velocity fields that satisfy boundary conditions on one sphere, each velocity field representing a reflection of the wave generated at the bead–medium interface. The problem is axisymmetric about the line of centers between the two beads, and can therefore be reduced to a two-dimensional problem. The method of reflections gives the solution as an infinite series. Calculating all the terms in this geometric series, one can analytically find the summation of all terms.

The correspondence principle allows one to replace the frequency-independent shear modulus and Poisson ratio of simple elastic materials by the complex shear modulus and complex Poisson ratio of linear viscoelastic materials, after the equations have been transformed from the time domain to frequency space. This identification is possible because linear viscoelasticity presumes a convolution integral for the stress tensor, whose Fourier transform yields the Stokes relation with complex (frequency-dependent) modulus. Therefore in the frequency domain, the linearized Cauchy equations (steady or unsteady) for a compressible viscoelastic material are equivalent to the linearized Cauchy equations for a compressible purely elastic solid. Whenever the linear elastic deformation problem can be solved, the analogous solution of the linear viscoelastic deformation problem follows.\textsuperscript{18–20}

Consider two particles of radius $R$ located a distance $L$ apart and moving with velocities $v_{R(t)}$ and $v_{b(t)}$ in an unbounded viscoelastic solid. In the absence of the particles the medium is not deformed; the motion of the particles produces a velocity field in the medium $v(r, t)$. The particles are at least a few diameters apart. We assume following\textsuperscript{3,5,6,16} that $L$ is independent of the motion of the beads, which implies that the displacements of the beads are small compared to $L$. The relation between the motion of the two beads and the force exerted by the viscoelastic solid on one of the particles is,

$$F_{(1)}(t) = \frac{-\zeta_0}{1 - \frac{\omega}{A_1(\omega)}} \left( \frac{v_{R(t)}}{A_1(\omega)} - \frac{v_{b(t)}}{A_1(\omega)} + \frac{\dot{v}_{R(t)}}{v_{R(t)}} \right) \delta(t),$$

where,

$$\zeta_0 := \frac{4\pi G^s(\omega)}{\int_0^\infty R(ik_0(\omega)R^2 \left[ \frac{1 - ik_0(\omega)}{2(ik_0(\omega)R^2)A_1 + ik_0(\omega)R^2} \right] \left[ 1 - ik_0(\omega) \right] A_1 - 2[\dot{v}_{R(t)}(\omega)R - 1]A_1 \right) \right) .$$

is the one-sided Fourier transform $[i.e., \zeta(\omega) \equiv \mathcal{F} \{ \zeta(t) \} := \int_0^\infty \zeta(t) e^{-i\omega t} dt]$ of the frequency-dependent friction coefficient including the Basset force term and the mass of material dragged by a single bead embedded in a viscoelastic compressible medium. We indicate the Fourier transform by $A$ and $b$ (\textsuperscript{17}) the number, and the symbols $\perp$ and $\|$ indicate the directions perpendicular and parallel to the line of centers of the two beads, respectively. $A_1$ and $A_4$ are defined by,

$$A_4 = 3 - 3ik_0(\omega)R + (ik_0(\omega)R)^2$$

$$A_1 = 3 - 3ik_0(\omega)R + (ik_0(\omega)R)^2.$$
$k(t)$ and $k'(t)$ are frequency-dependent, complex wave-numbers for the transverse and longitudinal waves respectively,

$$
k_i(t) = -\omega \sqrt{\frac{\rho}{G^*(t)}} k_i(0) = -\omega \sqrt{\frac{\rho(2\nu^*(t) - 1)}{2G^*(t)(\nu^*(t) - 1)}}
$$

where $\nu^*(t)$ is the bulk modulus, and $G^*(t)$ is the dynamic modulus. Note that the real part of the complex Poisson, $\nu'(t)$, ratio is always bounded between $-1$ and $1/2$ and its imaginary part, $\nu''(t)$, between $0$ and smaller than $1/2$. These inherent bounds of the Poisson ratio make it especially convenient to characterize the compressibility of a material by significantly reducing the parameter space that has to be explored during data analysis.

To calculate the reflections we assume that particle 1 is located at a relatively large distance (several diameters) from particle 2. We then compute the translational effect of particle 1 by assuming that: (i) it generates the same force as that produced by a point force located at the center of the particle; (ii) the drag resulting from the field reflected at a given particle can be approximated by considering the field to be equivalent to a uniform velocity field with the same magnitude and direction as would exist at the location of the particle center if it were not present. As long as $L/R$ is sufficiently large the assumptions should be safe. Then the functions $A_i(Q, \omega)$ and $A_j(Q, \omega)$ are given by

$$
A_i(Q, \omega) = \frac{i\omega QC_i(\omega)}{4\pi G^*(t)} \left\{ \frac{2}{ik_i(\omega)L} e^{ik_i(\omega)L} - \frac{2}{ik_i(\omega)L} e^{ik_i(\omega)L} \right\}
+ \frac{i\omega QC_i(\omega)}{4\pi G^*(t)} \left\{ \frac{1 - 2\nu^*(t)}{2 - 2\nu^*(t)} \right\} e^{ik_i(\omega)L} - \frac{2}{ik_i(\omega)L} e^{ik_i(\omega)L}
+ \frac{2}{ik_i(\omega)L} e^{ik_i(\omega)L} \right\},
$$

where $Q := R/L$ is the bead radius to bead separation ratio. The detailed derivation of eqn (7) and (8) is given in Section VII.

