Microrheology: a review of the method and applications

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A set of local mechanical probes has been developed over the last ten years, allowing a kind of dynamical mechanical testing known as microrheology. This paper provides a short introductory review of these methods of performing rheology, comparing them to conventional rheometry, and highlighting the major advantages. The authors also share their outlook on some of the most promising and fastest developing areas that are being studied though microrheology, in the areas of biophysics and soft matter.

1. Introduction

Rheology is an interdisciplinary subject, spread between different communities including chemical engineers, physicists, material scientists and chemists.[1] It is also remarkable as a subject of extreme industrial importance; a very wide range of technologies, from paints to foods, from oil recovery to processing of plastics, all rely heavily on understanding the flow of complex fluids.

"Simple" (Newtonian) fluids are characterized by a viscosity and have a negligible elasticity. "Simple" (Hookean) solids do not flow, and are characterized by an elastic modulus. These two limiting behaviors clearly cannot describe a vast number of soft materials that are both viscous *and* elastic over the timescale at which they are probed.† Traditionally, viscoelastic materials have been studied with mechanical rheometers, in various deformation geometries depending on the extent of strain and the magnitude of moduli to be measured. Microrheology is a term that does not describe one particular technique, but rather a number of approaches that attempt to overcome some serious limitations of traditional bulk rheology, such as the range of frequency and moduli that can be probed, the sample size and heterogeneity, and cost. The "micro-" in the term refers to the size of the stress/strain probe, which is typically a micron-sized colloidal particle, but also indicates that this type of rheology can be carried out on very small volumes, of the order of a micro-litre. The advantages offered by microrheology approaches, summarized in Table 1 and described in greater detail below, have made these measurements very popular over the past decade, and have opened up new fields of investigation. The unconventional geometry and conditions encountered in microrheology experiments have also raised a number of theoretical challenges that are still not fully resolved.

In this short review paper our aims are limited to: a) providing an overview for newcomers to this topic, and b) giving our personal perspective of where this technique is proving most important, and where the most promising future developments lie. There are other recent reviews that can serve as extensive references to the microrheology methods and the spectrum of applications, in particular ref. 2 and 3. Details of the fluid-dynamics aspects of microrheology are presented in ref. 4. These references are recommended to

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† Even the "simple" behavior of Newtonian or Hookean systems is always confined to some frequency range.

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Table 1 Summary of the main advantages and drawbacks of microrheology compared to traditional methods of rheometry. These points are expanded in section 4 of the text.

Advantages	Drawbacks
An extremely wide frequency range, extended in particular towards high frequency. For example, fluctuation modes up to 10^6 Hz are accessible in gels.	Difficult to make use of microrheology to study the non-linear response.
Local probe, ideally suited to spatially heterogeneous systems or multiphase solutions, where bulk methods give only average distributions.	Limited to materials that are at least partially transparent to light.
Samples can have very low viscosity (like water) and very low elasticity (tenuous gels).	Very computationally intensive.
Applicable to small volumes, and non-conventional geometries: thin films, interior of biological cell, membranes *etc*.	Challenging to apply to very stiff or viscous materials.
Price of equipment.	

anyone wanting to become fully active in this field.

2. Rheology

The rheological (flow) response of complex fluids can be linear or non-linear as a function of the applied stress. Non-linearity is usually a sign of structural rearrangement in the system, caused by the applied stress or deformation. Non-linear rheology is of extreme practical importance, since most industrial processing occurs in non-linear regimes. However it is very difficult to make general models of non-linear behavior, because it is, by its nature, dependent on the history of the system and the specific details of the flow. For systems in thermodynamic equilibrium, there is always a linear response regime for small enough strains (or stresses). The existence of a linear response region is a more delicate issue for systems not in equilibrium, such as for example colloidal glasses, pastes, foams *etc.* In any case, much attention has focused on the linear rheology of complex matter. This is not just because there is a better hope of providing successful models, it is also because in many cases a strong link can be made between the system's linear response and the underlying structure. For example, the physical quantities of a system, like the length distribution in a polymer solution, or the persistence length in a filament gel, can be measured by studying the linear dynamic response.

