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Microrheology of complex fluids

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Abstract

The field of microrheology is concerned with how materials store and dissipate mechanical energy as a function of length scale. Recent developments in the theory and instrumentation of the microrheology of complex fluids are reviewed. Equal emphasis is given to the physical phenomena probed, advances in instrumentation, and specific experimental systems in which this field has already had an impact. The inversion of the compliance data, measurement of sample heterogeneity, high frequency viscoelasticity, effects of shear flow, single molecule experiments, surface viscoelasticity and time evolution studies are considered. The techniques highlighted include particle tracking microrheology, diffusing wave spectroscopy, laser tracking, magnetic tweezers and atomic force microscopy. Specific examples of complex fluid systems are chosen from the fields of polymers, colloids and biological assemblies.

(Some figures in this article are in colour only in the electronic version)

Contents

1. Introduction

1.1. Description of the field

Rheology is the study of the viscoelasticity of materials. Microrheology extends this definition to consider how the dynamic behaviour of these materials changes with length scale. The separation of a material into 'component' or 'structure' is often artificial and morphologies commonly exist at a whole range of length scales in complex fluids. Thus a rich range of theoretical and experimental questions are presented to the prospective microrheologist (MacKintosh and Schmidt 1999).

Microrheology relates to the phenomena involved in the storage and dissipation of mechanical energy in soft materials at the micrometre or sub-micrometre level (MacKintosh and Schmidt 1999). A technical hurdle for optical methods of mechanical spectroscopy is typically set at the micrometre level, which dictates the prefix in the title. There has also historically been a distinct biological bias in the field, since cells operate at the micrometre level, providing a strong impetus to drive the research (Berg 1993).

Practically microrheology has long been identified with the resolution limit of an optical microscope operating at its largest degree of magnification, $\sim 0.5\,\mu$m, and optical techniques continue to dominate the field. However, the area is rapidly enlarging and advancing with a series of technical and theoretical barriers being overcome. Within this enlargement there has been the creation of new sub-fields which are the subject of more specialist reviews; magnetic tweezers (Strick *et al* 2003), force microscopy (Mukhopadhyay and Granick 2001), particle tracking (Tseng *et al* 2002a, 2002b), surface viscoelasticity (Meyer *et al* 1998), microfluidics (Hansen and Quake 2003) and diffusing wave spectroscopy (DWS) (Harden and Viasnoff 2001). In this paper the basic universal principles of the techniques are discussed and more modern developments are reviewed.

The field of complex fluids is an emerging area of physics. It coexists with such conventional areas as condensed matter and fluid mechanics. There is an extremely wide range of sample chemistries, topologies and geometries that are investigated in complex fluids, in many ways richer and more demanding than those included under more traditional physical classifications. In particular, complex fluids (polymers, colloids and biological materials) are expected to demonstrate behaviour intermediate between solids (completely elastic) and fluids (completely viscous), and accurate methods are thus required to quantify the phenomena associated with their viscoelasticity. The industrial applications of complex fluids are vast. How do you choose the viscosity of a new shampoo in a new formulation, a drug delivery system as it is transported through the body, a fruit cake mixture as it experiences multiple phase transitions during cooking or a grade of cement in the construction of a new building (Larson 1999)? There is thus a wide range of fields that will benefit from new rheological probes driving the current research.

Microrheology is closely connected to the field of microfluidics, which considers such phenomena as those involved in ink jet printing, microelectrophoresis on a chip, microvalves, and the kinetics of protein crystallization (Hansen and Quake 2003). The change of emphasis, which separates the two fields, is the extension to the consideration of viscoelasticity with microrheology. The overlap is thus quite strong and the fluid mechanics of materials in confined geometries is a common area to the two research fields.

The subject of biological motors has recently been revolutionized by new microrheology techniques (Howard 2001). The understanding of the viscoelasticity of the motors at the molecular level has been improved, which directly relates to questions of muscular dysfunction vitally important to the medical industry. Intensive research funded from studies into

cardiac disease with optical/magnetic tweezers and atomic force microscopy (AFM) has been transferred to more general areas of complex fluids research.

There is a long history of colloidal probe particles (Mhetar and Archer 1996) being used as tracers to define flow kinetics in engineering studies of bulk viscoelastic materials. Here a number of assumptions are made; the probe particles are isokinetic with the motion of the material investigated, there are no interparticle interactions involved between the probes, and there is no restructuring of the viscoelastic material by the probes (Solomon and Lu 2001). The experimental situation with these tracer studies is thus much more complicated than typically found with particle tracking microrheology (PTM) and many of these assumptions need to be revisited if engineers wish to quantify their measurements in more detail.

1.2. Scope of the techniques

Historically the roots of microrheology can be found in the observation of the Scottish biologist, Robert Brown (1827) that pollen grains (his probe particles) moved incessantly on the surface of water. This phenomenon at first sight appears abnormal; what is the origin of the force driving the motion? Such behaviour was later theoretically analysed by Albert Einstein (1905), who established the molecular nature of matter by explaining Brown's results in terms of a statistical analysis of the collisions of pollen with the surrounding solvent molecules. The calculations of Einstein were subsequently shown to be quantitatively correct by the painstaking experiments of Jean Baptiste Perrin (1948). Perrin demonstrated that the mean-square-displacement (MSD) of 0.37 μm gutta-percha particles in water is directly proportional to time with a constant of proportionality that describes the frictional dissipation of the particles. Another historical land mark is seen in early attempts to create active magnetic microrheometers to manipulate micrometre-sized ferromagnetic particles (Crick and Hughes 1950), although the first suggestion of such a device is much earlier (Seifriz 1924). After a period of relative inactivity, there followed modern developments such as particle tracking video-microrheology (Mason *et al* 1997), AFM (Mahaffy *et al* 2000), DWS (Pine *et al* 1988) and optical tweezers (Starrs and Bartlett 2003). These methods have principally been driven by the availability of cheap computing power and intense coherent monochromatic light sources (lasers).

In the present day, the case for microrheology as a new analytical technique for complex fluids is very strong. For the simplest available technique, PTM, the apparatus is relatively cheap (<£10 k), and sufficiently simple that internationally competitive apparatus can be adapted and constructed without advanced technical ability. Some of the specific advantages of microrheological methods will be enumerated to make a clear case for the range of techniques that are reviewed and to emphasise some unifying features:

(1) *Combinatorial chemistry.* Microrheology allows the rapid characterization of microlitre quantities of material, enabling detailed phase diagrams to be quickly established (Amis and Schubert 2004, Breedveld and Pine 2003). An order of magnitude increase in throughput has recently been demonstrated with particle tracking of viscoelastic peptides, when compared with previous bulk rheology measurements. Further increases in throughput are possible, that will scale with improved computing speed in the case of tracking experiments (Breedveld and Pine 2003). Microrheology presents an ideal method for probing permutations of reactants during the synthesis of a new viscoelastic material.

(2) *Agreement with bulk values.* Measurements obtained on expensive low shear rate solution rheometers have been shown to agree with those taken on budget microrheology equipment with a subset of complex fluids (see section 2.3). A consensus on how the sample chemistry and heterogeneity relate to the question of micro/bulk agreement needs to be constructed

in full, but there are good prospects that the range of overlap can be improved using two-particle cross-correlation techniques (Crocker *et al* 2000).

(3) *High frequency response.* The low inertia of colloidal probe particles allows the measurement of the high frequency response of soft materials with methods such as DWS (Mason and Weitz 1995) and optical tweezers (Schnurr *et al* 1997). There are no prospects for such large frequency windows being developed with conventional bulk rheometers due to the large forces and torques required.

(4) *Minimization of sample volumes.* Reducing the amount of viscoelastic material necessary to perform a measurement of a complete viscoelastic spectrum causes a concurrent reduction in cost and increase in the flexibility with which the sample geometry can be chosen. For example, the characterization of specimens from medical trials necessitates the use of minute histology samples and microrheology could provide a diagnostic method in these cases.

(5) *Length scale.* The viscoelasticity can be characterized as a function of length scale within the sample. Complex fluids typically exhibit hierarchical structures on a series of separate length scales and measurements at each scale would enable a complete picture of their viscoelasticity to be developed. Specifically, techniques such as particle tracking video-microscopy, optical/magnetic tweezers and AFM, allow the viscoelasticity to be quantified as it varies from point to point (Tseng and Wirtz 2001).

(6) *Sizing of particles*. This can be performed using microrheological measurements and offers a method for characterizing the molecular structure of a complex fluid. The intrinsic viscosity of the solution is plotted against the particle concentration and subsequent analysis can provide a radius of gyration for the particles (Goodman *et al* 2002). This process could compete with other standard methods of particle characterization such as light scattering techniques (Chu 1991) and bulk viscometry (Kulicke and Clasen 2004), since it requires much smaller sample volumes, i.e. microlitres compared with millilitres.

(7) *Phase diagrams.* Measuring the viscoelasticity rapidly at a wide range of concentrations allows phase diagrams to be mapped. Modern scaling theories allow these phase diagrams to be motivated for a range of complex fluids and microrheology offers a relatively fast route for their determination. Investigations are not restricted to the sample chemistry; new insights into the physics are also possible, e.g. the dynamic modes that control the viscoelasticity of a polymer solution: Zimm, Rousse, sticky Rousse, or reptation (Rubinstein and Colby 2003).

(8) *Cellular function.* Microrheological techniques provide new methods for probing intracellular function. Many cellular processes involve changes in the viscoelasticity of biomaterials at the micron level such as phagocytosis and motility. Microprobes can be introduced into living cells to quantify their motility without completely disrupting their function (Lau *et al* 2003).

(9) *Delicate probes.* Thermal energies ($\sim kT$) on colloidal particles provide a delicate probe of the structure and dynamics of fragile soft condensed matter systems. Materials which are damaged during measurement on bulk rheometers often fare better with microrheological techniques, which typically apply forces on the order of a few piconewtons. It is then possible to be certain that measurements are in the linear rheological regime.

(10) *Single molecule experiments.* The theoretical analysis of the fluctuation spectra of micrometre sized probe particles relates directly to a range of single molecule force spectroscopy techniques. For example, microrheology experiments are required to calibrate optical/magnetic tweezers and atomic force microscopes in such studies. Furthermore a second generation of single molecule experiments are investigating the dynamics of soft condensed matter systems on an individual molecular basis. Rheological

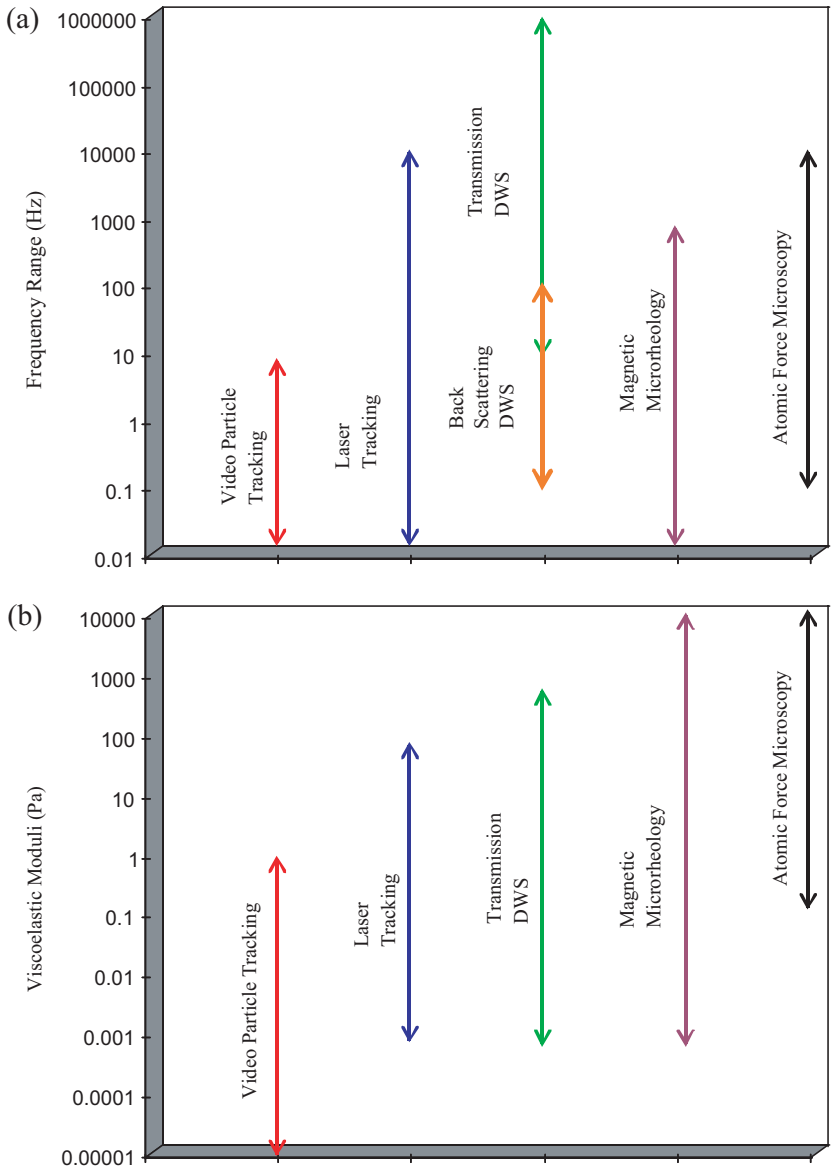

Figure 1. Typical ranges of the (*a*) frequency and (*b*) shear modulus (G', G'') calculated in microrheology techniques during measurement of the linear viscoelasticity of complex fluids. The response at low moduli has not been investigated in detail except with video particle tracking.

concepts are required to understand the response of these viscoelastic specimens (Okajima *et al* 2004).

Before entering into a discussion of specific experimental techniques, it is useful to try to get a broader outline of the range of frequencies and moduli that can be measured. For example, how is it possible to measure the linear viscoelastic spectrum of a new specimen of a complex fluid (figure 1)? Here there is a sharp dichotomy between passive and active microrheological techniques, where the probe particles are subjected to thermal energy or an

external field, respectively. Passive techniques are typically more useful for measuring low values of predominantly viscous moduli, whereas active techniques can extend the measurable range to samples containing significant amounts of elasticity.