It is important to note that in viscoelastic media, unlike purely viscous or purely elastic media, the waves produced at the bead–medium interface have frequency-dependent, complex wave-numbers $k_i(t)$ and $k_i'(t)$, for the waves propagating in the transverse and longitudinal directions, respectively. We previously considered incompressible fluids where only shear waves are produced; a detailed characterization and dimensional analysis for the shear waves propagating through a viscoelastic medium has already been presented elsewhere. Although the characterization and dimensional analysis is analogous in this work we restate it for the longitudinal waves propagating through a compressible viscoelastic solid. The wavelength of the longitudinal wave penetrating into the viscoelastic solid from the bead surface is defined as,

$$
\lambda_i(t) := \frac{1}{|k_i(t)|}
$$

where $k_i(t)$ is the real part of the frequency-dependent wave number. We may also define the penetration depth of the wave as,

$$
\Delta_i(t) := \frac{1}{k_i'(t)}
$$

where $k_i'(t)$ is the imaginary part of the frequency-dependent complex wave number. From eqn (9) and (10) we can see that,

$$
-ik_i(t)L = \frac{L}{\Delta_i(t)} + \frac{iL}{\Delta_i(t)}.
$$

The first term on the right hand side of eqn (11), which involves the penetration length, determines how fast the wave decays. The second term, which involves the wavelength, characterizes the oscillatory part of the memory function tensor. If the distance $L$ between the two beads is much larger than half the penetration depth, $\Delta_i(t)/2$, then the reflected waves decay significantly before reaching the other bead and therefore the effect of reflections is small. However if the distance between the beads is comparable to the penetration depth the reflected waves from bead 2 will have an important effect in the motion of bead 1 and vice versa.

Based on eqn (11) we propose two simple dimensional arguments that can serve as guides to determine whether medium inertia and high order hydrodynamic reflections should be taken into account when determining the compressibility of a viscoelastic solid with the two-point microrheology technique. The Brownian motion of two micron-sized beads near each other in a viscoelastic medium produce waves at the bead–medium interfaces. These waves will travel a distance equal to their penetration length before dissipating significantly. If the distance between the two beads is comparable to, or smaller than, the penetration length, then bead 1 will feel the presence of bead 2, and vice versa. This effect is called “hydrodynamic interaction,” and its observable manifestation is the appearance of cross-correlations in the statistics of the beads’ displacements. Additionally, when the wave produced by bead 1 reaches bead 2 it gets reflected, bouncing back towards bead 1. This reflection is the so-called “first-order reflection” of the hydrodynamic waves. If the distance between the beads is sufficiently small, then these first-order reflections will return to bead 1 before dissipating; the same holds for bead 2. Therefore in such a situation the beads feel the first-order reflections. These in turn, have an observable effect on the cross-correlations of the beads’ displacements. Moreover if the beads are brought closer together, then higher-order reflections are felt by the beads before dissipating.

Fig. 1 shows a dimensional-analysis phase diagram for the waves propagating through a 4-mode Maxwell viscoelastic solid during a two-bead microrheology experiment. For the wave that forms in the bead–medium interface to decay before producing
multiple reflections it is required that \( \frac{2L}{\Delta_1(\omega)} \gg 1 \). The dashed line in parts C and D represent \( \frac{2L}{\Delta_1(\omega)} = 1 \), which means that, in region I, below this line higher-order reflections should be negligible. In region II where \( \frac{2L}{\Delta_1(\omega)} \ll 1 \) it is safe to neglect medium inertia. In region III both inertia and high order hydrodynamic reflections may have a measurable effect in the dynamics of the shear and longitudinal waves propagating through the viscoelastic medium. Frequency is made dimensionless by the shortest relaxation time of the material, \( \lambda_{\text{min}} \).

We turn now to the effects of medium inertia. Inertia causes a vortex-like flow surrounding a localized disturbance at short times which leads to enhanced correlations in the thermal velocity fluctuations in liquids. A well-studied consequence of these correlations is the slow, decay of velocity correlations, known as the long-time tail.\(^{11,12}\) In viscoelastic media the velocity autocorrelation functions, specifically their oscillatory character, becomes much more pronounced with increasing elastic component of the shear modulus.\(^3\) At a more microscopic level the main effect of medium inertia is that it can significantly affect the velocity at which the waves generated at the bead–medium interface propagate through the medium. In traditional microrheology analysis one assumes that hydrodynamic waves propagate instantaneously between the beads. However, for sufficiently large bead separation, or sufficiently high frequencies, the lag time for wave propagation can be important. Therefore to analyze these effects it is useful to define the propagation speed of the longitudinal wave (speed of sound) produced at the bead–medium interface as,

\[
c_l(\omega) = \frac{\omega A_l(\omega)}{c_l(\omega)}.
\]

Where \( A_l(\omega) \) is the wavelength of the wave, that was defined in eqn (9). Therefore the time it takes for the wave to travel from one bead to the other is \( \frac{L}{c_l(\omega)} \) At time scales much longer than this the propagation of longitudinal stress through the medium can be assumed to be instantaneous and therefore inertia can be safely neglected from the analysis of the longitudinal waves. Therefore for medium inertia is negligible if \( \frac{\omega L}{c_l(\omega)} \ll 1 \). At high frequencies inertia becomes important because the time the wave takes to travel between the beads becomes comparable to the relaxation or delay time of the fluid being measured at a frequency \( \omega \). Opposite to what is required to reduce the effect of reflections, to reduce the effect of inertia a large separation between the beads is required. This is because the shorter the distance between the beads, the shorter the time it takes for the
waves to propagate between them and therefore the assumption that the propagation occurs instantaneously is more easily approached. The solid lines in the phase diagrams shown in Fig. 1 represents \( \frac{\delta L}{\delta t} = 1 \). In region II, above this line, the waves can be assumed to be traveling infinitely fast. In region III the magnitude of the effect of both inertia and reflections on the motion of the waves propagating through the viscoelastic medium becomes important.