Microrheology has been used up to now as a probe for the linear response of a system. In its "passive" implementation it relies on measuring the motion in the system as caused by thermal noise or weak induced stresses. In contrast, "active" microrheology relies on inducing controlled external stresses, usually stronger than the ones resulting from thermal forces. To understand the various flavors of microrheology, it is useful to remind the reader of the classical experiments used to characterize viscoelastic fluids: stress relaxation following a fast deformation measures $G(t)$; creep under constant stress measures the compliance $J(t)$; oscillatory stress (or strain) is a measure of $G^*(\omega)$. Within the linear response region, these experiments are all equivalent, *i.e.* there is a well defined framework for converting one measured quantity into any of the others. For example, $G^*(\omega)$ is proportional to the one-sided Fourier transform of $G(t)$, and $J(t)$ is related to $G^*(\omega)$ by the inverse Laplace transform *etc.*[1] Converting raw microrheology results into a form of data that can be compared to mechanical experiments depends on performing transformations between these quantities.

3. Methods

"Microrheology" indicates a family of methods, the most common experiments involve video-particle tracking, laser tweezers or magnetic tweezers.

3.1 Light scattering: DLS and DWS

Dynamic light scattering (DLS) is the ancestor of today's microrheology.[5,6] Much of the theoretical and practical framework for microrheology has its roots in the light scattering literature. Light scattering is intrinsically a bulk-average technique, and that is a key difference that sets it apart from microrheology. The measurement of the scattered light's time-correlation function can be used to extract not just the viscosity but also the elastic modulus of viscoelastic material. By measuring at different scattering angles, the spectrum of different modes is probed. This has been known since the 1970 s, when complex fluids began to be investigated this way, with or without the addition of tracer (scattering) particles.[7] This experiment can be thought of most simply as a stress relaxation, with thermal fluctuations acting as a local source of stress. The time-correlation function can be fitted as a stress relaxation. Traditional DLS is limited to quite transparent samples, requiring more than 90% of light to be transmitted unscattered in order to avoid the complication of multiple scattering. This was a serious limitation for the study of complex fluids, until it was realized that in the opposite limit of multiple scattering the time-correlation function of scattered intensity also contained useful information.[8] This extension of light scattering to opaque systems, known as diffusive wave spectroscopy (DWS), allowed the study of dynamics in colloidal pastes and foams.[9] DWS has the additional advantage of extending measurements to very high frequencies and very good spatial resolution, but it is however still a bulk technique, with the limitations of large (millilitre) sample size and inability to resolve spatial heterogeneity.

3.2 Video-particle tracking

Video analysis of trajectories of tracer particles can yield a complete characterization of the linear viscoelasticity of the matrix. This idea was given a firm foundation by Mason and Weitz.[9] The well-known Stokes–Einstein equation

$$D(a) = \frac{k_B T}{6\pi\eta a}, \qquad (1)$$

relates the diffusion coefficient D to the bulk viscosity η (the single parameter that describes a Newtonian fluid) and a the colloid radius. In the simple case of a Newtonian viscous fluid, colloids are observed to undergo a random motion.

The mean square displacement $\langle \mathbf{r}^2(t) \rangle$ travelled from time $t = 0$, which we shall also abbreviate as MSD, will grow like

$$\langle \mathbf{r}^2(t) \rangle = 4Dt, \tag{2}$$

with $\mathbf{r}$ lying in a two dimensional plane as in optical microscopy observations.

In the opposite case of a purely elastic medium of modulus G_0, the embedded particles are "tied" to their initial position by a spring-like potential, and their position fluctuates by an amount:

$$\langle \mathbf{r}^2(t) \rangle = \frac{2k_B T}{3\pi a G_0}. \tag{3}$$

Complex fluids have both a viscous and elastic character, and furthermore there may be complex dynamics in the material that lead to the viscous and elastic modulus exhibiting frequency dependence. Mason and Weitz extended the analysis of the motion of a tracer particle to the general viscoelastic case, where the shear modulus may be frequency dependent. They proposed a "generalized Stokes–Einstein" equation, which for motion in 2-dimensions is:

$$\langle \tilde{\mathbf{r}}^2(s) \rangle = \frac{2k_B T}{3\pi a s \tilde{G}(s)} \tag{4}$$

where s is the Laplace frequency, and $\tilde{\mathbf{r}}^2(s)$ and $\tilde{G}(s)$ are the Laplace transforms of $\mathbf{r}^2(t)$ and $G(t)$.