It is instructive to compare microrheology with the field of inelastic scattering, which is a standard method for studying the wide range of dynamic processes that occur in complex fluids. Inelastic and quasi-inelastic scattering of light (Berne and Pecora 2000), x-rays (Grubel and Zontone 2004) and neutrons (Higgins and Benoit 1994) provide complementary information to rheological studies. The common bond between microrheology and scattering is that both allow non-invasive measurements of the time response of fragile complex fluids. This is not true with many electron/x-ray/confocal microscopy, and bulk rheological methods. Furthermore both microrheology and scattering offer a length scale sensitive measure of the dynamics of complex fluids. Microrheology is starting to compete on this front with inelastic scattering techniques, in contrast to previous bulk rheological methods, which typically provide time averaged measurements over many millilitres of material. Rheological measurements have the advantage that they quantify how materials store and dissipate energy, and thus directly relate to every day macroscopic observables, whereas scattering methods are connected with less physically tangible intensities in reciprocal space.

2. Physical phenomena

2.1. Introduction

It is useful to consider some of the general principles of the physics which underpin the microrheological techniques described. A rich variety of phenomena are observed in low Reynolds number dynamics. The phenomena have has been investigated in detail in molecular biophysics with respect to the motility of cells and microorganisms. The reader is directed to two clear and insightful pedagogic accounts (Berg 1993, Purcell 1977) for an introduction. The low Reynolds number approximation to the dynamics of small colloidal particles can often be invoked in microrheology experiments and can be crucial to provide tractable data analysis.

The *Reynolds number* for the motion of a small particle in a fluid ($\Re$, inertial force/friction force) is given by:

$$\Re = \frac{v R \rho_{\mathrm{m}}}{\eta}, \tag{1}$$

where η is the viscosity of the fluid, v is the particle velocity, R is the particle radius and ρ_{m} is the relative density. This dimensionless group ($\Re$) determines when it is possible to neglect the inertial force terms ($m\,\mathrm{d}v/\mathrm{d}t \cong 0$) in a particular system. From a basic analysis of Newton's second law for a particle moving in a viscoelastic material (mass m), the simplified equation of motion (no viscoelastic memory) is

$$m\frac{\mathrm{d}v(t)}{\mathrm{d}t} = F_{\mathrm{Sto}}(t) - \gamma\frac{\mathrm{d}x(t)}{\mathrm{d}t} - \kappa x(t), \tag{2}$$

where $F_{\mathrm{sto}}(t)$ is the stochastic thermal force acting on the system, γ is the drag coefficient, κ is the elastic constant, and $x(t)$ is the displacement of the particle as a function of time. It can be shown that in most passive microrheological experiments the viscous forces of the surrounding medium dominate the dynamics of the probe particles and the particles will coast to a halt in a few Angstroms if there are no forces actively driving their motion (Berg 1993). Although the average particle displacement of the probes is zero, they are constantly fluctuating due to thermalized collisions with surrounding solvent molecules, $\langle x^2(t)\rangle \neq 0$.

The *buoyancy* of the probe particles is another important practical factor to be considered in making accurate rheological measurements. The particle dynamics need to be examined

unmodified by the effects of sedimentation, far away from the perturbing effects of boundaries, to perform meaningful measurements. For example, probe particles dropping into and out of the plane of detection in particle tracking experiments can introduce high levels of low frequency noise drastically reducing the quality of measurements.

Using Archimedes principle, the effective mass (m') of a particle suspended in a fluid is given by its actual mass (m) minus the mass of the fluid it displaces (particle volume (V)$\times$ fluid density (ρ)).

$$m' = m - V\rho. \tag{3}$$

The number density of the particles $N(z)$, as a function of their height (z) above the bottom of the container, is given by a Boltzmann distribution when the particles are in thermal equilibrium (Berg 1993).

$$\frac{N(z)}{N(0)} = \mathrm{e}^{-m'gz/kT}, \tag{4}$$

where z is the height of the particles, m' is the mass adjusted for the buoyancy, $N(0)$ is the particle density when z is 0, g is the acceleration due to gravity and kT is the thermal energy scale. The concentration of probe particles in a container will thus change exponentially with height in thermal equilibrium.

The velocity (v_{sed}) adopted by the particles as they sediment also needs to be considered, i.e. the rate at which they approach equilibrium (Berg 1993). A simple analysis of the equation of motion of a non-interacting single particle in a purely viscous fluid gives an expression for the sedimentation velocity

$$v_{\mathrm{sed}} = \frac{2a^2 m' g}{9V\eta}, \tag{5}$$

where a is the particle radius, η is viscosity, g is gravity, and V is the particle volume.

For example, a polystyrene sphere of 1 μm radius and specific gravity $\rho_{\mathrm{s}} = 1.05\,\mathrm{g\,cm^{-3}}$ (iron oxide particles have densities as high as $5.2\,\mathrm{g\,cm^{-3}}$) will sediment in water with a velocity (v_{sed}) of $1.1 \times 10^{-5}\mathrm{cm\,s^{-1}}$. It will take about 2 h for such spheres to sediment 1 mm making tracking experiments in water possible. In contrast iron oxide particles require only 1 min to travel this distance making passive techniques difficult with such probes. It is a tacit assumption that low rates of sedimentation (v_{sed}) do not affect the fluctuation spectra measured in the perpendicular directions in PTM experiments and thus dense particles can be used for measurements in highly viscous materials. There continue to be a series of theoretical questions in the hydrodynamics of sedimentation that are not well understood (Dufresne *et al* 2000).

The question of buoyancy in PTM sets a number of experimental challenges; how are the probe particles to be prevented from dropping out of solution or how can the experiment be performed before this happens? One solution is to increase the viscoelasticity of the materials examined, but this can cause problems if the probe fluctuations are reduced below a detectable level. An ideal solution is when the sedimentation can be adjusted with an applied potential *in situ* with active microrheology techniques, such as optical and magnetic tweezers (Gosse and Croquette 2002, Starrs and Bartlett 2003). An optical or magnetic force can thus be used to balance the gravitational force.

Diffusion is a process whose conceptual complexity is often underestimated by a superficial examination of the basic mathematics involved. The diffusion coefficient in two dimensions (calculated, for example, by a time series of digitized camera images) of a particle in a purely viscous fluid allows the amplitude of particle fluctuations to be quantified.

The mean square fluctuation of a particle in n dimensions (Berg 1993) ($\langle r^2 \rangle$) depends linearly on time (t) with a proportionality constant D defined to be the diffusion coefficient

for *translational motion*

$$\langle r^2 \rangle = 2nDt \tag{6}$$

and $\langle r^2 \rangle = \langle r_x^2 \rangle + \langle r_y^2 \rangle$ in two dimensions ($n = 2$), the sum of the mean square fluctuations in the x and y components of the MSD.

The linear time dependence $\langle r^2 \rangle \sim t^\alpha$, with α equal to 1 in equation (6), corresponds to the case of diffusion in a purely viscous material, e.g. water or glycerol. Introducing an elastic element in the complex fluid causes the value of the exponent to reduce at short times and sub-diffusion is observed, $\alpha < 1$. Quantitative analysis of this subdiffusive motion can allow the calculation of the rheological properties of the material and this is the key concept behind many of the microrheological techniques that will be discussed (section 2.2).

The diffusion coefficient (D) of a probe particle (units $\mathrm{cm}^2\ \mathrm{s}^{-1}$) can be calculated from the fluctuation dissipation theorem in its simplest form i.e. it is inversely related to the frictional coefficient (f) of the particle scaled by the thermal energy (kT)

$$D = \frac{kT}{f}, \tag{7}$$

where f for a colloidal sphere can be calculated from Navier–Stokes equations and is given by:

$$f = 6\pi\eta r_\mathrm{h} \tag{8}$$

and thus the Stokes–Einstein relationship can be formed by combining equations (7) and (8)

$$D = \frac{KT}{6\pi\eta r_\mathrm{h}}, \tag{9}$$

where η is the viscosity of water, r_h is the hydrodynamic radius and kT is the thermal energy.

Rotational diffusion can also be considered in a similar fashion to translational motion to study the response of a complex fluid. In this case the fluctuations in the rotational displacement of a particle in three dimensions are given by the formula

$$\langle \theta^2 \rangle = 6D_\theta t, \tag{10}$$

where $\langle \theta^2 \rangle$ is the mean square fluctuations in the angle of rotation of the particle, D_θ is the rotational diffusion coefficient (units s^{-1}) and t is the time.

For a rigid sphere with stick boundary conditions, the rotational diffusion coefficient (D_θ) can be calculated by the fluctuation dissipation theorem equation (7), where in this case the rotational frictional coefficient (f) is $8\pi\eta r_\mathrm{h}^3$.

$$D_\theta = \frac{kT}{8\pi\eta r_\mathrm{h}^3}, \tag{11}$$

where η is the viscosity, and kT is the thermal energy. The dependence of the rotational fluctations on time become sublinear with the introduction of an elastic component in the material, in a similar manner to translational fluctuations.

The fluctuation spectra of minute particles embedded in a complex fluid are thus seen to be a measure of its viscoelasticity. The next challenge covered in section 2.2 is how to bridge the gap from the microscopic behaviour to macroscopic measures of viscoelasticity (G', G'', $J(t)$, etc). The relevant measures of bulk viscoelasticity are described in detail in section 3.1. Although the emphasis is often made in microrheology on the measurement of shear moduli (G', G''), it is also possible to examine longitudinal moduli (E', E'') with microdynamic mechanical testing apparatus (μDMTA), i.e. AFM.

2.2. *Generalized Stokes–Einstein equation*

A recurrent question in the analysis of the fluctuation spectrum of particle displacement in *passive* microrheology experiments is how to transfer between the compliance (proportional to the mean square amplitude of the particle fluctuations, equation (34)) as a function of time and the linear viscoelastic spectra (shear modulus) as a function of frequency. Measurement of a wide time range is required to provide the corresponding width in frequency space. A number of different methods are indicated in the literature for calculating this transformation and it is a well-defined class of mathematical 'inverse problem', having general features in common with such classic techniques as the Fourier transform (Bracewell 1986).

Historically the generalized Stokes–Einstein (GSE) equation was initially put forward on an ad hoc basis to analyse the thermal fluctuation spectrum of probe spheres (Mason and Weitz 1995). It was later placed on firmer theoretical foundations (Levine and Lubensky 2000). The algebraic form resembles that of the Stokes–Einstein equation (9), although it now involves Laplace transformed quantities

$$\tilde{D}(s) = \frac{kT}{6\pi a s \tilde{\eta}_s}, \tag{12}$$

where a is the radius of the probe sphere, $\tilde{\eta}_s$ is the Laplace transformed frequency dependent viscosity, $\tilde{D}(s)$ is the Laplace transformed frequency dependent diffusion coefficient and s is the Laplace frequency.

The GSE equation applied to colloidal hydrodynamics is thought to be valid if the inertial effects of the probe particles, the inertial effects of the fluid and the longitudinal compression mode of the fluid can all be neglected over the frequency range of the measurements. The effect of the particle inertia is shown to be negligible if the frequency obeys the inequality (Levine and Lubensky 2000)

$$\omega \ll \left(\frac{9G(\omega)}{2a^2\rho_f}\right)^{1/2}, \tag{13}$$

where $G(\omega)$ is the frequency dependent shear modulus, a is the particle radius, and ρ_f is the density of the particle. This introduces an upper bound on the frequency of a measurement, on the order of 10 MHz in a typical system (polystyrene spheres of 0.2 μm diameter in polyethylene oxide (PEO) solutions).

Similarly, the fluid inertia can be neglected if the measurement frequency obeys the inequality

$$\omega \ll \left(\frac{\pi^2 G(\omega)}{4a^2\rho_f}\right)^{1/2}. \tag{14}$$

This frequency is again on the order of 10 MHz.

The longitudinal compressive modes are typically on the order of 10 Hz for a polymeric material providing the lower frequency limit of the measurements. Thus there is a wide frequency range over which the GSE can be used with accuracy for colloidal hydrodynamics in practical situations (10–10^7 Hz).

Although they are in principle equivalent for well-defined MSD data sets, it is useful to make a comparison of the different *inversion methods*, which are required in order to transfer between the shear moduli and the compliance. Figure 2 shows a graphical representation of the possible methods of data inversion. Fourier transform methods (power spectral density $\langle r^2(\omega)\rangle$) may be useful for data sets over a wide range of frequencies with a wide range of features (Schnurr *et al* 1997). However for the limited data sets typically encountered in practice with microrheology experiments the numerical method using analytic continuation is

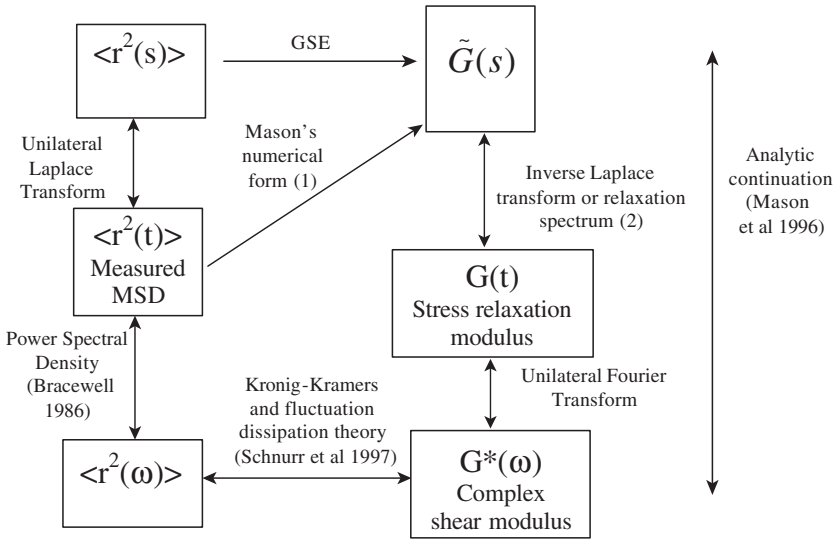

Figure 2. Possible methods of data inversion to provide the complex viscoelastic shear moduli (G', G'') from the compliance (or mean-square particle fluctuations $\langle r^2(t)\rangle$). s is the complex Laplace frequency, and ω is the experimental frequency.

(1) Mason and Weitz's numerical form for the shear modulus as a function of frequency (Mason 2000)

$$G(\omega) \approx \left.\frac{2kT}{3\pi a\langle\Delta r^2(\tau)\rangle\Gamma(1+(\mathrm{d}\ln\langle\Delta r^2(\tau)\rangle/\mathrm{d}\ln\tau))}\right|_{\tau=1/\omega},$$

where kT is the thermal energy, a is the probe radius, $\Delta r^2(\tau)$ is the MSD calculated at frequency time $\tau = 1/\omega$ and Γ is the gamma function.