Note that the phase diagrams shown in Fig. 1 differ from the phase diagrams we have presented before\(^\text{23-24}\) for viscoelastic fluids in that there is no region where medium inertia and high order reflections become negligible at the same time. This region appears for viscoelastic fluids at low frequencies and small bead-radius-to-bead-separation ratio. This is because for viscoelastic fluids, the equilibrium modulus \( g_e \) is zero \( (g_e = 0) \) and therefore each component of the dynamic modulus is \( G' \sim \omega^2, G'' \sim \omega \) at the low-frequency terminal zone, so that \( \mathcal{A}(\omega) = \mathcal{A}(\omega) \sim \omega^{1/2} \). On the other hand, for viscoelastic solids, the equilibrium modulus is finite \( (g_e > 0) \) and therefore \( G' \sim \omega^0 \) so \( \mathcal{A}(\omega) \) is much larger than \( \mathcal{A}(\omega) \) at low frequencies.

The preceding analysis, which was carried out for a compressible viscoelastic solid, with a complex-valued, frequency dependent Poisson ratio, is an alternative to results previously presented by Levine and Lubensky\(^\text{9}\) for two-fluid models. In two-fluid models at high frequencies the compressible network is dissipatively coupled to the incompressible fluid (solvent). Therefore, the longitudinal mode of the network plays no role in the high-frequency bead dynamics. Moreover at large enough frequencies the fluid will carry the larger part of the stress in the material, while below some crossover frequency the network shear modulus is the dominant contributor to the mechanical properties of the two-fluid material. A simple calculation shows that this crossover frequency is on the order of \( 10^8 \) Hz,\(^\text{9}\) which is well above experimentally accessible frequencies, so that the network shear modulus is typically the principal contributor to the two-fluid shear modulus.

### III Two-point high-frequency compressible generalized Langevin equation

In this section we write the equations of motion for the two probe beads of radius \( R \) and separated by a distance \( L \) embedded in a viscoelastic solid. The dynamics of micron-sized beads embedded in a viscoelastic solid are known to be described by a generalized Langevin equation (GLE),\(^22-24\)

\[
\frac{d\mathbf{p}_b(t)}{dt} = -\mathbf{H}_c \cdot \delta \mathbf{r}_b(t) - \int_{-\infty}^{t} \mathbf{F}_b(t', \mathbf{p}_b(t')) \frac{dt'}{m} + \mathbf{f}_b(t).
\]

Where,

\[
\delta \mathbf{r}_b = \begin{pmatrix}
\frac{\partial \mathbf{r}_{b(1)}}{\partial \mathbf{r}_b} \\
\frac{\partial \mathbf{r}_{b(2)}}{\partial \mathbf{r}_b}
\end{pmatrix}
\]

is the bead displacement vector and \( \mathbf{p}_b = m \frac{d\mathbf{r}_b(t)}{dt} \) are the beads’ momenta. The Brownian forces satisfy the fluctuation-dissipation theorem (FDT),

\[
\langle f_{ab}(t) f_{b'(t')} \rangle_{eq} = k_B T \delta(t - t').
\]

If the fluctuations of the beads’ positions are small compared to \( L \) the solutions to the linearized Cauchy’s equation such as eqn (1) can be used to write a GLE with a bead-position-independent memory kernel. We can use eqn (1) and (2) to write expressions for the components of the memory function tensor, \( \mathcal{A}(\omega) = \mathcal{A}(\omega) \) (where \( \mathcal{A}(\omega) \) is the inverse one-sided Fourier transform),

\[
\mathcal{A}(\omega) = \begin{pmatrix}
1 & 0 & -A_{11}(\omega) & -A_{12}(\omega) \\
0 & 1 & 0 & A_{12}(\omega) \\
-A_{11}(\omega) & 0 & 1 & 0 \\
-A_{12}(\omega) & 0 & 0 & 1
\end{pmatrix}
\]

Note that, although the two beads are correlated, the \( \perp \) and \( \parallel \) directions are decoupled. The expression for \( A_{11}(\omega) \) and \( A_{12}(\omega) \), which include all reflections, medium inertia and compressibility where given in eqn (8) and (7) respectively.

\( \mathbf{H}_c \) is the so-called frequency matrix involving only purely elastic elements. We have shown before that for viscoelastic solids there exists a frequency matrix \( \mathbf{H}_c \) outside of the memory function.\(^22\) For a compressible viscoelastic solid the frequency matrix is given by,

\[
\mathbf{H}_c = \mathbf{H}_c = \begin{pmatrix}
1 & 0 & -A_{e11} & -A_{e12} \\
0 & 1 & -A_{e12} & A_{e12} \\
-A_{e11} & 0 & 1 & 0 \\
-A_{e12} & 0 & 0 & 1
\end{pmatrix}
\]
where
\[ H_e = \frac{24\pi R_g(r_e - 1)}{6r_e - 5} \]
and
\[ A_{e\parallel} = 6Q(4r_e - 3), \quad A_{e\perp} = \frac{3Q(4r_e - 3)}{2(6r_e - 5)}. \]  
(18)
(19)

Where \( g_e = G^*(\omega) = 0 \) and \( r_e = r^*(\omega) = 0 \). Eqn (17)-(19) are obtained by solving the steady linearized Cauchy equation for two beads embedded in an infinite purely elastic medium and keeping only first-order terms in \( Q \).

For a homogeneous isotropic medium the mean-squared displacement tensor will be symmetric with four distinct components. The diagonal component involving only auto-correlations of the first particle in the direction perpendicular to the line of centers is given by,
\[ \langle \Delta \delta r_{b(1,\perp)}^2(t) \rangle_{\perp} = 2\langle \delta r_{b(1,\perp)}(0)^2 \rangle - \langle \delta r_{b(1,\perp)}(t)\delta r_{b(1,\perp)}(0) \rangle, \]
with a similar definition for the mean-squared displacement (MSD) in the direction parallel to the line of centers. Similar definitions also hold for the second probe particle. The off-diagonal components of the mean-squared displacement tensor involve only cross-correlations, one of them in the direction perpendicular to the line of centers,
\[ \langle \Delta \delta r_{b(1,2)}^2(t) \rangle_{\perp} = -2\langle \delta r_{b(1,\perp)}(t)\delta r_{b(2,\perp)}(0) \rangle, \]
and an equivalent cross-mean-squared displacement (CMSD) in the direction perpendicular to the line of centers.