An equivalent and simple way to consider a video-tracking experiment is in analogy to a creep experiment, measuring the compliance. It was shown recently that the mean square displacement can be written as:[10]

$$\langle \mathbf{r}^2(t) \rangle = \frac{k_B T}{\pi a} J(t), \tag{5}$$

where $J(t)$ is the compliance of the material. Eqn (5) is another form of the "generalized Stokes–Einstein" eqn (4) originally proposed by Mason and Weitz.[9]

From a practical point of view, the most challenging aspect of a video-tracking experiment can often be the image analysis, *i.e.* the process of acquiring the trajectory of a number of particles. Video analysis is usually broken down into two steps: a) individual frames are analyzed to extract the coordinates of all the particles in the frame; and then b) the particles are matched through subsequent frames to produce data of trajectories. These methods are described very clearly in ref. 11. Particle tracking of multiple objects imaged using a microscope is very convenient because good statistics can be gathered by making sufficiently long videos, or introducing the right number of tracer particles. However the frame rate is limited by camera and computer technology, and more importantly there is enormous redundancy in the data that is acquired. This data has to be stored and analyzed offline.

An alternative strategy is to image/track one or two particles at a time, either within an optical tweezer setup,[12] or by imaging the light scattered off one particle.[13] Tracking a few particles with these methods, the advantages are a very high sampling frequency (limited only by the particle inertia, which becomes important around 100 kHz), and the lack of redundancy in the data, meaning fewer problems with data storage and the possibility of online analysis. In either case, the spatial resolution of these methods is extremely good, and is not a limiting factor in experiments on most soft systems. The position of a micrometre-sized particle can be measured to better than 10 nm[2,14] *via* the optical methods just described.

3.3 Two-particle correlation

The particle-tracking approach described above assumes that the probe particles do not affect the system locally. This is not always the case, for example a probe particle in a solution could become surrounded by either a depletion layer or a more dense layer of the molecules in solution. If either of these problems occur, and in general if there is a specific interaction between the probe particle and the matrix, then it is necessary to image and calculate the fluctuations as a function of the distance between pairs of particles. These 2-particle correlations are not affected by the local environment around each bead, and provide instead an unbiased probe of the response of the bulk matrix.[15]

Furthermore the material can have a heterogeneous structure on a wide range of lengthscales, so that by analysing the correlated motion of particles at a fixed distance, the mode structure can be recovered. A recent example of this can be found in ref. 16.

In fact the only (important) downside of 2-particle correlation *vs.* single-particle, is that a much more extensive amount of video data needs to be recorded and analyzed in order to have good statistics for many pairs of particles at many distances.

3.4 Numerical aspect of data analysis

To illustrate the method further, it may be useful to consider a simple (but not as trivial as eqn (2) and eqn (3)) case, where the mean square displacement and the frequency dependence of the elastic moduli can be compared. The Maxwell model consists of an elastic element with modulus E and a viscous element of viscosity η, in series. For this ideal model of a viscoelastic liquid all the linear elastic moduli and the response of the system can be calculated analytically. Subject to a stress σ, a colloid in a Maxwell fluid will undergo motion with an MSD(t) that grows linearly with time, following[1]

$$\mathrm{MSD}(t) = \frac{\sigma}{E} + \frac{\sigma}{\eta} t. \tag{6}$$

A characteristic time is given by

$$\tau = \frac{\eta}{E}, \tag{7}$$

and the shear moduli are given by

$$G'(\omega) = E\frac{\omega^2\tau^2}{1+\omega^2\tau^2} \quad \text{and} \quad G''(\omega) = E\frac{\omega\tau}{1+\omega^2\tau^2}. \tag{8}$$

We consider the response of this Maxwell model in Fig. 1, where we have chosen τ = 0.1 s. The analytical data (exact) is shown as solid lines. Fig. 1 also shows values of $G'(\omega)$ and $G''(\omega)$ (solid and open symbols) obtained by analyzing an artificial MSD data set that would be found in an experiment on a Maxwell system. The artificial MSD dataset is made of 5000 points, linearly spaced from t = 0.01 to 100 s, which is similar to data in video-tracking experiments. What is shown here is a test of the numerical framework for data analysis. These results highlight the problems at low frequencies that are introduced by the Laplace transform. Less severe problems are also present at high frequency.