(2) The shear modulus ($G(s)$) as a function of the Laplace frequency (s) can be fit to the functional form

$$\sum_j \left(\frac{G_j s}{s + 1/\tau_j}\right),$$

$G(t)$ is reconstructed as the sum of exponentials amplitude G_j and time constant τ_j.

becoming a standard tool (Mason *et al* 1997), since the Fourier transform method can provide noisy data with spurious high frequency fluctuations (Schnurr *et al* 1997). The Provencher algorithm is a readily available robust implementation of a numerical inverse Laplace transform, to construct the stress relaxation modulus from $\tilde{G}(s)$ (equation (12)), but analytic continuation is a faster tool for smooth data sets with few distinct features (Mason 2000).

The GSE equation in terms of the Laplace-transformed modulus $\tilde{G}(s)$ takes the form

$$\tilde{G}(s) = s\tilde{\eta}(s) = \frac{s}{6\pi a}\left[\frac{6kT}{s^2\langle\Delta r^2(s)\rangle} - ms\right], \tag{15}$$

where m is the particle mass, and $\Delta r^2(s)$ is the Laplace-transformed MSD. The additional subtracted term ms is included for completeness as an inertial correction. In the limit of a freely diffusing particle at low frequencies in a purely viscous fluid there is a simplified expression for the Laplace-transformed MSD (Mason 2000)

$$\langle\Delta r^2(s)\rangle = \frac{6D}{s^2} \tag{16}$$

and the frequency independent viscosity is recovered $\eta = kT/6\pi aD$ equivalent to the Stokes–Einstein equation (9).

Table 1. Classifications of homogeneous and heterogeneous microrheology systems typically encountered. Materials can transform from homogeneous to heterogeneous as a function of concentration, e.g. DNA in dilute solutions is effectively homogeneous (Goodman *et al* 2002) and heterogeneous in semi-dilute solutions (Chen *et al* 2003).

Homogeneous (1 point microrheology measurements typically give agreement with bulk rheology)	*Heterogeneous* (viscoelasticity of the material varies dramatically from point to point)
Neutral polymers in good/theta solvents, e.g. polystyrene in toluene/decalin (Starrs and Bartlett 2003), PEO in water (Mason *et al* 1997, Schnurr *et al* 1997)	Chemically cross-linked polymeric gels (charged, uncharged), e.g. actin with cross-linker proteins, poly(vinyl alcohol) (Narita *et al* 2001)
Charged flexible polymers in good/theta/bad solvents, e.g. polyacrylic acid (good), polystyrene sulphonate (bad), titin (theta) (Di Cola *et al* 2004), hyaluronic acid (good)	Physically cross-linked biopolymer gels, e.g. carragenans, pectins, collagens (Velegol and Lanni 2001), starches (Heinemann *et al* 2004)
Charged semi-flexible polymers, e.g. DNA (Goodman *et al* 2002), actin (Xu *et al* 1998), myosin, *de novo* peptide (Aggeli *et al* 2001)	Associating polymers, e.g. ionomers, hydrophobically modified polyelectrolytes (Di Cola *et al* 2004), hydrophobic/hydrophilic block copolymers (Lu and Solomon 2002)
Molecular liquids, e.g. water (Crocker and Grier 1996), glycerol, ethylene glycol	Gelled and jammed colloids, e.g. cheese, cement, jammed micelles
Colloidal fluids, e.g. PMMA spheres, polystyrene spheres (Sohn *et al* 2004), tomato bushy stunt virus, silica particles	Biological assemblies, e.g. cells (Fabry *et al* 2001, Lau *et al* 2003)
Lyotropic liquid crystals, e.g. surfactants (Cardinaux *et al* 2002), tobacco mosaic virus, actin	

Theoretical questions have been raised concerning the applicability of the generalized Stokes–Einstein equation (12) to charged sphere suspensions. Due to the long-range nature of electrostatic interactions, a charged tracer sphere experiences particularly strongly the discontinuous nature of its environment which could cause a break down of the GSE equation (Nagele 2003). However experimentally good agreement between micro and macrorheology has been found for highly charged linear polyelectrolytes with single PTM (section 4.1.2), so further experimental and theoretical work is required for a range of different charged complex fluids to properly understand this behaviour.

The analysis of the unconstrained motions of fluctuating particles has been considered in this section, i.e. passive microrheology techniques. The fluctuations in the motion of a trapped colloid (magnetic/optical tweezers) require consideration using the Langevin equation (36) and are discussed in section 3.2.3.

2.3. Heterogeneity

Many materials contain heterogeneous/inhomogeneous structures in nature, i.e. they are structured on a range of length scales greater than that of their molecular arrangement at the Angstrom scale. This phenomenon provides a challenge for the microrheologist, since the heterogeneities are typically on the length scale of the size of the probe particles and they can be the dominant factor determining the result of a viscoelastic measurement (Levine and Lubensky 2000).

Practically, the question of heterogeneity relates to an important problem; is the response of one probe particle a true measure of the bulk rheology? Forces between probe particles can sensitively affect their dynamics and a wide range of mesoscopic forces are possible with complex fluids (Evans and Wennerstron 1994).

Table 1 includes a list of materials, which are typically homogeneous or heterogeneous at the micron level with microrheology experiments. A familiar example of a heterogeneous

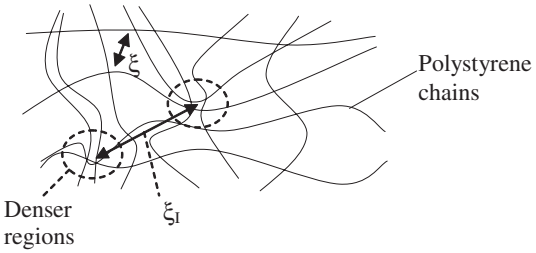

Figure 3. Schematic arrangement of polystyrene chains in a good solvent deduced from small angle neutron scattering measurements. Two length scales, ξ the mesh size and ξ_I the size of the heterogeneities are required to fully describe the system (Bastide and Candau 1996, de Luca *et al* 2004).

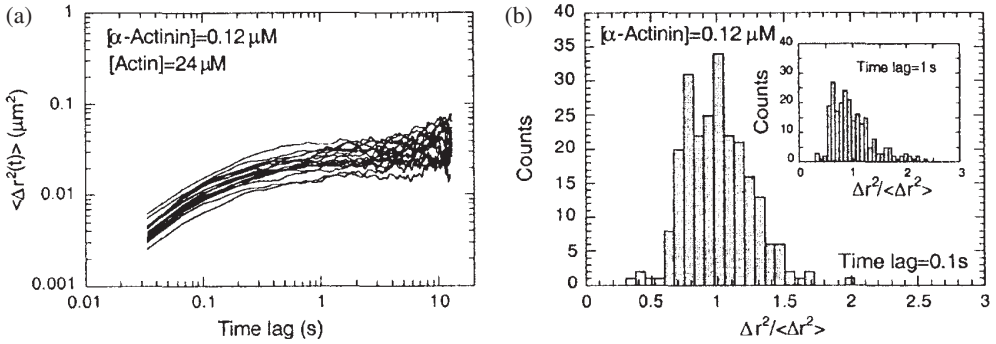

Figure 4. (*a*) Mean-square fluctuations of the particle displacement of colloidal probes embedded in partially cross linked actin, (*b*) histogram of the incidence (counts) of particle fluctuations of a particular amplitude for a time lag of 0.1 s (inset shows time lag 1 s) (Tseng and Wirtz 2001).

material is table jelly (jelo), in which relatively fluid sections (low number of collagen cross links) are interspersed with dense elastic regions (high number of crosslinks) at the microscopic level (Velegol and Lanni 2001). However, heterogeneous viscoelasticity is not specific to gel systems, any complex fluid in which the size of the probe particle is of the length scale of inhomogeneities in its structure, could cause the material to dissipate energy differently from point to point.

Even with some 'standard homogeneous fluids', as defined in table 1, such as polystyrene in a good solvent, two correlation lengths (ξ and ξ_I) are sometimes required to describe static neutron scattering experiments. There are heterogeneities on two simultaneous scales, the 10 nm length scale and the nanometre scale, even with this well-characterized, well-behaved complex fluid system, see figure 3 (Medjahdi *et al* 1991).

A challenge with PTM is encountered in the study of associating polymers which is practically closely connected with the examination of heterogeneity. This relates to the stabilization of the associating system against flocculation when mixed with the probe spheres (Lu and Solomon 2002, Valentine *et al* 2004). Flocculation would halt any serious microrheological investigation and is the extreme limit of strong probe/fluid attraction with the sphere perturbing the mesh.

Histogram methods are a useful tool for quantifying the heterogeneity in the compliance from multiple one-particle tracking experiments, in which the magnitude of particle fluctuations for a particular time step is plotted against the number of particles in an ensemble experiencing the fluctuations (figure 4). Large fluctuations correspond to less dense regions of the polymeric

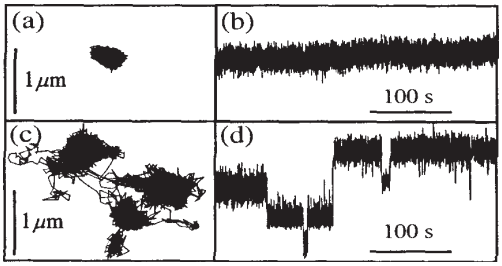

Figure 5. (*a*) Trajectory of a trapped fluctuating particle, (*b*) *y* coordinate of (*a*) as a function of time, (*c*) jumps between trapped trajectories over long times, and (*d*) *y* coordinate of (*c*) as a function of time (Wong *et al* 2004).

network and vice versa. There is an open theoretical question on how to relate the statistical distribution of the particle fluctuations to both the mesostructure and bulk rheology of a sample (Tseng and Wirtz 2001).

Further questions related to multiple particle tracking experiments in polymeric meshes have been examined (Wong *et al* 2004). The importance of the ratio of the probe particle radius (a) to the mesh size (ξ) in determining the results of one-particle microrheology experiments was demonstrated. When the size of the particle is on the order of the mesh size ($a \sim \xi$) anomalous subdiffusive dynamics were observed in the tracked particle trajectories due to probe spheres jumping between cages created by the actin mesh (figure 5). Thus for both one and two-particle experiments using materials containing large mesh sizes, care must be taken to account for such cage hopping artefacts. The statistics of the histograms of such particle motions (figure 4) have not yet been quantitatively related to the statistical mechanics of the activated diffusion in a particular system, although the framework of the mathematics required in terms of the probability of a particle jumping between cages has been sketched out (Wong *et al* 2004).

A new technique that overcomes some of the problems caused by heterogeneity has recently been introduced using the cross correlation of the motion of two thermally fluctuating probe particles (Crocker *et al* 2000, Levine and Lubensky 2000). Through examinination of the viscoelasticity of guar gels, it was established that the one-particle fluctuations were larger than the fluctuations measured from cross correlation (figure 6(*a*)). The linear viscoelasticity calculated from the one-particle technique largely underestimates the bulk value by a factor of 5. More importantly the cross-correlation method showed good agreement with standard bulk rheology techniques (figure 6(*b*)) and the method has now been established with a series of other complex fluid systems (DNA (Chen *et al* 2003), living cells (Lau *et al* 2003), and actin (Crocker *et al* 2000) etc).

The diffusion coefficient (D_{rr}) for correlated fluctuations of two-particle motions along the line connecting them takes the form of a GSE equation (12) rescaled by a factor of 3

$$D_{rr}(r, s) = \frac{kT}{2\pi r s G(s)}, \tag{17}$$

$$D_{\theta\theta} = D_{\phi\phi} = \tfrac{1}{2} D_{rr}, \tag{18}$$

where kT is the thermal energy, r is the particle separation, $D_{\theta\theta}$ and $D_{\phi\phi}$ are components of the diffusion tensor corresponding to the transverse components of the two-particle fluctuations in spherical coordinates.

A demanding test of the analytic machinery to quantify heterogeneity using two-particle cross correlation has been observed in the application of PTM techniques to living cells

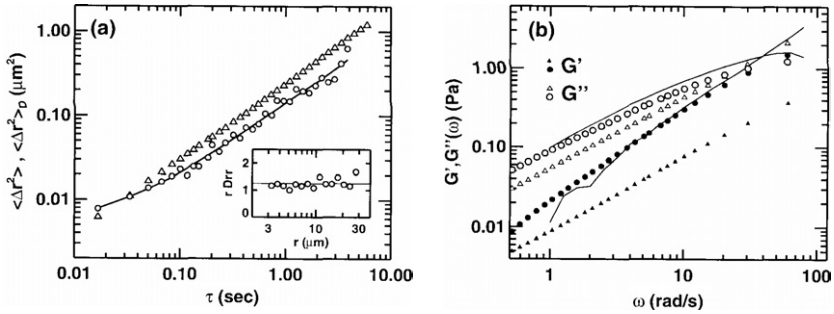

Figure 6. Comparison of the data from single-particle and two-particle cross-correlation tracking experiments with guar; (*a*) mean square particle fluctuations, triangles correspond to single particle and circles the cross-correlated particles (inset shows the behaviour of two-point correlation function versus particle separation where the $1/r$ behaviour is emphasized, or comparison with equation (18)). (*b*) The linear viscoelastic shear moduli calculated from the MSDs, circles correspond to the two-particle technique and triangles one-particle technique. Continuous lines refer to bulk measurement of the shear moduli in fair agreement with data from cross-correlated particle motions (Crocker *et al* 2000).

(Lau *et al* 2003). The internal cellular stress fluctuations were found to have a ω^{-2} power spectrum, as expected for a material with a slowly evolving internal stress.

Optical tweezer techniques (Starrs and Bartlett 2003) to measure the rheology of heterogeneous matter have subsequently been demonstrated using the same idea of cross correlation between the motion of the particles, but require a slightly different mathematical formalism. Here the fluctuations of two colloidal particles held in two separate optical traps are cross correlated and the analysis invokes a Langevin equation (36) with an explicit trapping potential. The microrheology of the correlated motion of the two trapped spheres is considered in terms of their individual friction coefficients. Newton's second law is applied to each particle relating the acceleration to the applied force (Starrs and Bartlett 2003). For example, for the displacement (x_1) of particle 1 it is found that

$$m\frac{\mathrm{d}u_1(t)}{\mathrm{d}t} = \int_{-\infty}^{t} \xi_{11}(t-t')u_1(t')\,\mathrm{d}t' - \int_{-\infty}^{t} \xi_{12}(t-t')u_2(t')\,\mathrm{d}t' - kx_1(t) + f_1^{\mathrm{R}}(t), \qquad (19)$$

where $f_1^{\mathrm{R}}(t)$ is the stochastic thermal force, m the colloidal mass, u_1 and u_2 are the velocity of particles 1 and 2, respectively, k is the spring constant of the optical trap, t is the time, ξ_{11} and ξ_{12} are, respectively, the force acting on one moving sphere when the second sphere is stationary and the force generated on one sphere by the motion alone of the secondary sphere. Such optical tweezer techniques can provide the high frequency rheology ($\sim$10 kHz) of heterogeneous materials (Starrs and Bartlett 2003).