The following relationship between the one-sided Fourier transform of the mean-squared displacement in the direction perpendicular to the line of centers and the memory function tensor can be found by using the FDT, eqn (15), and the solution of the GLE, eqn (13), in the frequency domain,
\[ \langle \Delta \delta r_{b}^2(\omega) \rangle_{\perp} = \frac{2k_B T}{i\omega} \left( H_{e,1,1} - \frac{1}{i\omega}(m_{11} + \zeta_{11}) \right). \]
(22)

An equivalent relation can be written for the direction parallel to the line of centers with the replacements \( \zeta_{1,1}[\omega] \rightarrow \zeta_{1,2}[\omega], H_{e,1,1} \rightarrow H_{e,2,2} \) and \( H_{e,1,3} \rightarrow H_{e,2,4} \). The following relation between the CMSD and the components of the memory tensor, can also be obtained from the solution of the GLE and the FDT in the frequency domain,
\[ \langle \Delta \delta r_{b(1,2)}^2(\omega) \rangle_{\perp} = \frac{2k_B T}{i\omega} \left( H_{e,1,3} - \frac{1}{i\omega}(m_{13} + \zeta_{13}) \right). \]
(23)

Here again an equivalent equation can be written for the direction parallel to the line of centers with the replacements \( \zeta_{1,1}[\omega] \rightarrow \zeta_{2,2}[\omega], H_{e,1,1} \rightarrow H_{e,2,2} \) and \( H_{e,1,3} \rightarrow H_{e,2,4} \).

Eqn (22) and (23) and their equivalent equations in the direction parallel to the line of centers, used together with eqn (16) and definitions for the functions \( A_{\perp}(\omega) \) and \( A_{\parallel}(\omega) \), eqn (8) and (7) relate observable two-bead statistics to linear viscoelastic properties (including compressibility) of the solid. These relations include bead and medium inertia as well as an infinite number of reflections.

If higher-order hydrodynamic reflections and medium and bead inertia are neglected the following simple relation between the Poisson ratio and the two-bead cross-correlations can be derived by taking the limits \( m \to 0, \rho \to 0 \) and only the first-order terms of a Taylor series expansion in \( Q \) of eqn (22) and (23),
\[ \rho^*(\omega) = 1 + \frac{1}{4} \left( \frac{\Delta \delta r_{b(1,2)}^2(\omega)}{\Delta \delta r_{b(1,\perp)}^2(\omega)} - \frac{\Delta \delta r_{b(1,\perp)}^2(\omega)}{\Delta \delta r_{b(1,2)}^2(\omega)} \right). \]
(24)

Eqn (24) was originally presented by Levine and Lubensky and has been used by Gardel et al. and Pelletier et al. to calculate the complex Poisson ratio of F-actin and actin composites. In Sections IV and V we test the relations derived in this section for inferring the high-frequency compressibility of viscoelastic solids from two-bead cross-correlations. Additionally we illustrate the magnitude of the errors that can be introduced in the measured compressibility if eqn (24) is used to analyze two-bead cross-correlations obtained under experimental conditions where medium inertia or higher-order reflections are expected to be important. We also evaluate, using our generalized Brownian dynamics (GBD) simulations the possibility of using the two-point micro rheology technique to elucidate the microstructural origins of enhanced compressibility in biopolymer composites.

IV Sensitivity of the two-point micro rheology technique to medium compressibility

In this section we evaluate the sensitivity of the cross-correlations of bead positions to changes in the Poisson ratio of the medium. We consider both statics and dynamics of a time-dependent Poisson ratio. For the sample calculations that follow we consider a discrete relaxation spectrum, which gives the following form for the dynamic modulus of the medium,
\[ G^*(\omega) = g_e + \sum_{j=1}^{N} \frac{g_j \lambda_j \omega^j}{1 + \lambda_j \omega^j}. \]
(25)

To illustrate the effect of reflections and finite bead size on the solutions derived above, we consider the 4-mode relaxation spectrum, \( H^* = \{4, 3, 2, 1\} \) and \( \lambda^* = \{1, 3, 9, 27\} \) where \( H_j := 6\pi R_g \) and the asterisk indicates that they have been made dimensionless by using \( \sqrt{k_B T/H_e} \) as the characteristic length scale, where \( H_e = 6\pi R_g \) is the incompressible \( H_e \). The smallest relaxation time, \( \lambda_1 = \min[\lambda^*] \) is used to make time dimensionless. For the following illustrations the density of the bead and the density of the medium are assumed to be the same, the dimensionless bead mass is set to \( m^* = m/\lambda_1^2 H_e = 0.0001 \). Therefore \( \sqrt{m^*} = 0.01 \) is the ratio between the smallest inertial
time scale of the system and the shortest relaxation time of this material.

The complex Poisson ratio of a viscoelastic solid can also be spectrally decomposed as,

$$p^*(\omega) = \rho_e + \sum_{j=1}^{2N} \rho_j \theta_j i\omega$$

(26)

where,

$$\rho_e - \rho_g = \sum_{j=1}^{2N} \rho_j$$

(27)

here $\rho_e = p^*(\omega = 0)$ and $\rho_g = p^*(\omega = \infty)$ are the equilibrium and glassy (or instantaneous) Poisson’s ratios, respectively, and the $\theta_j$ are the delay times, while the $\rho_j$ are the associated lateral contraction ratios, i.e., the strengths of the spectral lines that compose the discrete distribution of delay times. 19

The real part of the one-sided Fourier transform of the two-bead cross-correlations calculated using eqn (23) and its equivalent equation in the direction parallel to the line of centers of the beads is shown in Fig. 2A. The Poisson ratios used to calculate these two-point cross-correlations are shown in Fig. 2B, the black line corresponds to the incompressible solid, while the red lines correspond to the compressible solid. The upper line is the real part and the lower line is the imaginary part of the complex Poisson ratio. This particular compressible solid has a high compressibility at low frequencies ($\rho_e = 0.1$) and becomes incompressible at higher frequencies ($\rho_g = 0.5$).