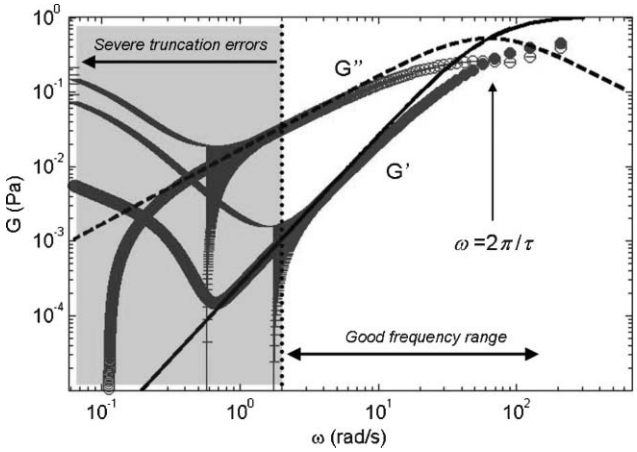

Fig. 1 Comparison of expected theoretical (lines) and modeled G′ (•) and G″ (○) responses, assuming a Maxwell model (an elastic element with modulus E and a viscous element of viscosity η, in series, see text for details of parameters used). The error bars correspond to the theoretical uncertainty that propagates into G' and G'' for an error on the MSD raw data that is 10^{-3} of the MSD, a realistic estimate. The truncation problems, particularly severe at low frequency, and to a lesser extent at high frequency, were acknowledged right from the early work on microrheology (*e.g.* ref. 13) and are of a similar type as encountered in other areas of data analysis in rheology.[1]

The results also show that there is good quantitative agreement over the intermediate range of frequencies, and also that the crossover frequency is identified correctly. Various strategies have been proposed to extend the frequency window over which data can be inverted, but there are drawbacks in each one, as discussed further in ref. 2.

4. Comparison to traditional rheology

4.1 Advantages

1) An extremely wide frequency range, extended in particular towards high frequency; 10^3 Hz can be reached with full-frame multi particle tracking, and 10^6 Hz can be reached with photodiode position detection, but limited to tracking just a few beads; displacement frequencies of 10^9 Hz can be reached with DWS, but this is an ensemble averaging method.

2) It is a local probe, ideally suited to heterogeneous systems where bulk methods give average distributions that are often very difficult if not impossible to deconvolve. Microrheology (in its particle-tracking forms) is not constrained to ensemble averages. Furthermore, careful analysis of 2-particle correlated motion can give lengthscale/timescale resolution of dynamical modes.[15,16] It can also give information on other forms of non-uniformity, such as directional anisotropy,[17] see Fig. 2 and 3.

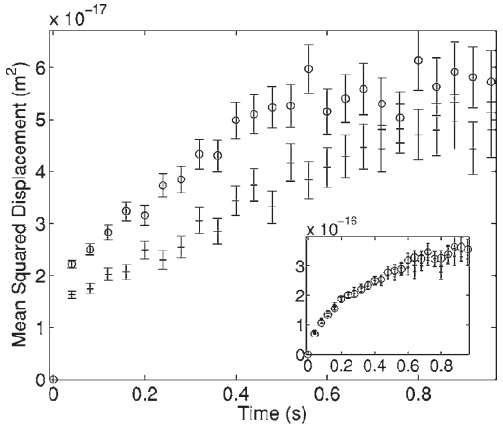

Fig. 2 The MSD along principal and minimal directions, for a sheared DNA solution (11 mg ml^{-1} at 298 K). Crosses (+) correspond to displacements parallel to the optical director, and circles (○) to displacements perpendicular to this. Inset: MSD for the same DNA at 4.9 mg ml^{-1}, where there was no visible birefringence and no visible anisotropy in the MSD. Reprinted with permission from ref. 17, copyright 2007, American Physical Society.

If spatial heterogeneity is found to be present, careful statistical analysis is required to test the significance of it. Various approaches have been attempted. At the most basic level, heterogeneity may be assessed qualitatively by simply comparing the tracks of different particles in the sample by eye, but this is rarely adequate. For an ensemble of tracer particles, all in the same environment, the distribution of one-dimensional displacements at lag time τ (not to be confused with a material's characteristic time, defined earlier) should be Gaussian, since the particle path is simply a random walk. If all the tracer particles are in equivalent environments, then the distribution of the x displacements after lag time τ of all the particles in the field of view of $x(\tau)$, will also be Gaussian, with the same width. The simplest quantitative method measures the kurtosis of the overall distribution $x(\tau)$. Kurtosis is a measure of the 'peakedness' of a distribution.[18] The excess kurtosis of a distribution is a measure of its 'peakedness' relative to a Gaussian distribution, and is one measure of heterogeneity, see Fig. 4.