Stress relaxation has been used to measure the heterogeneity in living cells by means of magnetic probes and active pulsed magnetic fields (Bausch *et al* 1999). The stress fields can be mapped across the cellular microstructure and then related to its biological function. Similarly the viscoelasticity across a cell can be mapped using magnetic cytometry by exerting a torque on ferromagnetic particles (Fabry *et al* 2001).

The disagreement in one-particle oscillatory magnetic microrheology with bulk rheology experiments has been examined (Schmidt *et al* 2000). It has been demonstrated that microrheology experiments with sinusoidally oscillated magnetic beads underestimate the viscoelasticity of actin solutions.

There is the intriguing possibility of using cross correlation of driven magnetic particles to study the local rheology of highly viscous samples in a manner similar to two-particle

optical tweezer and particle tracking experiments to provide better agreement with bulk rheology results. Here the cross-correlated motion would need to be calculated between active and passive particle motions, i.e. magnetic beads and surrounding inert probe colloids (Evans 2004). This cross-correlation method holds the prospect of a high modulus rheometer for heterogeneous complex fluids. A further idea is to examine the cross correlation of the rotational motion of rod-shaped particles (Reichert and Stark 2004), e.g. anisotropic ferromagnetic colloids experiencing a torque from a magnetic field and passive anisotropic particles.

Dynamic heterogeneous rheological phenomena such as shear banding (Olmsted 1999) and phase separation (Sohn *et al* 2004, Tanaka 2000) could be areas for future research once the behaviour of static heterogeneous systems have been thoroughly investigated.

2.4. High frequency viscoelasticity

The high frequency mechanical spectroscopy of complex fluids has only recently been subject to serious examination. The sole bulk technique available is the use of torsional oscillation, which provides measurements up to 10 kHz but only at a series of fixed frequencies (Fritz *et al* 2003), and is consequently not widely used. In comparison high frequency microrheology techniques, primarily DWS (Mason and Weitz 1995) and optical tweezers (figure 1), offer measurement of the linear rheology over a continuous range up to megahertz frequencies. There are a number of new novel dynamic processes which have been demonstrated and there are good prospects for relating the observed high frequency phenomena to the molecular dynamics of the components of the material. The theory to explain the new results is being developed hand in hand with the experiments and important advances have been made (Morse 1998). High frequency microrheology can provide relaxation times for the internal modes ($\sim$10 ns) of individual molecules in solution, which are typically unavailable from conventional solution-state bulk rheology (Xu *et al* 1998).

The crucial facet of the new microrheological measurements is that the small inertia of the probe particles facilitates the measurements, i.e. the probe particle motion can be rapidly reversed as required with high frequency oscillatory readings. Rapid reversal of the applied torque is not possible within conventional bulk rheometers, which typically have an upper frequency limit of 100 Hz for measurements of continuous spectra.

The inertia of the solvent at high frequencies with solution state complex fluids is observed as a correction to the dissipative shear modulus $G'' - \omega\eta$ (Ferry 1980, Larson 1999, Massa *et al* 1971) (ω is the frequency, and η is the viscosity of the solvent). A breakdown of the standard assumptions of the coarse-grained nature of the frictional coefficient of the solvent is thought to occur at still higher frequencies (above megahertz frequencies) (Morris *et al* 1988). This breakdown has, as yet, not been observed in microrheology techniques up to 10 MHz frequencies. The experimental evidence is from oscillatory electrical birefringence experiments that indicate that the rotational motion of solvents associated with polymers depends on their concentration (Lodge 1993).

Measurements of non-linear rheology have been achieved using DWS on colloids under shear (Uhomoibhi and Earnshaw 2000). Interpretation of the correlation functions from the experiments is still at an early stage of development.

Predictions exist for the high frequency viscoelasticity of polymers for free-draining and non-free-draining flexible chains in solutions, but as yet have only been well tested for intermediate frequencies (Ferry 1980). Claims have been made for the novel behaviour of the high frequency viscoelasticity of PEO in water, but this needs to be verified by further experiment (van Zanten *et al* 2004).

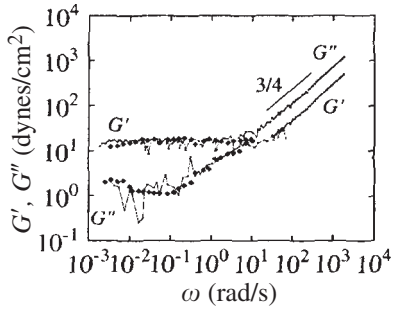

Figure 7. The linear viscoelasticity of semi-dilute semi-flexible polymers (actin). The high frequency $\omega^{3/4}$ modes for the shear moduli are shown for both the elastic (G') and dissipative (G'') components (Xu *et al* 2002). Dots indicate DWS data and filled diamonds are from bulk rheology.

Semi-flexible polymers are a testing ground for microrheological methods (figure 7). Further, as yet unseen, ultra-fast dynamic modes (less than nanoseconds) are now predicted for the high frequency fluctuations of semi-flexible polymers (Liverpool and Maggs 2001). These timescales may well be overlapping with those at which non-coarse-grained specific solvent/polymer interactions occur (Lodge 1993).

The GSE relation (equation (12)) has been tested with hard sphere colloids at high frequencies and the relevant dimensionless groups have been examined (Sohn *et al* 2004). The GSE equation works very well in this case.

The development of the double-trap optical-tweezer cross-correlation technique allows the measurement of the high frequency viscoelasticity of heterogeneous systems as described in section 2.2. DWS in contrast is only able to measure the ensemble averaged one-particle response (figure 7).

2.5. Geometry

The field of microfluidics has demonstrated many phenomena in which the geometry of a fluid system relates directly to the application, e.g. electrophoresis on templated structures (Hansen and Quake 2003), dying textile fibres (Quere 1999) and ink jet printing (Hansen and Quake 2003, Probstein 1994). To date there have only been a small number of microrheology experiments in confined geometries, but there is a large scope for the extension of the methods, driven by the possible applications.

The hydrodynamic interaction between two isolated colloids has been measured using in-line optical tweezers (Bartlett *et al* 2001) (figure 8). The hydrodynamic interaction with a surface has been studied (Dufresne *et al* 2000). These optical tweezer methods also allow accurate measurements of interparticle potentials in confined geometries, although experiments are limited to optically transparent materials.

Depletion (Verma *et al* 2000), bridging (Kampf *et al* 2004) and steric forces (Meyer *et al* 1998) are intimately related to the geometry of the confinement of a complex fluid and will have a direct impact on microrheology measurements. Silica spheres were found to have a depletion attraction in optical tweezers experiments when placed in DNA solutions in agreement with the Asakura/Oosawa model (Verma *et al* 2000).

There is a wide range of dynamic physiochemical effects, which are related to surface tension, such as coating flows and the Rayleigh instability (Probstein 1994). Such behaviours are only just starting to attract attention from the microrheological community. The change in viscoelasticity across a liquid–liquid interface has been considered (Sohn *et al* 2004). This

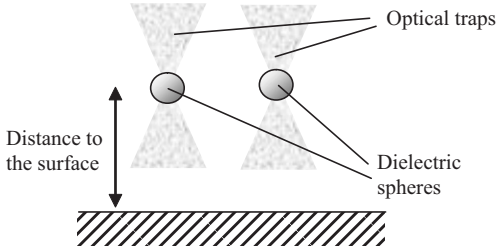

Figure 8. The hydrodynamic interaction between two colloidal spheres near to a surface can be measured using in line optical tweezers (Dufresne *et al* 2000).

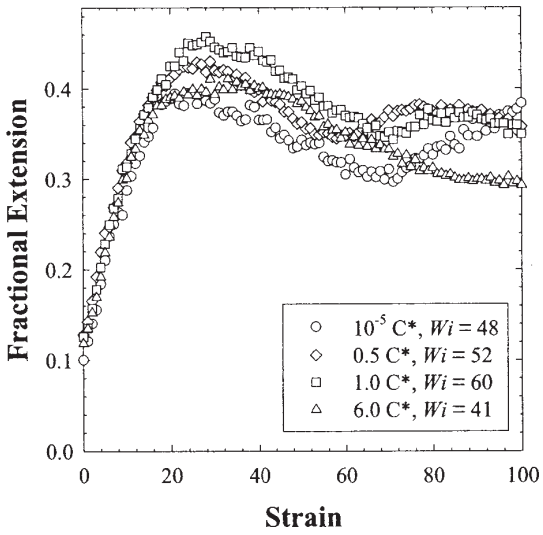

Figure 9. The degree of extension of single DNA molecules both above and below the overlap concentration (c^*) from fluorescence microscopy studies. The Weissenberg number (Wi) is included for each concentration (Hur *et al* 2001).

elegant study uses single optical fibre DWS to measure the rheology in a phase-separated polymer mixture.

2.6. Shear flow

Only a few studies on microrheology in shear flow have thus far been reported. They are direct analogues of bulk measurements, i.e. the complex viscosity (η^*) is calculated as a function of the shear rate ($\dot{\gamma}$).

Single DNA molecules have been examined in shear flow (Hur *et al* 2001, Larson *et al* 1997, LeDuc *et al* 1999, Perkins *et al* 1997, Smith *et al* 1996). DNA dynamics were probed as a function of the Weissenberg number (Hur *et al* 2001); the ratio of the diffusive time scale (t_D) of the translational motion of the chain to the characteristic flow time scale ($1/\dot{\gamma}$). Furthermore, the degree of extension of the DNA molecules could be measured as a function of strain (figure 9) (Hur *et al* 2001). An additional study by this group demonstrated the behaviour of chains in elongational flows using a crossed-slot shear-flow geometry (Perkins *et al* 1997). Another fluorescent DNA experiment combined with an optical trap was used to test a non-linear elastic dumbbell model for the chain hydrodynamics (Larson *et al* 1997).

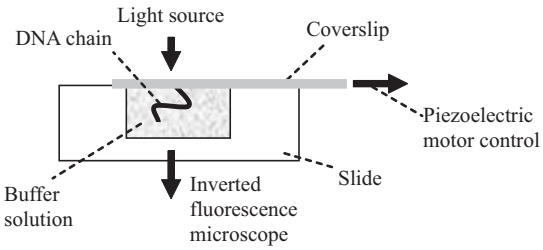

Figure 10. Dynamics of single biological macromolecules under shear were investigated by moving a coverslip over a microscopy slide controlled with a piezoelectric motor. The fluorescing tags attached to the macromolecule are observed with an inverted microscope containing a suitable light source (LeDuc *et al* 1999).

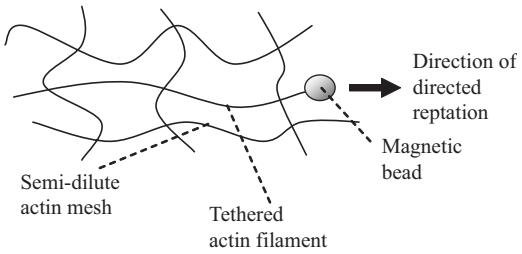

Figure 11. Actin filaments can be forced through a semi-dilute mesh using magnetic microrheology (Dichtl and Sackmann 2002).

Another simple, experimentally elegant method was designed to measure the response of a single DNA chain (LeDuc *et al* 1999). Here a DNA chain was attached to a microscope slide and the corresponding coverslip was glued to a piezoelectric motor stage as shown in figure 10. The dynamics of the chains under shear could be followed by imaging a fluorescent tag on the molecules using an inverted microscope with a suitable light source, such as a correctly filtered mercury lamp or a laser.

A sophisticated method to measure motion in shear flow uses magnetic tweezers (Dichtl and Sackmann 2002). Forced reptation of semi-flexible actin molecules in semi-dilute solutions was examined using this method (Dichtl and Sackmann 2002) (figure 11). The force on a single actin fibre can be studied and the viscoelastic relaxation of the motion of the fibre is represented using an equivalent circuit (figure 12). Anomalously high values of the frictional coefficient of the longitudinal motion of the fibres were found. They were not compatible with predictions from simple reptation theory and more work is needed to understand the measurements. Such experiments are subject to many of the same questions concerning the effects of microheterogeneity (section 2.3) as standard thermally activated one-particle tracking measurements. For example, how are the probe particle fluctuations activated by their hopping motions through the matrix of a heterogeneous complex fluid?

Optical tweezers are more limited for shear flow experiments, since the optical trap typically cannot be moved over such a wide length range limiting the applied stress. Furthermore the small trapping forces available with optical tweezers provide low measurable shear rates (see section 3.2.3) even in low viscosity materials such as water. DWS experiments have been applied to sheared colloidal motion, but the data analysis still offers a number of intriguing questions (Hebraud *et al* 1997, Uhomoibhi and Earnshaw 2000).

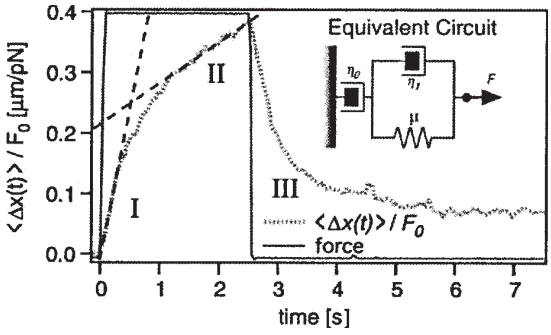

Figure 12. The stress response as a function of time of a single actin fibre experiencing forced reptation followed by relaxation. The equivalent circuit used to model the data is shown as an inset with two viscous dashpots and an elastic element (Dichtl and Sackmann 2002).