Notice that the cross-correlations in the direction parallel to the line of centers are practically identical for the incompressible and compressible viscoelastic solid. The inset shows the only frequency region where a small difference can be observed. In data with noise, which will be the case in any situation of practical interest, this difference might be too small to be detected. When inertia and high order reflections are neglected from the calculations it can be shown analytically that the cross-correlations in the direction parallel to the line of centers are independent of the Poisson ratio. 19 Therefore the small difference that can be observed in the inset of Fig. 2A between the cross-correlations in the direction parallel to the line of centers are purely due to differences in the speed at which the hydrodynamic shear and longitudinal waves travel through the medium from one bead to the other. Since the compressibility of the medium does not have an observable effect on the two-bead cross-correlations in the direction parallel to the line of centers this data can be used to extract the dynamic modulus of the material using the time-domain methods that we have previously described 19 for incompressible materials.

On the other hand in the direction perpendicular to the line of centers the cross-correlation for the beads embedded in the compressible solid are shifted upwards from the incompressible case. At high frequencies as the Poisson ratio of the compressible media approaches 0.5 the difference between the cross-correlations in the perpendicular direction decreases until it eventually vanishes completely as can be observed in Fig. 2A. We show in Section V using GBD simulations that the vertical shift in the perpendicular direction to the line of centers caused by compressibility can be accurately detected in systems where microrheology is usually applied.

Having established that changes in the compressibility of a viscoelastic solid may be detected in the direction perpendicular to the line of centers, we now consider sensitivity to changes in the dynamics of the Poisson ratio. Fig. 3 shows the cross-correlations in the direction perpendicular to the line of centers for two beads embedded in compressible viscoelastic solids with the same glassy ($\rho_g$) and equilibrium ($\rho_e$) Poisson ratios, but with different distributions of delay times ($\theta_i$). The differences in the dynamics of the Poisson ratio for the three different model solids are significant. The maximum in $v''(\omega)$ are about an order of magnitude of frequency apart. However the differences that can be observed in the two-point cross-correlations are very small. For most of the frequency range plotted in Fig. 3A no differences can be detected between the
cross-correlations corresponding to the three different solids. The only region where some effect of the dynamics of the Poisson ratio can be observed is shown in the inset of Fig. 3A. The effect is too small to be detected in data with noise; moreover it occurs in a very narrow frequency range.

From the two-point cross-correlations in the direction perpendicular to the line of centers of the beads for a 4-mode Maxwell solid as a function of frequency, eqn (23), normalized by $2kT/\mu$. The bead radius to bead separation ratio, $Q$, was set to 0.2 in this calculations. Inset: zoom of the frequency range where the larger difference between the compressible and incompressible cross-correlations is observed. (B) The complex Poisson ratios, eqn (26), corresponding to the cross-correlations shown in part A. The black lines correspond to an incompressible solid and are given as reference. The dashed lines indicate the location of the shortest relaxation time ($\lambda_t$) and the characteristic inertial time ($\sqrt{M^*}$) for this systems.

### V Application: actin–microtubule composite networks

Recently Pelletier et al.\textsuperscript{7} used two-point passive microrheology to measure the Poisson ratio of F-actin and actin–microtubule composite networks. They found, in agreement with Gardel et al.,\textsuperscript{26} that pure F-actin is incompressible with $\nu = 1/2$. By contrast, in the composite network, $\nu$ was found to be unambiguously less than $1/2$ at longer times, and is closer to 0.3.

The different filament types influence each other through their viscoelastic responses. Actin filaments are a prototypical example of semiflexible polymers, and entangled, uncrosslinked actin filaments have been shown to follow the worm-like-chain model.\textsuperscript{25} Microtubules are the stiffest element in cells. Their fluctuations, although much smaller than those of actin filaments, are important for deployment of polymerization forces and for the search and capture mechanism used to position the mitotic spindle. Microtubules have a persistence length of a few mm, and in solution might be expected to behave as rigid rods. Macroscopic linear rheology of microtubule solutions has shown an elastic plateau modulus of $\sim 1$ Pa, with a weak frequency dependence and no terminal relaxation over a frequency range extending as low as $\omega = 6.3 \times 10^{-3}$ rad s$^{-1}$.

In an attempt to interpret the experimental results reported Pelletier et al.,\textsuperscript{7} Das and MacKintosh\textsuperscript{28} developed a model for the mechanical response of a composite material consisting of rods in an elastic matrix using a mean-field approach and a dipole approximation for the rod-like inclusions. The elastic matrix under consideration is treated as an effective medium that is made of the bare elastic medium (e.g., the F-actin matrix) and a collection of rods (microtubules) embedded in it. Their approach is similar to what has been used to model aligned fiber-reinforced composites.\textsuperscript{28} Consistent with the experiments of ref. 7, they found that the addition of rigid rods can lead to enhanced compressibility of a nearly incompressible medium. Specifically, they found that for matrices characterized by Poisson’s ratio $1/4 < \nu < 1/2$, the addition of rods reduces $\nu$, while for $\nu < 1/4$, stiff rods increase $\nu$. In this way, $\nu = 1/4$ can be thought of as a stable fixed point of such a composite.