Alternatively one may consider $\mathrm{MSD}(\tau)_{\mathrm{single}}$, *i.e.* the MSD($\tau$) calculated for individual particles. Having decomposed the overall MSD into the MSD for single particles the result may simply be a spread of values around the overall MSD, and the larger the spread, the greater the degree of heterogeneity. However, in some cases it may then be possible to group the particles by setting some threshold MSD at a particular lag time. For example, this sort of analysis has been applied to the motion of vesicles in live cells, allowing vesicles to be classified as mobile or immobile.[19] This simple analysis gives some indication of the degree of heterogeneity in the sample which is lost if one just considers the total MSD averaged over all particles.

3) The sample can have very low viscosity and very low elasticity. Pure "simple" liquids like water give a signal that is too weak for the majority of rheometers. On the contrary, microrheological data are easier to acquire

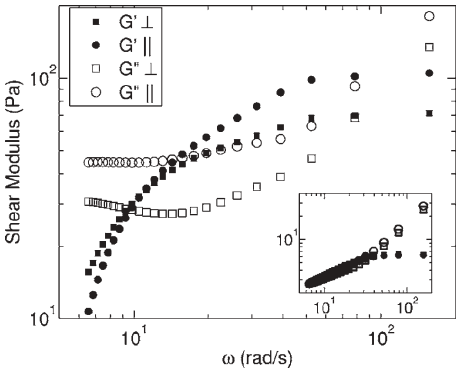

Fig. 3 Elastic modulus, G' and loss modulus, G'', along calculated eigenvectors for dense (11 mg ml^{-1}) sheared DNA. Filled circles correspond to G' along the axis approximately parallel to the optical director, and filled squares to G' perpendicular to this. Open symbols refer to G'' in these directions. Inset: Moduli of the same DNA at a lower concentration (4.9 mg ml^{-1}), where the sample is isotropic in its mechanical response. Reprinted with permission from ref. 17, copyright 2007, American Physical Society.

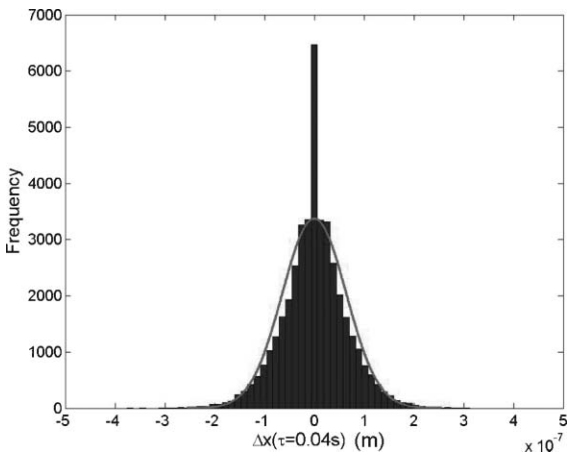

Fig. 4 Distribution of Δx at $\tau = 0.04$ s, averaged over all the particles in the same hectorite clay sample (ρ = 1.0008 g cm^{-3}) as Fig. 6, after approximately 24 hours ageing. The curve is a Gaussian fit (mean of zero and standard deviation calculated from the data), illustrating that the distribution is *not* Gaussian at this stage.

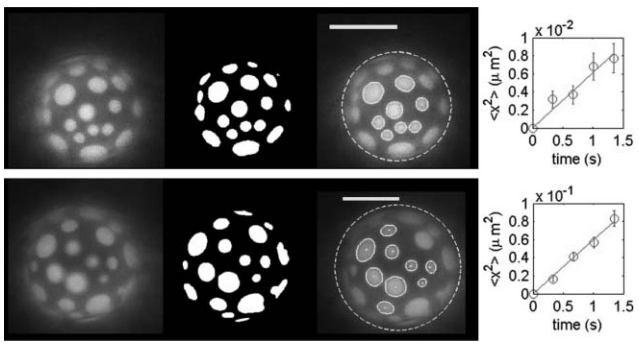

Fig. 5 Giant unilamellar vesicle showing coexisting fluid domains in a fluid background. Through a series of image analysis steps, the white regions are identified as domains and tracked through successive frames. Mean square displacement data for domains with radii of 1–1.5 μm are shown on the right. Both vesicles have a composition of 1 : 2 DOPC/DPPC + 30% cholesterol; temperature is T = 10 °C (top) and 20 °C (bottom). Note the factor of 10 difference in diffusion coefficients. The scale bar is 40 μm. Reprinted with permission from ref. 20, copyright 2007, American Physical Society.

the smaller the elasticity and the viscosity of the material. The presence of colloidal probes undergoing thermal motion is also unlikely to break-up a tenuous structure. This makes microrheology ideal for monitoring spontaneous gelation over time, where at the onset of the gel the mechanical moduli are very small.