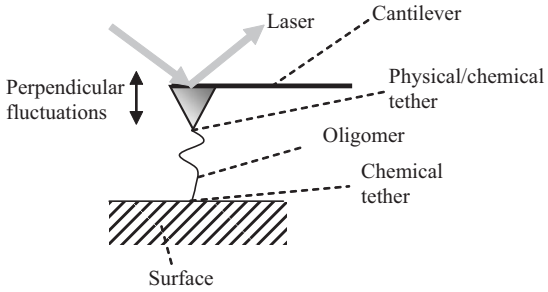

Figure 13. Schematic arrangement of an AFM single molecule microrheology experiment (MacKintosh and Schmidt 1999). The viscoelasticity of a single oligomer can be examined tethered between a surface and the cantilever.

2.7. Single molecule experiments

Following the success of single molecule force studies to examine tensile properties with AFM, and optical/magnetic tweezers, an obvious extension is to measure the dynamic modes of the specimens. With AFM this research is at a fairly early stage of development with questions existing on the analysis of data as a function of the cantilever geometry and the analysis of the statistics of the motion of a material on a molecule by molecule basis.

As described in the previous section, fluorescent labels attached to single molecules are typically used to image their dynamics. These fluorescent methods tend to be restricted to the study of giant biopolymers such as DNA (LeDuc *et al* 1999), titin (Tskhovrebova and Trinick 2002) and actin (Dichtl and Sackmann 2002). Similarly, optical and magnetic tweezers are typically restricted to the motions of large molecules (DNA, actin, titin, etc) and in this respect AFM has an advantage; it can be used to study oligomers (figure 13).

With AFM, optical- and magnetic-tweezer single-molecule experiments, an important question is whether the chemistry used to tether the molecules to the surface/probe affects the particle dynamics. A large amount of effort is thus expended in preparing protocols for correct sample adhesion.

In AFM, single molecule studies are often statistical in nature. An ensemble of molecules are stretched one after another using an automatic motorized routine. The experimentalist needs to decide which scans are characteristic of a molecule and which contain artefacts due to

the attachment of more than one molecule, the failure of cohesion or impurities. Attempts at data analysis with dynamic AFM on oligomers tend to be model dependent, i.e. the fluctuation spectrum of a cantilever is calculated from a molecular model.

The dynamics of partially stretched protein molecules were examined with a specially adapted AFM in which both the molecule and the surface could be sinusoidally oscillated (Okajima *et al* 2004). This allowed the viscoelasticity of the proteins to be examined during stretching.

Optical tweezer experiments (Svoboda and Block 1994) can be used in either probe/surface or probe/probe geometry (in-line tweezers) enabling complicated surface interactions to be avoided.

Cheap magnetic tweezers have enabled parallelized measurements on the single molecular elasticity of DNA chains (Assi *et al* 2002). This could allow combinatorial chemistry on the single molecule level (perhaps with biotechnological sequencing applications) or the examination of a large number of high modulus specimens (Amis and Schubert 2004).

Theory with regard to the structure and dynamic response of single polymeric chains has taken some recent advances (Dobrynin *et al* 1995, Farge and Maggs 1993). The behaviour of polymeric chains has been modelled in detail including the effects of both chirality (Moroz and Nelson 1998) and semi-flexibility (Liverpool and Maggs 2001).

2.8. Surface viscoelasticity

The dynamics of complex fluids often change dramatically when they are confined near a surface (Meyer *et al* 1998). The interfacial permutations of gas, liquid and solid interfaces (i.e. gas/liquid etc) all require individually optimized methods for the measurement of the surface viscoelasticity.

Both active and passive microrheological techniques (MacKintosh and Schmidt 1999) are possible to probe the viscoelasticity of a surface. Particles can be embedded in a surface and their thermally generated motility quantified using particle tracking microscopy techniques (Saxton and Jacobson 1997). Alternatively magnetic or optical particles can be attached to the interface and their dynamics probed using magnetic or optical tweezers, respectively.

For *liquid/liquid* interfaces, the viscoelasticity of membranes has been probed using single-trap optical tweezers (Helfer *et al* 2001). A large range of interactions are observed with cytoskeletal components (figure 14) and the wide range of possible viscoelastic responses are of direct relevance to the biological function of the membrane. Predictions for the in-plane and out-of-plane fluctuations of the membrane motions were made, related to the complex shear modulus of the material and compared with experiment.

Particle tracking applications in membrane dynamics (*liquid/liquid* interfaces) have been reviewed (Saxton and Jacobson 1997). The diverse range of interactions of bilayers with cytoskeletal proteins on the subdiffusive dynamics of tracked particles have been highlighted.

Liquid/solid interfaces were studied using the interaction of optically trapped spheres in a liquid as they approach a solid surface (Dufresne *et al* 2000). Corrections were found to the hydrodynamics of the trapped sphere depending on the distance from the surface.

Quartz crystal microbalances are used to probe the viscoelasticity of thin films adsorbed on quartz (*liquid/solid* interfaces). This is a resonance technique and as such is confined to a series of fixed frequencies for rheological measurements, although high frequency measurements can be performed (Buckin and Kudryashov 2001).

With AFM, the attachment of a colloidal sphere (figure 15) to the cantilever of the microscope provides a mechanism to simplify the probe geometry, better define its chemistry and thus provide more accurate measurement of the hydrodynamic interaction between the

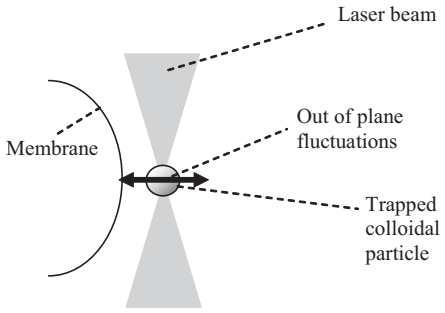

Figure 14. High frequency fluctuations of membranes can be measured using optical tweezers with a single trapped colloidal sphere (Helfer *et al* 2001).

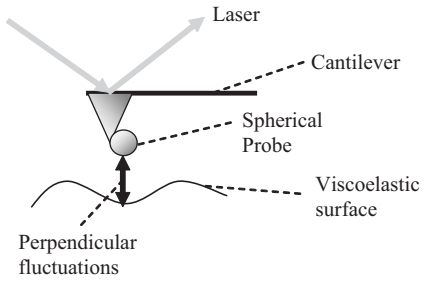

Figure 15. Out of plane fluctuations of the surface of a complex fluid can be measured with an atomic microscope using a colloidal probe attached to the cantilever (Mahaffy *et al* 2000).

surface and the probe. In this case the Hertzian approximation can then be invoked to calculate the viscoelasticity (Mahaffy *et al* 2000). This would seem to be the most flexible approach for the analysis of surface viscoelasticity, since it provides direct measurement of the complex longitudinal modulus (E', E''). These rheological functions can be subsequently modelled with standard rheological theory and compared with bulk measurements. However, the case of AFM microrheology is still far from being well developed. Problems exist with the tractability of calculations with regard to the surface forces and, in much the same way that particle tracking experiments are affected by bead chemistry, so too will cantilever, bead and surface chemistry affect dynamic AFM. The possibility of using unmodified tips as indenters is covered in section 3.2.5 (Alcaraz *et al* 2003, Benmouna and Johannsmann 2004).

Tribology, the study of the frictional properties of surfaces (typically *solid/solid* or *solid/liquid/solid* interfaces), is a field that could profit greatly from new microrheological methods (Meyer *et al* 1998, Scherge and Gorb 2001). Classic problems such as the frictional properties of cartilage (driven by medical questions concerning osteoarthritis) are hampered by the non-planarity of natural samples on the millimetre length scale. Nanotribology measurements using flexible probe geometries could revolutionize this area of research allowing frictional properties to be correlated with diseased states of the material. However, current microtribological methods are often not well-defined physically. The normal force is often not accurately measured, which is important to calculate friction coefficients (Amontons law defines the frictional coefficient (μ) to be the ratio of the frictional force to the normal force $F/N = \mu$) and care must be taken during analysis with this additional parameter. Surface force apparatus (SFA) can provide accurate values of the friction coefficient in constant (Kampf *et al* 2004) or oscillatory shear mode (Mukhopadhyay and Granick 2001), but these

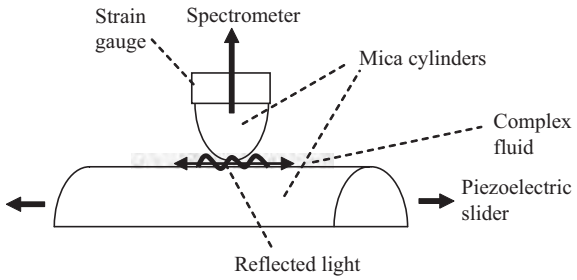

Figure 16. SFA can be used to measure surface microrheology by oscillating crossed mica cylinders (Boschkova *et al* 2001). The relative displacement of the surface is measured using ellipsometry.

measurements are for large areas of complex fluids adsorbed on to plane mica surfaces, limiting the range of problems that can be studied.

SFA can measure the frictional properties of fluids confined between two solid surfaces (*solid/liquid/solid* interfaces (Meyer *et al* 1998)), figure 16. Interesting surface effects have been described for the rheology of polymer melts. The polymer melts were found to become glassy below a critical film thickness due to the pinning of the chains on the mica surfaces (Luengo *et al* 1997). Another SFA study examined the shear moduli of confined soap films (G', G'') as a function of cylinder separation in the range 1–100 Hz. The films were found to have a dominant elastic component in contrast to their behaviour in the bulk (Boschkova *et al* 2001). With SFA the high frequency limit is again set by the large inertia of the apparatus, limiting the range of measurements.

The rheology of *gas/liquid* interfaces has been studied using a rotating magnetic rod rheometer (Bantchev and Schwartz 2003, Brooks *et al* 1999). The diameter of the rods is typically $\sim$100 μm, on the border of the microrheological regime. There is nothing limiting the size of the magnetic rod other than the available torque and, if scaled down further with a compensating increase in the sensitivity of the measurement of the particle deflection, this could provide a useful method for studying interfacial microrheology.

The nanorheology of a single perfluoropolyether meniscus bridge from a polymeric material was examined (Choi and Kato 2003) using two 20 μm glass spheres. The shear moduli were measured for bridges of length in the range 0–100 nm, at frequencies from 1 to 1000 Hz.

Practically, in most cases experimental studies of surface microviscoelasticity should be considered an order of magnitude more difficult than bulk microrheological studies. However such measurements are of fundamental importance to a series of fields, including membrane dynamics, phase separation, adhesion and tribology.

2.9. Time evolution

Complex fluids have a memory, but this memory can change over time i.e. the viscoelasticity can evolve with time (Ferry 1980, Larson 1999). For example, this could be in a gelation process (cooling a gelatine/water mixture), a nematic–isotropic phase transition (such as in the liquid crystalline display of a computer) or a biochemical process in a cell (e.g. contraction of muscle cells in the arm). To measure these processes places a further restriction on a microrheological measurement; a full spectrum must be acquired in a short time period, to provide detailed information on the evolution of the viscoelasticity of the material. This feat has been achieved in a multiple particle tracking study (Tseng *et al* 2002a, 2002b). The time evolution of the viscoelasticity of F-actin combined with cross-linking proteins was measured

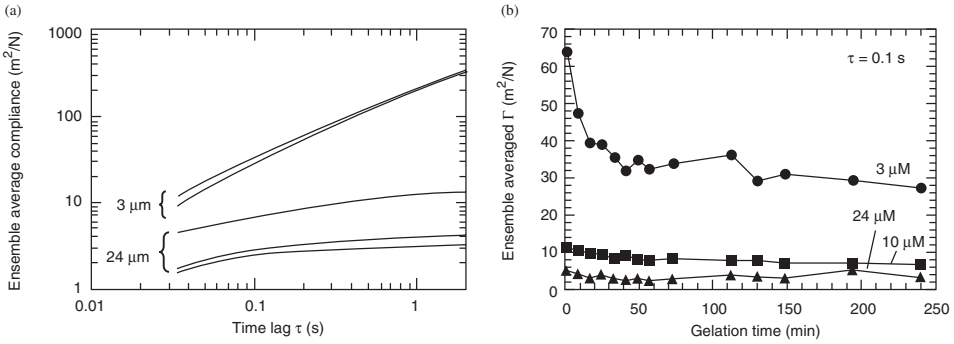

Figure 17. The ensemble averaged network compliance from PTM of actin gels. (*a*) Time lag dependence of the mean compliance (Γ), after the initiation of actin polymerization. (*b*) Evolution of the ensemble averaged compliance evaluated at a time lag of 0.1 s, during gelation of the solution (Tseng *et al* 2002a, 2002b).

with an 8 min time step (figure 17). This novel study indicates that it is the degree of network homogenization which controls the rate of gelation of F-actin networks (Tseng *et al* 2002a, 2002b). Thus the heterogeneity of the network reduces over time as the system approaches thermal equilibrium.

Phagocytosis (Feneberg *et al* 2001), the process by which a cell envelopes and transports food particles, has been studied dynamically with PTM. A cellular slime mould was examined as it interacted with folate covered polystyrene beads. Three phases of phagocytosis were found each of duration $\sim$10 s. The time resolution for the capture of an individual spectrum was of the order of 2 s.

Experiments with molecular motors also examine time evolution processes. Myosins (inch worm molecular motors) interact for well controlled time steps as they power themselves along actin filaments. Both the time steps and lengths of the working strokes have been measured for the interaction of single actin fibres with myosin using sophisticated dual-beam optical tweezers (Rief *et al* 2000).

The gelation of charged polystyrene colloids destabilized by the addition of salt was examined using DWS (Romer *et al* 2001). The averaging of non-ergodic correlation functions measured from the colloids was considered to obtain the high frequency moduli from the DWS experiments (figure 18). Practically, DWS is limited to sampling rates on the order of $\sim$1 min^{-1} to obtain well-defined correlation functions, which depend on sample absorption, laser power and detector efficiency. In principle, DWS can be used to investigate the evolution of features in the high frequency viscoelasticity of complex fluids at this rate.

3. Instrumentation

3.1. Types of measurement

Some standard types of bulk rheological measurement have their analogues in microrheological techniques (Goodwin and Hughes 2000). It is possible to calculate a number of measures relating to the linear viscoelasticity of a complex fluid including G', G'' (shear moduli), E', E'' (longitudinal moduli), J', J'' (complex compliance) and the Poisson ratio (ν) with suitable apparatus.