We first summarize the main elements of the model proposed by Das and MacKintosh.\textsuperscript{28} We then incorporate this model into our GBD simulations of two-bead microrheology with the purpose of evaluating the sensitivity of the two-bead microrheology technique to the changes in the compressibility of the actin network upon addition of microtubules. For an isotropic and homogeneous elastic compressible material with shear modulus $G$ and Poisson ratio $\nu$, the displacement field $u$ at a position $r$ in the medium due to a force $F$ acting at a point $r'$, is equal to $u = \alpha \cdot F$ where,

$$
\alpha(r) = \frac{1}{8\pi G l} \left[ \delta_0 \delta_r \left( 1 - \frac{2\nu - 1}{2(\nu - 1)} \right) + \delta \left( 1 + \frac{2\nu - 1}{2(\nu - 1)} \right) \right].
$$

where $\delta_u$ is the unit vector in the direction of $r$, and $\delta$ is the unit tensor. The change in the response function and Lamé time-domain data analysis, how accurately a constant Poisson ratio can be extracted from the cross-correlations.
coefficients upon addition of rods is calculated as follows. Consider a single rod of length \( a \) embedded in the elastic medium. The presence of the rod represents a constraint on the displacement field induced by the applied force. For a force applied at the origin of the coordinate system, the net displacement of the ends of the rod with end-to-end vector \( a \) is given by,

\[
\Delta u(r) = u(r + a/2) - u(r - a/2).
\]

(29)

The constraint of an incompressible rod is approximated by a dipole at its center of mass. This induced (tensile) dipole is oriented along the rod and its strength is chosen so as to enforce a constant end-to-end distance of the rod: \( G \pi a (\mathbf{a} \cdot \mathbf{d}u) \). By keeping only leading order terms in \( a \), which is assumed to be smaller than all other length scales in the system, the resulting displacement field allows calculation of the change in the linear response functions. In the effective medium approach the change in response arises from a cloud of induced dipoles in the elastic continuum.

The changes in the parallel and perpendicular response functions with the addition of rods are:

\[
\delta \alpha_\parallel = -\frac{\pi}{30} na^2 \alpha_\parallel
\]

and

\[
\delta \alpha_\perp = \left[ \frac{7 + 4r(4r - 5)}{8(r - 1)^2} \right] \alpha_\perp, \tag{31}
\]

where \( n \) is the rod number density and average has been taken over rod orientation. Therefore for a small increment \( dn \) in added rods, the following differential equation can be written for the change in the Poisson ratio, \( \nu \), upon addition of the rigid rods in the compressible elastic matrix

\[
\frac{d\nu}{dn} = \frac{1}{60} n^2 \pi (1 - 6r + 8r^2).
\]

(32)

By solving eqn (32) with initial condition \( \nu(n = 0) = \nu_a \), where \( \nu_a \) is the Poisson ratio of the soft matrix (actin), we obtain an expression for the Poisson ratio of the composite network (actin–microtubule)

\[
\nu_c = -\frac{1 - e^{-\frac{1}{2} \tau_\xi}}{2 - 4r_a e^{-\frac{1}{2} \tau_\xi}} - 4r_a e^{-\frac{1}{2} \tau_\xi} \frac{1 - e^{-\frac{1}{2} \tau_\xi}}{2 - 4r_a e^{-\frac{1}{2} \tau_\xi}}
\]

\[
= \frac{1 - e^{-\frac{1}{2} \tau_\xi}}{2 - 4r_a e^{-\frac{1}{2} \tau_\xi} - 8r_a e^{-\frac{1}{2} \tau_\xi}}
\]

(33)

where, \( \xi \) is the mesh size of the microtubule network. The mesh size \( \xi \) is related to the rod density \( n \) by \( 1/\xi^2 = na \). The Poisson ratio of the composite network as a function of the ratio \( a/\xi \) is shown in Fig. 4, for various values of the pure F-actin Poisson ratio. For soft matrices with Poisson ratio close to 0.5 the addition of the rigid rods (increasing \( a/\xi \)) causes an increase in the compressibility (or decrease in the Poisson ratio) of the composite network. On the other hand for highly compressible elastic matrices the addition of rigid rods causes an increase in the Poisson ratio on the composite network. In general, the model predicts a stable fixed point for the Poisson ratio at \( \nu_c = 1/4 \).

To simulate the passive two-bead microrheology experiment in the actin–microtubules network we make use of eqn (33) with the Poisson ratio of the soft viscoelastic matrix, the F-actin, set to a value very close to \( \nu_a \approx 0.5 \). As explained in Section IV here we consider only viscoelastic solids with a constant Poisson ratio. We performed GBD simulations\(^\text{15} \text{14}\) of the two-point passive microrheology of composite networks with different values of the \( a/\xi \) ratio. According to Fig. 4 the compressibility of this nearly incompressible solid matrix will increase upon the addition of rigid rods (an increase the \( a/\xi \) ratio). The two-bead cross-correlations calculated from the simulations are shown in Fig. 5. Notice that in the direction parallel to the line of centers all the data points collapse to a single curve. This is because, as was pointed out in Section IV, the cross-correlations in this direction are not significantly sensitive to changes in the velocity at which the longitudinal wave travel through the medium. Therefore one can use the cross-correlations in the direction parallel to the line of centers to extract the dynamic modulus of material as if analyzing the data from an incompressible medium. We do this using the time-domain data analysis strategy that we have previously proposed.\(^\text{13} \text{14}\)

The method involves fitting the CMDS in the direction parallel to the line of centers (in the time domain), and then extracting the discrete relaxation spectrum of the medium from this fitted parameters. The line labeled as input in Fig. 6A shows the dynamic modulus that was used as input in the simulations. Output 1 illustrates the dynamic modulus that is obtained from analyzing the two-bead cross-correlations in the direction parallel to the line of centers as if they had been obtained from an incompressible medium. A very good agreement with the input dynamic modulus is obtained, as expected. The line labeled output 2 in Fig. 6A is the dynamic modulus obtained when the cross-correlations in the direction parallel to the line of centers are analyzed using the traditional inertialless generalized Stokes–Einstein relation (GSER), which does not account
for inertia or high order hydrodynamic reflections (a detail discussion of why the traditional GSER fails in a situation such as the one considered here has been given elsewhere).†