4) Price of equipment and sample. The cost of a state-of-the-art rheometer is around $100 000, whereas particle tracking can be carried out with a conventional optical microscope and a video camera for around a third of the price. Sample volume can be as small as 100 μl, compared to at least a few ml in traditional rheometers. This may also have the effect of reducing the cost of an experiment, or even making the experiment practically possible.

5) Applicable to non-conventional geometries, for example to measure the viscosity of two dimensional lipid bilayers, see Fig. 5,[20] or of Langmuir films on liquid interfaces.[21]

4.2 Limitations

1) It is difficult to make use of microrheology to study the non-linear response. 2) Microrheology is limited to materials that are at least partially transparent to light (although recent developments of X-ray photon correlation point to a new direction for measuring rheological properties from X-ray scattering).[2] 3) It is computationally intensive. Although this limit becomes less restrictive each year, it will be at least 5 years before real-time measurements become feasible. At present, a particle-tracking experiment might record up to 10 minutes of video, and the analysis of this data takes about 10 hours on a dedicated PC. 4) For very stiff or viscous materials it will be challenging to observe the very small motion of the probe particle. The limit of maximum moduli that can be measured depends on the resolution of the experiment. Given a resolution, the maximum moduli can be estimated *a priori* from eqn (2) and eqn (3).

5. Applications in biophysics and the life sciences

While the very first experiments proving the validity of the GSER approach were

performed on synthetic complex fluids, it was immediately clear that the advantage of small sample volumes plays a particularly important role with biological systems. When one attempts to reproduce certain biological structures i*n vitro*, the material has to be painstakingly extracted and purified from biological matter. The amount of material (in particular proteins) is often very limited, and under these conditions it may not be feasible to produce enough for 1 ml of solution (the typical volume for DLS).

An important target for microrheological investigation is the cytoskeleton of animal cells.[22] The cytoskeleton is a scaffold that serves various biological functions, one of which is to give cells their mechanical integrity. The cytoskeleton is composed of proteins assembled into filaments. The main types of filaments are composed of the proteins actin and tubulin, assembling to make F-actin and microtubule filaments, respectively. There are also other filaments, known collectively as ''intermediate filaments'' that occur in specialized cells. These types include neurofilaments, and they too are aggregates of proteins. Mechanics of the cytoskeleton has attracted the attention of researchers with a background in soft matter because of the clear links with rheological and mechanical models of entangled rods, themselves related to theories of flexible and semi-flexible polymer networks.[23] There is now a good understanding of this problem, and a body of work is collected in ref. 24. The first *in vitro* microrheology investigations involved entangled actin filaments, and gave mechanical moduli that are orders of magnitude smaller than in cells.[25,26] This pointed to the importance of cross-linkers between the rods, but the homogeneous cross-linked *in vitro* model systems could still not reproduce the high value of the biological modulus. The current understanding is that the presence of molecular motors, actively cross-linking and moving the filaments, induces both bundling and the onset of internal stresses in a cross-linked network. In this case the elastic modulus increases substantially because the stress–strain curve of the cross-linked rod system is highly non-linear.[27]

Overcoming the sample-volume problem is not the only reason to do microrheology. It can highlight physical processes in the material that would not be seen by any other bulk-averaged technique. Examples of the power of this method come from the study of inhomogeneous systems, where there can be a dependence of the rheology on the spatial lengthscale.[16] Another example is the case of directional anisotropy, as in dense sheared solutions of DNA.[17] Fig. 2 shows the mean square displacement of beads embedded in a dense DNA solution that has been subject to shear in one direction, causing permanent alignment of the DNA molecules. This data can be analyzed to resolve the mechanical response as a function of the direction, see Fig. 3. The magnitude of the moduli correlates with the local orientation of the DNA molecules measured by birefringence. Directional anisotropy of the mechanical response is a feature that can only be resolved with a microscopic probe.