3.1.1. Linear viscoelasticity. A standard linear rheology experiment is to apply a sinusoidal stress/strain and measure the corresponding response of the material through its corresponding

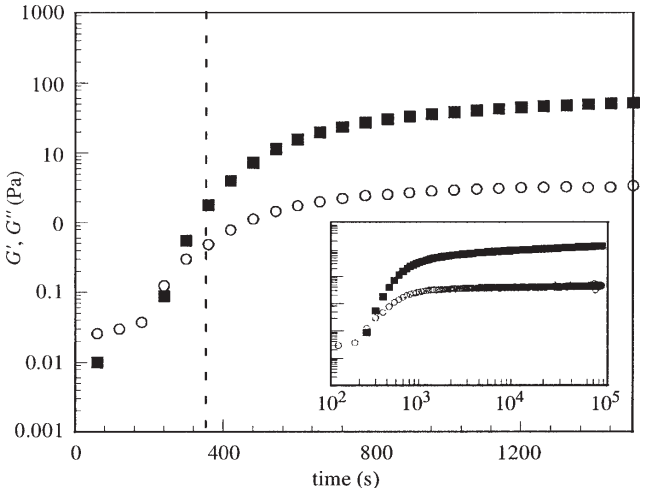

Figure 18. Evolution of the elastic and dissipative shear moduli (G', G'') from DWS as a function of time from the point that the charged colloids are destabilized. The inset shows the long time behaviour (Romer *et al* 2001).

strain/stress (Goodwin and Hughes 2000). For example, a shear strain (e) can be applied to a complex fluid as a function of time (t)

$$e = \mathrm{Re}(e_0 \exp[\mathrm{i}\omega t]), \tag{20}$$

where e_0 is the strain amplitude, and ω is the applied frequency.

The corresponding induced stress (σ) oscillates in time with a frequency ω, but is offset by a phase lag (δ)

$$\sigma = \mathrm{Re}(\sigma_0 \exp[\mathrm{i}(\omega t + \delta)]), \tag{21}$$

where σ_0 is a constant stress amplitude in Pascals.

The complex shear modulus G^* in Pascals is then defined by

$$G^* = \frac{\sigma}{e} = \frac{\sigma_0}{e_0}\mathrm{e}^{\mathrm{i}\delta} = \frac{\sigma_0}{e_0}(\cos\delta + \mathrm{i}\sin\delta). \tag{22}$$

The real and imaginary parts of the shear modulus can then be considered separately.

The *storage modulus* (real part) is a measure of the elastic energy stored in the system at a particular frequency and is given by

$$G' = \frac{\sigma_0}{e_0}\cos\delta. \tag{23}$$

Similarly, the *loss modulus* (imaginary part) is a measure of the energy dissipated as a function of frequency:

$$G'' = \frac{\sigma_0}{e_0}\sin\delta. \tag{24}$$

Active magnetic microrheological techniques typically follow the method of application of a stress followed by consideration of the resultant strain (the analogue of a bulk stress controlled rheometer). A strain controlled device has not yet been built, although it could be possible with particle tracking feedback control with magnetic/optical tweezers.

A method of comparing the storage and loss modulus is made by the calculation of the loss angle (δ), combining equation (23) and (24)

$$\tan\delta = \frac{G'}{G''}. \tag{25}$$

The viscosity is equal to the zero frequency limit of

$$\eta = \frac{G''}{\omega} \qquad \omega \to 0, \tag{26}$$

which often is equal to the zero shear rate limit of the bulk viscosity measured at a series of constant shear rates:

$$\eta = \eta(\dot{\gamma}) \qquad \dot{\gamma} \to 0. \tag{27}$$

The real creep compliance ($J(t)$) can be defined in terms of an incremental longitudinal strain ($\Delta e(t)$) and the resultant stress ($\Delta\sigma$).

$$J(t) = \frac{\Delta e(t)}{\Delta\sigma}. \tag{28}$$

In terms of bead deflections ($x_{\mathrm{d}}(t)$) in a microrheology experiment, bead radius (a) and an applied force ($f(t)$) the compliance can be calculated as

$$x_{\mathrm{d}}(t) = \frac{J(t)f(t)}{6\pi a}. \tag{29}$$

The complex creep compliance (J^*) can be defined with an oscillatory strain and resultant oscillatory shear stress. The complex compliance J^* is defined by

$$J^* = J_1 - \mathrm{i}J_2 = \frac{1}{G^*}. \tag{30}$$

The real Young's modulus for an elastic material is given by E

$$E = \frac{\sigma}{e}, \tag{31}$$

where σ is the applied longitudinal stress and e is the resultant longitudinal strain. It can be extended to a complex modulus as a measure of the viscoelasticity in much the same way as the shear modulus (G^*) and is measured in AFM microrheology experiments.

The Poisson's ratio (ν) provides information on how a material changes shape when strained (Lau *et al* 2003). For an isotropic material the ratio is given by

$$\nu = -\frac{e_{\mathrm{perp}}}{e}, \tag{32}$$

where e_{perp} is the linear strain in a direction perpendicular to the tensile stress producing the tensile strain e. The negative sign insures that the quantity is positive in most experimental situations. The Poisson ratio can be measured for viscoelastic specimens using two-particle cross correlation (Lau *et al* 2003).

The real shear modulus (G) for an isotropic elastic maerial is $G = \sigma_{\mathrm{c}}/\theta$ when the shear angle (θ) is very small and σ_{c} is a constant stress. The different elastic moduli are interrelated for a simple elastic material (Bower 2002).

$$G = \frac{E}{2(1+\nu)}. \tag{33}$$

It is possible to mathematically transform between different complex measures of the linear viscoelasticity such as J^*, G^*, E^* and the reader is referred to standard texts for their interrelationship (Ferry 1980, Goodwin and Hughes 2000).

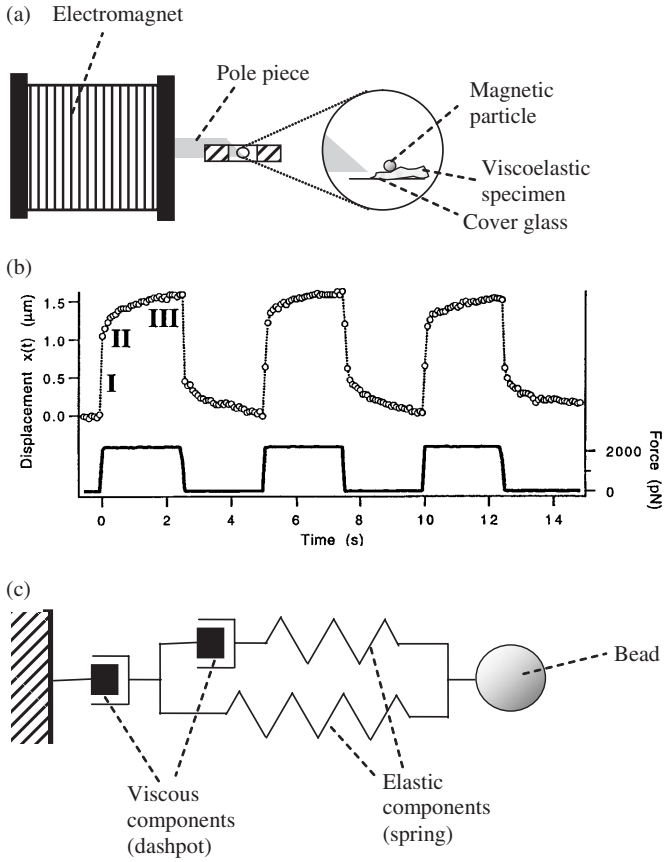

Figure 19. Stress relaxation experiments on the viscoelasticity of a material can be performed by the application of a stress field followed by measurement of the resultant strain response. (*a*) Schematic diagram of a single pole piece and magnetic probe (*b*) the applied magnetic field and the particle displacement in response and (*c*) the equivalent viscoelastic model (Bausch *et al* 1999).

3.1.2. Stress relaxation. Active microrheology techniques allow the option of measuring stress relaxation of a complex fluid under the influence of an external force. An example is the use of magnetic particles tethered to the surface of cells or embedded within them. The particles are subjected to a step-like magnetic field and their position with time is measured after the application of the force (figure 19). This is a less technically challenging microrheology experiment than that for probing the complex shear modulus, because the measurement of the phase information (δ) is not required. Only the measurement of the displacement of the particle in a correctly calibrated magnetic field is required. With a series of magnetic particles it is then possible to map out the stress relaxation across micrometre-sized samples using an optical microscope (section 2.2). Subsequently the data can be analysed by constructing an equivalent viscoelastic model (Maxwell, Kelvin, standard linear solid, etc) (Bower 2002, Goodwin and Hughes 2000) to fit the displacement relaxation observed (figure 19(*b*)). Such experiments are not constrained to step pulses, more complex stress functions could be envisaged whose form could be chosen to fit the application. Correct application of the stress fields requires linear amplification of computer generated signals and negligible inductance of the electromagnet coils (see section 3.2.4).

The stress relaxation of single molecules is considered in section 2.6 when actin fibres are subjected to forced reptation. Stress relaxation experiments are also possible using optical tweezers (section 3.2.3).

3.2. Techniques

A graphical comparison of the range of available apparatus to measure the linear rheology of complex fluids was made in figure 1. In the following section the practical requirements and limitations of these studies are considered, highlighting the major microrheological methods currently available. The techniques described require a degree of sample transparency to make microrheological measurements inside the materials.

3.2.1. Particle tracking microrheology. Particle tracking microrheology in its most basic form is a direct continuation from the oldest experimental microrheological study (e.g. Perrin). Probe particles are embedded in a viscoelastic specimen and the rheological characteristics are extracted from the thermal-fluctuation spectra measured using digital video-microscopy. Present-day studies tend to consider averaging over a large ensemble of simultaneously fluctuating particles embedded in a material, since this allows for better statistics on the subsequent rheological functions and facilitates analysis of the sample heterogeneity (Tseng and Wirtz 2001). The size of the probe particles is limited in standard video microscopy to optical wavelengths ($\sim$0.5 μm), as this is the diffraction limited resolution of an optical microscope.

Fluorescent particles can be used to decrease the probe size ($\sim$0.1 μm). This increases the spatial resolution in heterogeneity studies and increases the size of particle fluctuations ($\sim 1/r$). Fluorescent probe particles allow viscoelasticity to be probed in complex optical objects, e.g. allowing them to be easily located within living cells. However fluorescence studies can encounter challenges with respect to the chemistry of the fluorescent tags. Photobleaching gives a finite lifetime for the experiments and care is needed to avoid irrevocably changing the particle dynamics through the attachment of the probes. The measurable dynamic range in all these studies is defined by both the rate of data acquisition of the digital camera and the speed of data storage using either a CD writer or magnetic memory. It is typically of the order of 50 Hz (Chen *et al* 2003).

Anisotropic probes have not as yet experienced extensive consideration, one exception being the chains formed from magnetic nanocolloids ($\sim$10 nm radius) (Wilhelm *et al* 2003).

It is important to quantify the degree of noise inherent in particle tracking experiments, since this defines the largest moduli (smallest amplitude of particle fluctuations) that can be measured. Empirically it has been found that the tracked displacement of an immobilized colloidal sphere (e.g. glued to a coverslip) underestimates the errors that are included in tracked motions (Papagiannopoulos *et al* 2005). Superior calibration of the particle tracking experiments can be achieved with respect to the viscosity of a range of standard Newtonian fluids, e.g. glycerol/water mixtures at a range of concentrations. Figure 20 shows a typical range of fluctuation spectra measured as a function of time. Measurements with this experimental set-up (100$\times$ oil immersion lens, DVD recorder, Olympus BH2 microscope and Haitsu camera) are limited to glycerol concentrations below 80% glycerol or erroneous subdiffusive artefacts are introduced. Absolute calibration of the displacements in fluctuation spectra are typically performed by imaging a micrometer gauge onto the video microscope. An alternative method uses the displacement of a piezoelectric crystal and can improve the resolution to $\pm$1 nm in specific applications.

A related question is the accuracy with which the centre of the particles can be determined. Large particles subtend more pixels on the video camera and the centre of optical mass can

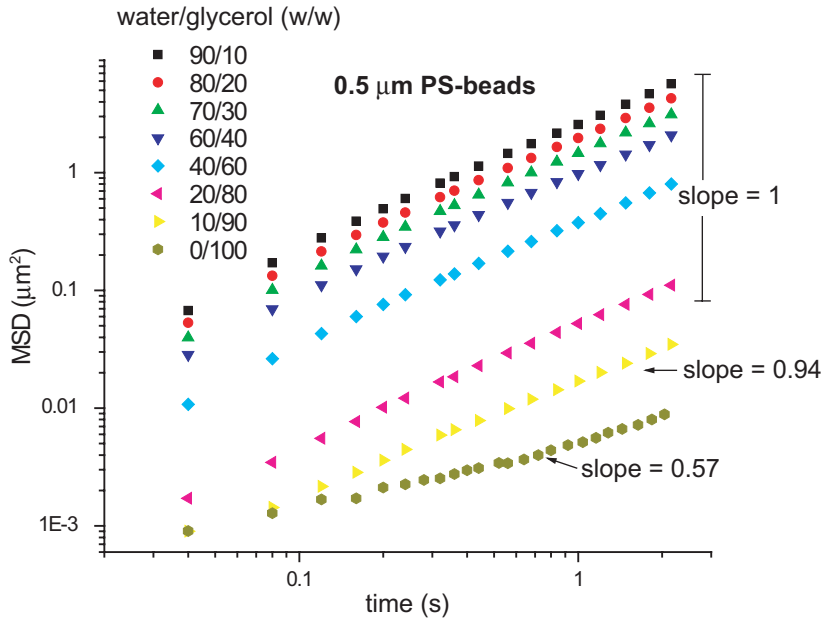

Figure 20. Mean square fluctuations (MSD) of 0.5 μm diameter polystyrene beads in glycerol mixtures of various compositions allow video PTM experiments to be calibrated (Papagiannopoulos *et al* 2005).

be obtained more accurately. Optical images of small probes are diffraction limited and steps need to be taken to deconvolve the diffraction effects. The accuracy with which the optical centre of a probe particle can be measured has been studied (Crocker and Grier 1996). It was calculated that image analysis techniques provide a resolution of ± 10 nm in the focal plane and ± 150 nm in depth with a $100\times$ oil immersion lens, S-VHS recorder, NEC TI-324A CCD camera and Olympus IMT2 inverted microscope.

The use of oil immersion optics is invaluable in providing high quality particle tracking movies free from the effects of particle/surface interaction, due to the increased working distance of the lens. Cavity slides (20 μl) can be purchased cheaply and the main limitation for minimization of the sample volume is that the fluctuations are analysed at a distance from the coverslip larger than that provided by Flaxen's law (Svoboda and Block 1994).