Once the dynamic modulus has been determined from the cross-correlations in the direction parallel to the line of centers, the Poisson ratio can be obtained by fitting a single extra parameter (in the case of a constant Poisson ratio) to the cross-correlations in the direction perpendicular to the line of centers. An approximation to the one-sided inverse Fourier transform of eqn (23) is fitted to the time-domain cross-correlation in the direction perpendicular to the line of centers, using as fitting parameter the Poisson ratio of the viscoelastic solid. The solid lines in Fig. 6B show $x(t)$ obtained from the values of the ratio $a/x$ used as inputs in the GBD simulations. We also calculate the Poisson ratios from the two-bead cross-correlations using eqn (24), which neglects inertia and high-order hydrodynamic interactions. Although this analysis correctly predicts a decrease in the Poisson ratio as the concentration of microtubules is increased, it can be observed that analyzing the cross-correlation data with this simplified equation leads to a frequency dependent Poisson ratio, even though the input Poisson ratio was constant. The simplified analysis works well in the lower frequency range but deviations from the input Poisson ratio start becoming significant at frequencies corresponding to the shortest relaxation time of the medium. According to this simplified analysis the material is compressible at low frequencies but becomes nearly incompressible at high frequencies. This is an important point since as we pointed out in Section IV the sensitivity of the two-point microrheology technique to changes in the dynamics of the

†Note that the sign of the second term of eqn (38b) of ref. 22 should be $\frac{\sqrt{\pi}}{2}$, instead of $+$ as printed. This is a typo, and all calculations have been done with the correct sign in our relevant papers, including Karim et al., Phys. Rev. E, 2012, 86, 051501.
bead trajectories when calculating the ensemble averages in eqn (22) and (23). With the proposed data analysis procedure the microstructural parameter \(a_\epsilon\) of the F-actin and microtubules composite can be estimated accurately using two-bead passive microrheology. Further the analysis correctly reproduces the theoretical results presented in Fig. 4, that is the increase in compressibility of the F-actin matrix as the concentration of microtubules is increased.

VI Conclusions

We have derived a generalized Stokes tensor for two hydrodynamically interacting beads embedded in a viscoelastic compressible solid. The tensors include the effects of medium inertia and consider an infinite number of reflections of the shear and longitudinal waves caused by the motion of the beads. A generalized Langevin equation for two hydrodynamically interacting beads embedded in a compressible viscoelastic solid was presented. Using the memory function tensor obtained from the generalized Stokes relation the GLE was solved in the frequency domain to obtain relations between the beads’ auto- and cross-correlations and the components of the generalized Stokes tensor.

Using these new theoretical developments we have systematically evaluated the sensitivity of two-bead cross-correlations to changes in the static and dynamic properties of the complex Poisson ratio of a model viscoelastic material. We find that the sensitivity of the cross-correlation in the direction parallel to the line of centers of the beads to the compressibility of the medium is very small. These effects which appear only at high frequencies, and are due to medium inertia, can be safely neglected in the data analysis. This means that the shear modulus can be inferred independently from the cross-correlations in the direction parallel to the line of centers even when inertia and high order hydrodynamic reflections are expected to have a relevant effect. This conclusion had already been reached with theoretical developments that neglect medium inertia and high order hydrodynamic reflections; here we show that it is still valid even when those effects are considered. On the other hand, the two-bead cross-correlations in the direction perpendicular to the line of centers show a detectable dependence on the compressibility of the material. A clearly distinguishable vertical shift can be detected in the cross-correlations obtained in a compressible medium with respect to those obtained in an incompressible one. However we have shown that the sensitivity of the cross-correlations in the direction perpendicular to the line of centers is practically undetectable to changes in the specific spectrum of delay times of the complex Poisson ratio. Therefore the passive two-point microrheology technique does not seem appropriate to accurately measure dynamic properties of the complex Poisson ratio.

Using generalized Brownian dynamics and a microscopic model for biological composite networks we simulated the two-bead microrheology experiment in F-actin and microtubules composites. We used the simulated two-bead cross-correlations to test data analysis formalisms for inferring microstructural properties of the composites. We find that commonly used data analysis which neglects inertia and high order hydrodynamic reflections will predict Poisson ratios close to 0.5 at high frequencies when actually the microstructural parameters used as input in the simulation give smaller Poisson ratios. On the other hand the time-domain data analysis procedure that can be constructed using eqn (23) can successfully recover the input complex Poisson ratio of the F-actin and microtubules composite network in all the frequency range considered here. The traditional data analysis algorithm and the one that accounts for medium inertia and high order reflections give comparable results at low frequencies, which indicates that medium inertia is the main cause for the failing of the simplified data analysis formalism.

There is a considerably large region of values for the radius-to-distance ratio and frequency, relevant to microbead rheology experiments, where the data analysis formalism that neglects medium inertia and higher-order reflections produces detectable errors in the dynamic modulus and the complex Poisson ratio inferred from two-point cross-correlations. At high frequencies the wavelength of the shear and longitudinal waves propagating through the viscoelastic solid becomes small compared to the distance between the beads and therefore the assumption that stress propagates instantaneously becomes invalid. Additionally reflected waves can be important at low frequencies, where the penetration depth of the waves can become larger than the distance between the beads. In experiments a large separation between the probe beads can reduce the effect of higher-order reflections and allow for statistically relevant sampling of larger microstructures. However a large separation will increase the effects of medium inertia and reduce the signal-to-noise-ratio in the CMSD. These factors constraint the experimental conditions under which the two-point technique is applicable. As has been illustrated with the F-actin and microtubules composite networks, the data analysis formalism presented in this work significantly expands the region of distances between the beads and frequencies at which the dynamic modulus and the complex Poisson ratio can be accurately measured using two-point passive microrheology.

VII Appendix A

Here we show the detailed derivation of the two-point hydrodynamic interaction scalar functions \(A_{\perp}\) and \(A_{\parallel}\), eqn (7) and eqn (8), respectively.