Of course, few systems are as spatially heterogeneous (and small in volume) as a cell itself, and there has been pioneering work by Wirtz and co-workers performing microrheology experiments on these objects, either by tracking features inside the cell or by micro-injecting tracer particles.[13,28,29] A comparison of four microrheology techniques, including active and passive methods, applied to cell dynamics is given in ref. 30. Wirtz and coworkers have shown that the region near the nucleus has much higher moduli than the peripheral region of the cell,[29] and this is an important example of an unexpected property arising from a quantitative measurement of a biological system.

6. Applications in soft matter and complex fluids

A range of systems have been studied with microrheology; one of the early papers, using DWS and the generalized Stokes–Einstein approach, already contained data on suspensions of hard spheres, polymer solutions and emulsions.[31] Since then, microrheology has been applied as a method of high-throughput screening, as demonstrated in ref. 32, where measurements on gelation of block copolypeptides and on surfactant micellar systems are presented.

More recently, the power to simultaneously probe the full spatial field of flow has been used to map (three-dimensionally) the flow in microscopic geometries. An array of microprobes can be simultaneously trapped and used to map out the fluid flow in a microfluidic device.[33]

Microrheology is particularly attractive for the study of gelation, a process characterized by extremely small initial moduli and fragile structures that are easily compromised in a typical stress-controlled bulk rheometer. For example, it has been used to study the *in situ* gelation of the clay hectorite. Because measurements of the MSD can be made at successive times, it is possible to follow how the ageing of the gel affects the motion of the particles – and hence extract from the measurements the viscoelastic moduli as a function of time. Fig. 6 shows data for a hectorite sample 1 hour and 24 hours after gelation commences.[34] It can be seen how a quasi-Newtonian response at short times switches to a very different behaviour as

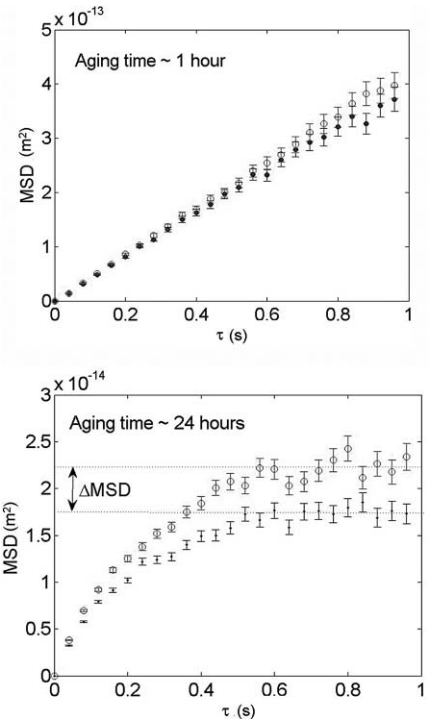

Fig. 6 The mean square displacement plotted as a function of lag time, up to a maximum lag time of 1 second, for a hectorite sample (ρ = 1.0008 g cm^{-3}) at the ageing times indicated, during the process of gel formation. For each lag time the MSD is plotted for the two perpendicular directions corresponding to the maximum (open circles) and minimum (filled circles) displacement axes found for each video recorded.

gelation sets in, with a plateau developing in the MSD. Fig. 4 shows the distribution of $\Delta x(\tau = 0.04$ s) after 24 hours ageing. The strong kurtosis illustrates how far the sample now deviates from a Gaussian fit for the distribution. This reflects the development of significant spatial anisotropy within the sample.

7. Outlook and conclusions

In this brief and introductory review we have shown examples of the use of microrheological techniques in biophysics and soft matter. We have drawn attention to the strength of this technique; the frequency and stress range of traditional rheometers is extended, but more dramatically completely new investigations are made possible, into heterogeneous systems, and live biological matter in particular. It is clear that these techniques will continue to evolve and become more popular in the life sciences, where they can be applied to a range of problems involving live cells, the effects of drugs and other external stimuli, *e.g.* mechanical.

In synthetic systems, and more generally soft matter, microrheology will continue to be applied in both its passive and active implementations. Microfluidics and other conditions involving small volumes of fluid (*e.g.* inkjet technology) are inaccessible to conventional rheology, and will inspire experiments aimed at understanding new complex fluids and at measuring the effect of confinement.

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