Software for PTM has been written on a number of platforms; IDL (http://glinda.lrsm.upenn.edu/∼weeks/idl/tracking.html), Labview and NIH have been the most popular. Multiple particle tracking and cross-correlation techniques are computationally intensive and have as yet only been implemented in IDL. The generic algorithms require the manipulation of large arrays and linked lists for the tracked particle motions, which require more advanced programming skills than that for single bead motion.

The dependence of the microrheological measurements on the colloidal probe size and on its chemistry need to be carefully considered; phenomena such as mesh hopping (Wong *et al* 2004) and depletion flocculation are not uncommon (Verma *et al* 2000). Thus in much the same way as a bulk rheologist considers their experimental geometry with relation to their measurements (Couette, cone and plate, etc (Goodwin and Hughes 2000)), a microrheologist must study the interactions of their probe bead with the complex fluids under examination.

Ideally, the bead chemistry should have a negligible influence on the result of a rheological measurement. A microrheologist needs to check that the probe particles are stable at the pH at

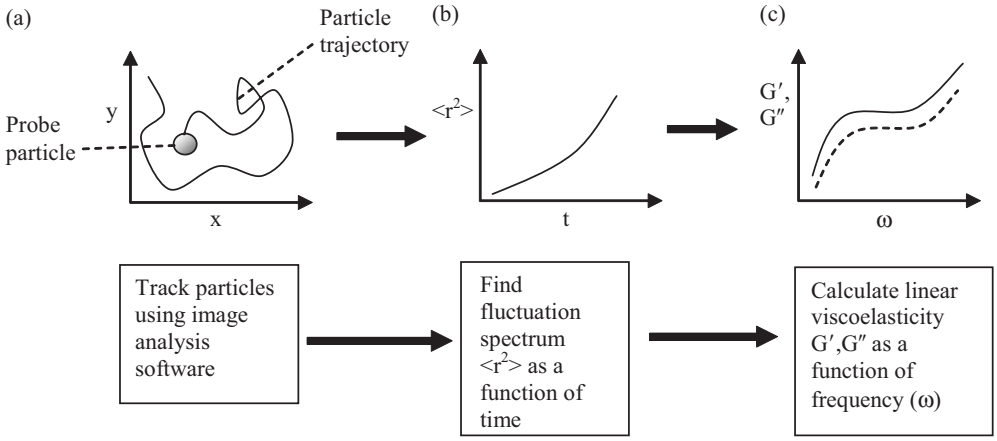

Figure 21. Particle tracking microrheology enables the linear viscoelasticity of low modulus materials to be extracted from the fluctuation spectrum. (*a*) Trajectory of the probe particle is measured, (*b*) the average fluctuation spectrum as a function of time t is calculated, and (*c*) the linear viscoelasticity as a function of frequency ω can then be found.

which the experiments are conducted and that there is negligible adsorption of the complex fluid on the beads surface, e.g. negatively charged fluids require negatively charged probes. Some work has already been published on sterically stabilized probes in organic solvents, but this requires careful choice of the complex fluid system limiting the range of applicability (Starrs and Bartlett 2003). Biological samples are restricted to aqueous chemistries, which leads to the standard use of pH sensitive charged colloids (polyacids, polybases). Experimentalists also require that there are no depletion effects destabilizing the probe particle/specimen mixtures.

A recent study has highlighted the effects of bead chemistry on measurements with biopolymers (Valentine *et al* 2004). Probe colloids were incubated with solutions of globular proteins (bovine serum albumin) to reduce adsorption of actin and fibrin. Interestingly the use of cross correlation between two particles reduces the effects of the bead chemistry on the linear rheology that is calculated.

This emphasis on bead chemistry can be considered an asset. Too many materials are placed into bulk rheometers with little thought for the molecular interaction between the sample and the cell. For example, depletion of the material at the walls of a rheological cell and dissolution of ionic impurities from the metals in the cell wall are both common artefacts with bulk rheology experiments on aqueous solutions. Particle tracking microrheology allows such interactions to be accurately measured.

The experimental steps for calculating the linear viscoelasticity using particle tracking are spelled out in figure 21. Particle trajectories are tracked, the mean-square amplitude of these fluctuations are calculated and the data is then transformed between rheological functions (typically the compliance is transformed into the complex shear modulus, section 2.2). It has been theoretically shown that the MSD of a probe particle (radius a, thermal energy kT) is directly proportional to the creep compliance ($J(t)$) (Xu *et al* 1998). The MSD data can thus be directly converted by rescaling into a standard rheological measure

$$\langle \Delta r^2(t) \rangle = \frac{kT}{\pi a} J(t). \tag{34}$$

It is a challenge to find neutrally buoyant probe particles for experiments in non-aqueous solvents. Polystyrene/toluene solutions were studied using PMMA tracers, and

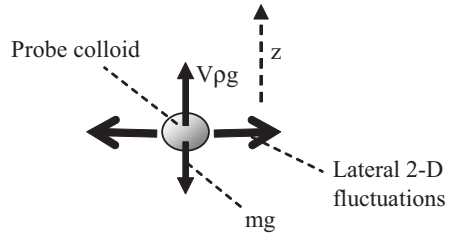

Figure 22. The sedimentation of a probe colloid in a viscoelastic solution (Berg 1993). At low Reynolds number it is assumed that the lateral fluctuations in particle displacement are decoupled from the sedimentation velocity.

good agreement was found with accepted rheological parameters at intermediate semi-dilute concentrations. However, sedimentation was a problem limiting the concentration range that could be measured and buoyant probes were not readily available. The terminal sedimentation velocity is a concern for PTM (figure 22). Problems are accentuated with complex fluids suspended in organic solvents whose density is often less than water (m' is large in equation (5)). Therefore the probes rapidly sediment, and make measurements of low modulus materials very difficult.

A major advantage of magnetic microrheology is that a vertical component of the magnetic force can offset the weight of the particle providing neutral buoyancy (section 3.2.4). The question of buoyancy is an important concern for measurement of low viscosity materials with dense metallic probes. Similarly optical tweezers can trap sedimenting probe particles, facilitating measurements with sedimenting probes (Starrs and Bartlett 2003).

Cross correlation of particle motions offers a new method for measuring sample heterogeneity. An experimental drawback with two-particle tracking experiments is that they require careful handling of the image analysis of long movies (∼30 min) and extremely well-defined sample environments to reduce high noise levels when compared with one-particle experiments (Crocker *et al* 2000).

The technique of particle tracking has been used to probe the dynamics of membrane systems. These allow the kinetics of reactions among membrane species to be measured (Saxton and Jacobson 1997).

3.2.2. Diffusing wave spectroscopy. The origins of DWS can be traced back to the seminal work of Weitz and Pine (Pine *et al* 1988). It was realized that the technique of photon correlation spectroscopy could be extended into the multiple scattering limit and there were a number of tractable methods of data analysis allowing the structure and dynamics of novel materials to be probed, e.g. foam coarsening (Hebraud *et al* 1997), and the aggregation process in cheese making (Horne 1989). Many of these subjects could not be analysed using standard light scattering methods, since the single scattering approximation is invalidated with these opaque materials (Stepanek 1993).

Subsequently, the DWS method was extended to become a new microrheology technique using multiple scattering from monodisperse colloidal probe particles embedded in a transparent viscoelastic material (Mason and Weitz 1995) (figure 23). This provides both the automatic ensemble averaging of a scattering technique and access to the ultra fast dynamics resolvable with commercially available photon correlator electronics (10 ns). Fast dynamics in a correlation function from dynamic light scattering (DLS) correspond to high frequencies in the complex shear moduli (∼10^6 Hz) when the data are mathematically transformed.

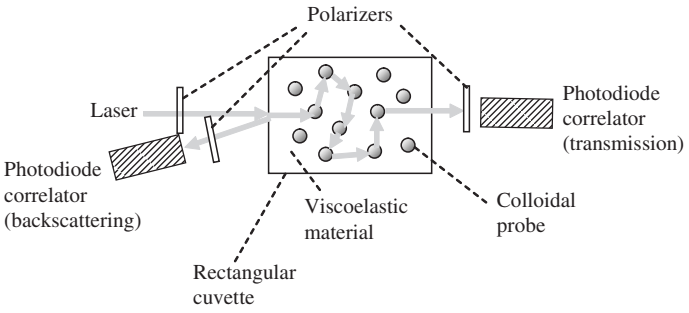

Figure 23. DWS can be used to measure the high frequency linear viscoelasticity of complex fluids using a laser with high frequency stability, probe particles in a transparent viscoelastic matrix and a fast correlator in transmission or back scattering geometries.

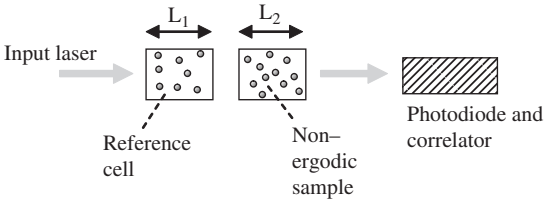

Figure 24. A reference cell placed in front of the non-ergodic sample of interest allows the correlation function to be physically averaged facilitating data analysis (Scheffold *et al* 2001).

Physically, the colloidal probes provide a strong contrast mechanism for light scattering. The large refractive index of the spheres masks the effects of the weakly scattering (non-contrast-matched) viscoelastic background, and establishes the accuracy of the mathematical approximations required for analysis of the correlation functions.

Back scattering and transmission geometry microrheology experiments are now fairly routine (Mason and Weitz 1995, Xu *et al* 1998). Practically it is deduced that an experiment is in the multiple scattering limit when the depolarized scattering is of the same magnitude as the polarized scattering ($I_{vv} = I_{vh}$). This is achieved by rotating a polarizer in front of the photodiode correlator and increasing the width of the sample cuvette or concentration of the probe particles until the depolarization condition is fulfilled. The DWS method is available to anyone with DLS equipment, square cuvettes and a flexible goniometer which can be placed at either 0° or $\sim 170^\circ$. Care is required to attenuate the signal in transmission geometry to avoid destroying the detector with the direct beam.

Experimentally a challenge to DWS is how to achieve rapid averaging of correlation functions over a series of sample volumes, with gelled or glassy non-ergodic specimens. This hurdle has now been overcome using the reference cell method (Scheffold *et al* 2001) (figure 24). A mathematical theorem (equation (35)) was established, the multiplication rule, which states that the ensemble averaged field autocorrelation function $g_1^{(2)}(L_1, L_2, \tau)$ of the double cell combination of the sample and opaque reference equals a product of the autocorrelation functions $g_1^{(1)}(L_1, \tau)$ and $g_1^{(1)}(L_2, \tau)$ corresponding to the individual cells. Thus, individual measurement of $g_1^{(2)}(L_1, L_2, \tau)$ and $g_1^{(1)}(L_1, \tau)$ followed by division provides the ensemble averaged autocorrelation function of interest $g_1^{(1)}(L_2, \tau)$.

$$g_1^{(2)}(L_1, L_2, \tau) \approx g_1^{(1)}(L_1, \tau) g_1^{(1)}(L_2, \tau). \tag{35}$$

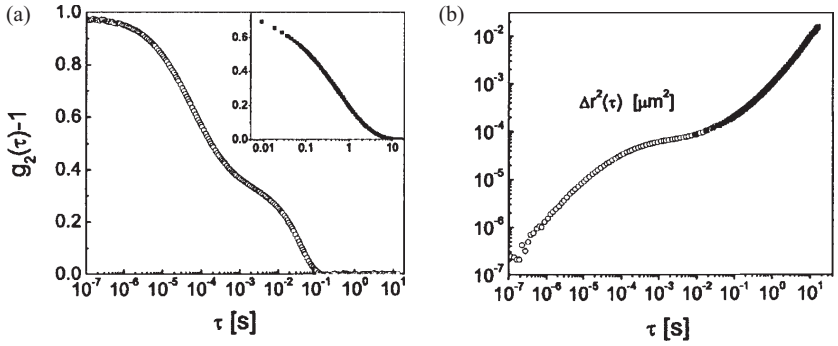

Figure 25. DWS experiments with polystyrene spheres embedded in worm like micellar solutions. (*a*) Correlation functions from the transmission and back scattering (inset) geometry, and (*b*) the composite compliance calculated for polystyrene spheres with both geometries (Cardinaux *et al* 2002).

The equation holds under certain well-defined circumstances; in particular scattering in the first reference layer should be much greater than the second sample one and there should be negligible losses of light at the interface. An alternative mechanical solution to obtain ensemble averaged correlation functions with non-ergodic samples is to oscillate the location of the incident laser beam (Nisato *et al* 2000) achieving averaging over a series of colloidal configurations.

CCD detectors with DWS (Cardinaux *et al* 2002, Furst and Gast 1998) are well matched to the study of the slow dynamics of glassy complex fluids, since they typically have very slow read out times and averaging over many pixels provides high resolution data. The back scattering geometry can be used to access slow relaxation processes, since the distribution of multiple scattering paths (length, s) is broad (probability distribution, $P(s) \sim s^{-3/2}$). A combination of CCD detectors and backscattering geometry extends the dynamic range that can be measured, figure 25.

DWS techniques have been used with single optical fibres to probe picolitre rheology with the detection of back scattered light (Popescu *et al* 2002) (figure 26). Many applications are foreseen (microphase separated materials, surface viscoelasticity and viscoelasticity in confined geometries) if the dependability of the technique could be established and protocols created for introducing the probe particles and fibres in a non-invasive manner. An exciting application of this picolitre DWS technique has been to measure the viscoelasticity across the boundary of a phase-separated polymer mixture (Sohn *et al* 2004) .

DWS has been used to examine the dynamics of sheared colloidal suspensions with light scattering through dense suspensions in a flow cell (Uhomoibhi and Earnshaw 2000). An additional study examined the *in situ* behaviour of the coarsening of Gillette shaving cream (Cohen Addad *et al* 1998). Experiments on emulsions subject to oscillatory motions have been studied using echo DWS (Hebraud *et al* 1997).

3.2.3. Laser particle tracking (optical tweezers). The fluctuation spectrum of a probe particle embedded in a complex fluid can be measured using the laser light forward-scattered from a single particle with a quadrant diode. The incident laser can lead to a tweezing action in the case of high fluxes of light, although this is not necessary for microrheological measurements (Svoboda and Block 1994). Thus laser tracking can lead to both ‘passive’ and ‘active’ types of microrheology.