A Introduction

We begin with the equation of motion for a compressible elastic solid

\[
\rho \frac{\partial^2 u(r,t)}{\partial t^2} = G\nabla^2 u(r,t) + (G + i\lambda)\nabla (\nabla \cdot u(r,t)) + f(r,t). \tag{34}
\]

where \(u(r,t)\) is the displacement field and \(f(r,t)\) is an external applied force field. The derivation is illustrated for a purely elastic solid, but in the frequency domain the results are equivalent for a viscoelastic solid according to the correspondence principle. The force is applied at particle 1 located at the origin \(r = 0\). We assume that it is given as the point force, that is,
where \( F(t) \) is the time-dependent part of the force. We seek to calculate the displacement field \( u \) at \( r \) where particle 2 is located. The coefficient of the force field and the displacement field gives the mutual compliance.

By taking the Fourier transform for both \( r \) and \( t \), eqn (34) becomes

\[
-\rho \omega^2 u(k, \omega) = -Gk^2 u(k, \omega) - (G + \lambda)kk \cdot (u(k, \omega) + f(k, \omega)),
\]

(36)
or, equivalently,

\[
\{(2G + \lambda)k^2 - \rho \omega^2\} \delta \delta_k + (Gk^2 - \rho \omega^2)(\delta \delta_k) \cdot u(k, \omega) = f(k, \omega).
\]

(37)

Therefore, the displacement field in the \((k, \omega)\)-domain is written as

\[
u(k, \omega) = \left[\frac{\delta \delta_k}{(2G + \lambda)k^2 - \rho \omega^2} + \frac{\delta \delta_k}{Gk^2 - \rho \omega^2}\right] f(k, \omega).
\]

(38)
The force field eqn (35) is written in the \((k, \omega)\)-domain as

\[
f(k, \omega) = F(\omega).
\]

(39)

By putting this into eqn (38) and by taking the inverse Fourier transform for \( k \), we have

\[
u(r, \omega) = H(\omega) \cdot F(\omega)
\]

(40)
where \( H \) is the Oseen tensor defined by

\[
H(\omega) = \frac{1}{(2\pi)^3} \int d^3 k e^{ikr} \left[\frac{\delta \delta_k}{(2G + \lambda)k^2 - \rho \omega^2} + \frac{\delta \delta_k}{Gk^2 - \rho \omega^2}\right].
\]

(41)

Note that \( \delta \) is the unit vector in the direction of \( k \), and \( \delta \) is the identity matrix.

**B Oseen tensor**

Since \( H(r) \) depends on the vector \( r \) only, it can be written in terms of the scalars \( A_\perp \) and \( A_\parallel \) and unit vector \( \delta_r \) parallel to \( r \) as

\[
H(\omega) = A_\perp \delta_r + (A_\parallel - A_\perp) \delta \delta_r.
\]

(42)

Therefore the scalars \( A_\perp \) and \( A_\parallel \) are determined from the two equations:

\[
\text{Tr}(H) = 2A_\perp + A_\parallel,
\]

(43)

\[
\delta \delta_r \cdot H \cdot \delta_r = A_\parallel
\]

(44)

By putting eqn (41), the left side can also be written as

\[
\text{Tr}(H) = \frac{1}{(2\pi)^3} \int d^3 k e^{ikr} \left[\frac{1}{(2G + \lambda)k^2 - \rho \omega^2} + \frac{2}{Gk^2 - \rho \omega^2}\right]
\]

\[
= \frac{f(k_1)}{2G + \lambda} + 2\frac{f(k_1)}{G},
\]

(45)

\[
\delta \delta_r \cdot H \cdot \delta_r = \frac{1}{(2\pi)^3} \int d^3 k e^{ikr} \left[\frac{(\delta \delta_r \cdot \delta_r)^2}{(2G + \lambda)k^2 - \rho \omega^2} + \frac{1 - (\delta \delta_r \cdot \delta_r)}{Gk^2 - \rho \omega^2}\right]
\]

\[
= \frac{g(k_1)}{2G + \lambda} + \frac{f(k_1) - g(k_1)}{G},
\]

(46)

where we have defined

\[
f(x) := \frac{1}{(2\pi)^3} \int d^3 k e^{ikr} \frac{1}{k^2 - x^2}.
\]

(47)

\[
g(x) := \frac{1}{(2\pi)^3} \int d^3 k e^{ikr} \frac{(\delta \delta_k \cdot \delta \delta_k)^2}{k^2 - x^2}.
\]

(48)

By solving eqn (43)–(46) for \( A_\perp \) and \( A_\parallel \), we have

\[
A_\perp = \frac{f(k_1) - g(k_1)}{2(2G + \lambda)} + \frac{f(k_1) + g(k_1)}{2G},
\]

(49)

\[
A_\perp - A_\parallel = -\frac{f(k_1) - 3g(k_1)}{2(2G + \lambda)} + \frac{f(k_1) - 3g(k_1)}{2G}.
\]

(50)

Where \( f(x) \) and \( g(x) \) are given by:

\[
f(x) = \frac{2\pi}{(2\pi)^3} \int_0^\infty dk_2 \frac{k_2}{k^2 - x^2} \frac{1}{1 - i} e^{-i\omega k_2}
\]

\[
g(x) = \frac{2\pi}{(2\pi)^3} \int_0^\infty dk_2 \frac{k_2^2}{k^2 - x^2} \frac{1}{1 - i} e^{-i\omega k_2}
\]

\[
\frac{2}{\lambda} e^{-i\omega k} + \frac{2}{(i\omega x)} (1 - e^{-i\omega k})
\]

(51)

(52)

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**References**


14. A. Córdoba, T. Indei and J. Schieber, Elimination of inertia from a generalized Langevin equation: applications to microbead rheology modeling and data analysis, *J. Rheol.*, 2012, 56, 185, note the prefactors in the right side of eqn (2) and (9b) were printed as 2 but should have been 1. In eqn (4) and (8) the prefactors in the right side were printed as 2 but should have been 2π. The prefactors in front of η0, ζ0 and μ0 in eqn (13), (14), (16) and (29) were printed as 2 but should have been 1. These were misprints, and do not affect any of the results presented in the paper.