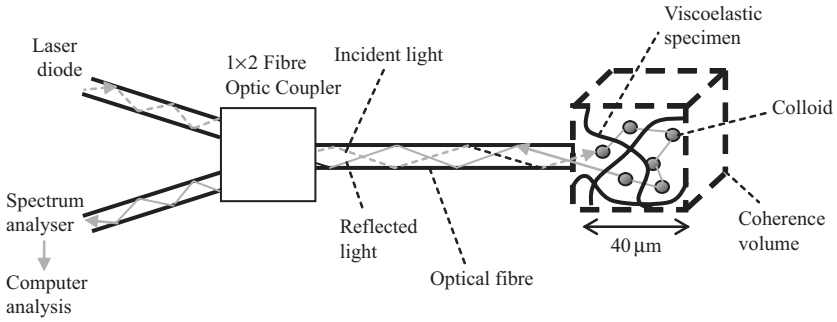

Figure 26. Optical fibre DWS can measure picolitre rheology using back scattered light from colloidal probes embedded in a viscoelastic material (Popescu *et al* 2002).

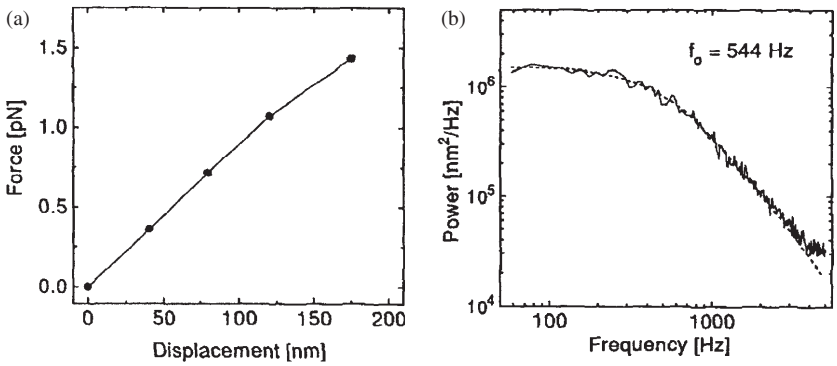

Figure 27. The power spectrum of optical tweezers in the frequency domain has a particular form derived from the Langevin equation (36) due to the interplay of the trapping potential and random thermal forces. (*a*) The force as a function of particle displacement in the optical tweezers, and (*b*) the fluctuation spectrum of the trapped sphere (Svoboda and Block 1994).

Quadrant diodes are a critical technology involved in optical tweezers and AFM. Differential photon-induced voltages are measured between pairs of the four diodes allowing accurate x–y positioning of the particle at high frequencies ($\sim$10 kHz). However, the operation of the noise function in quadrant diodes has only just begun to attract serious attention. It is thought to be a delicate interplay between electronic processing, semiconductor noise, and the quantum interaction of photons with semiconductors (Berg-Sorensen and Flyvbjerg 2004, Howard 2001).

The use of laser trapping for microrheology experiments complicates the analysis of microrheology data, since both the trapping force and the stochastic collisions with the viscoelastic sample effect the dynamics of the probe particles. A Langevin equation is required to analyse the fluctuations of the trapped beads. For low Reynolds number with a Newtonian fluid it is given by

$$-\eta\frac{\mathrm{d}x}{\mathrm{d}t} + \kappa x = F(t), \tag{36}$$

where $F(t)$ is the stochastic thermal force, κ is the elastic constant, and η is the drag coefficient. The particle fluctuations $\langle r^2 \rangle$ now have an upper bound at large times due to the trapping force (or low frequencies in figure 27(*b*)).

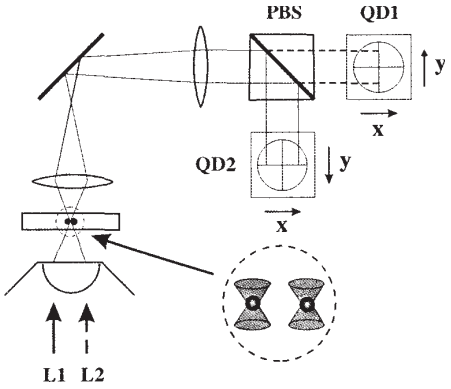

Figure 28. Schematic arrangement of the apparatus required for dual trap optical tweezers for viscoelastic studies. L_1 and L_2 are two orthogonally polarized laser beams, QD1 and QD2 are quadrant diodes, and PBS is a polarized beam splitter (Starrs and Bartlett 2003).

Optical tweezer apparatus used in force measurement (figure 27(*a*)), often employ microrheological techniques to calibrate the strength of the laser traps. Analysis of the Brownian motion of a trapped colloidal sphere can provide the strength of a trap with sub 1% error using statistical analysis of the Langevin equation and well characterized photomultiplier tubes (Berg-Sorensen and Flyvbjerg 2004). An alternative calibration method is to push fluid through the sample cell containing the optical trap and probe sphere. The trapped particle becomes dislodged from its optical potential well when the Stokes frictional force, equation (8), becomes equal to the trapping force. Such shear flow experiments (section 2.6) are thus commonplace with optical tweezers.

Recently in-line dual trap optical tweezers have been used to study the viscoelasticity of complex fluids (Bartlett *et al* 2001). The traps can be constructed from a single laser divided using a beam splitter and their position adjusted in the focus of an optical microscope using a mirror. A typical arrangement for a dual beam trap is shown in figure 28 (Starrs and Bartlett 2003).

The cornering frequency (f_0 on figure 27(*b*)) measured from the fluctuation spectrum of a trapped bead provides an accurate method to calibrate optical traps. Theoretically it can be calculated from a Fourier transform of the Langevin equation, equation (36). The power spectrum ($\sum_x$) as a function of the frequency (f) is found to be given by

$$\sum_x (f) = \frac{kT}{\pi^2 \gamma (f_0^2 - f^2)}, \tag{37}$$

$$f_0 = \frac{\kappa_x}{2\pi\gamma}, \tag{38}$$

where kT is the thermal energy scale, γ is the drag coefficient, and κ_x is the effective lateral spring constant.

Viscous corrections to the coupled motion of two particles near to a flat surface (Dufresne *et al* 2000) have been measured with dual optical traps. Corrections to the fluctuation spectra of the trapped particles persist to surface/particle separations of tens of particle radii, emphasizing the need for the isolation of particle dynamics away from surfaces to provide correct microrheological analysis. Flaxen's law for simple liquid hydrodynamics can be invoked for one-particle interaction with a surface and this consideration is useful in a range of microrheology techniques (Svoboda and Block 1994). The law requires that the ratio

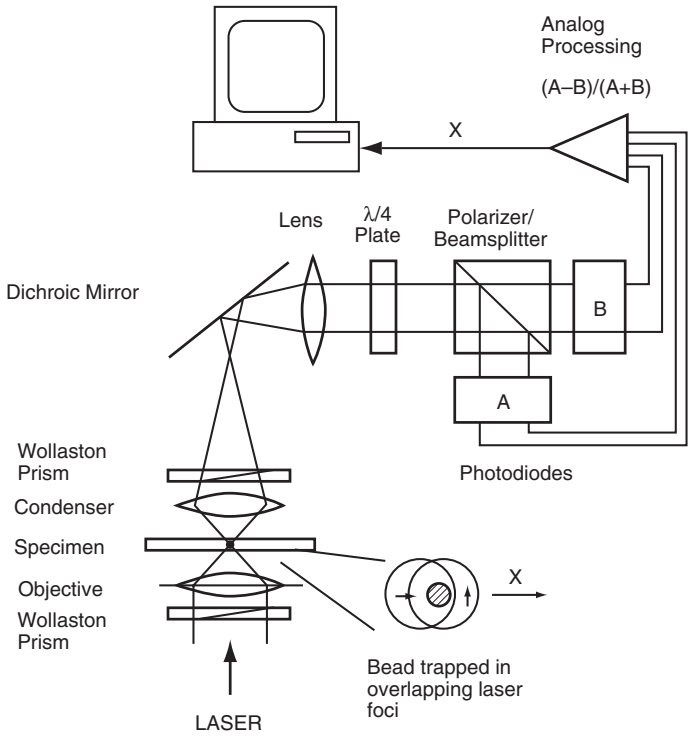

Figure 29. Setup of a back focal plane interferometer to measure the linear viscoelasticity of complex fluids (Schnurr *et al* 1997).

of the distance to the surface (h) to the colloidal diameter (a) is greater than fifty ($h/a > 50$), for the hydrodynamic corrections in water of the particle motion to be less than 1%. It provides a useful practical limit for probing bulk viscosities in confined complex fluids, e.g. samples contained in microscope slide cavities.

The fluctuations of membranes associated with cytoskeletal proteins have been studied using optical tweezers (Helfer *et al* 2001). The measured shear moduli for the composite membranes were consistent with measurements from actin solutions.

DLS experiments have been performed with optically trapped micrometre spheres using a second laser orthogonally incident to the first (Viana *et al* 2002). It is not evident whether the increased complexity in setting up the coincident lasers is offset by the ease of calibration of the trap using DLS and the increased dynamic response.

The possibility for tailored optical potentials (saw-tooth functions, etc) through modulation of the trapping laser beam implies that more sophisticated rheological experiments are possible, such as stress relaxation. Oscillating tweezers have been used to extract the storage and dissipative shear moduli of complex fluids (Hough and Ou-Yang 1999). Furthermore, polarized optical traps can induce rotation in dielectric spheres. This effect could also be used to explore viscoelasticity, but only a small torque is provided by the absorption of the angular momentum from photons, limiting measurements.

Back focal plane interferometry is an accurate method for measuring particle fluctuations in laser particle tracking, figure 29 (Allersma *et al* 1998). The laser beam is split into two oppositely polarized components, and focused on the probe particle. The relative changes in polarization upon scattering provide information on the particle displacements. The technique

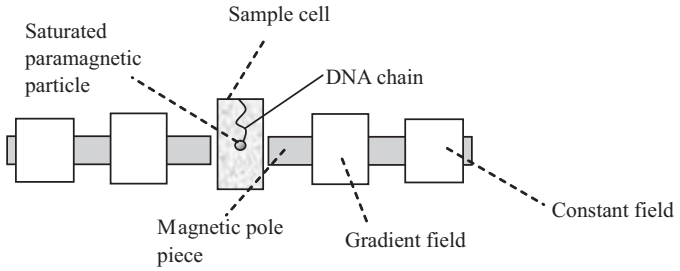

Figure 30. One-dimensional magnetic tweezers for force–extension/stress relaxation experiments (Haber and Wirtz 2000). Two sets of electromagnetic coils provide a gradient field over a constant background magnetic field.

has the advantage that the displacement detection is biaxial, has higher resolution than the standard arrangement, and is independent of the position of the laser focus in the specimen plane.

Thermal noise imaging has been achieved in three dimensions by tracking an optically trapped sphere with a photonic microscope (Rohrbach *et al* 2004). The three-dimensional positioning of the particle has been measured using both scattered laser light (Rohrbach *et al* 2004) and off-focus imaging of the fluorescent particles (Speidel *et al* 2003).

3.2.4. Magnetic tweezers. Magnetic tweezers (figure 30) and microrheometers fall into the 'active' category of microrheological techniques. Relatively large forces (2000 pN) can be applied to complex fluids extending the measurable range of moduli above that available with passive particle-tracking methods (the thermal forces are typically sub-pN in value). Indeed viscosity measurements on non-Newtonian fluids have been obtained up to 10^7 Pa using a falling magnetic needle in a strong magnetic field (Chu and Wang 1992). The frequency response could not be measured with the falling needle technique, the data are output as the viscosity plotted as a function of the shear rate over a limited range (0.007–10 Hz).

Oscillatory magnetic microrheology has the advantage that it can use heterodyne detection with video recording techniques, since there is a controllable driving force. Thus the maximum resolvable frequency is set by the speed of the camera shutter ($\sim$1 ms) and the induction of the magnetic coil (Fabry *et al* 2001). It is not the time between subsequent frames ($\sim$10 ms) as with passive techniques (figure 1).

Magnetic tweezers also have the advantage over their optical counterparts that they generate no heat in the sample examined, can have a uniform force field over the entire field of view (μm^2 s) and can orient objects regardless of their geometry (Strick *et al* 2003). They do have the disadvantage that it is difficult to make multiple independent traps.

There is an important choice in the strategy for the construction of magnetic tweezers, which is dependent on the nature of the magnetism in the probe particles. The main division is between ferromagnetic and super paramagnetic tweezers. Practically the distinction depends on both the size and chemistry of the particles. Particles whose size is reduced below the Weiss domain of the material are superparamagnetic and those above are ferromagnetic (Trepat *et al* 2003). Usefully for single molecule experiments, super paramagnetic particles do not experience a large torque, they rapidly adjust their induced dipole moment to avoid rotational motion and this facilitates the data analysis of their translational motion.

The calibration of magnetic tweezers holds some interesting questions, which are solved by a number of sophisticated methods. The fluctuation spectrum of the spheres in the trapping

potential can be considered in much the same fashion as with optical tweezers, but the strength of the magnetic field can also be studied *in situ* from point to point using Hall probes (Gosse and Croquette 2002). A complicating factor is that the magnitude of the induced magnetic dipole in the probe can depend on the magnitude of the applied field.

The potential energy (U) of a magnetic dipole (m) placed in a magnetic field (B) is given by the scalar product

$$U = -\underline{m} \cdot \underline{B}. \tag{39}$$

Thus a free permanent dipole experiences a torque as it minimizes the energy by aligning the dipole with the magnetic field.

The corresponding magnetic force (F) is the gradient of the potential (∇U) and for superparamagnetic spheres $m \cong M_{\max}$.

$$F = -\nabla(\underline{m} \cdot \underline{B}) \tag{40}$$

$$F \approx M_{\max} V \frac{\mathrm{d}B}{\mathrm{d}x}, \tag{41}$$

where V is the particle volume, and $M_{\max}$ is the saturated magnetization. The torque on a large ferromagnetic particle (4 μm) can be quite considerable ($\sim$1000 pN μm) and magnetic tweezer cytometry has found applications in determining the elasticity of cells adhered to magnetic beads (Laurent *et al* 2002).

Magnetic forces can be used to construct tweezers in an analogous manner to their optical counterparts through creation of magnetic field gradients in three dimensions (equation (39)) (Gosse and Croquette 2002). Accurate methods to calculate the position of the particle in three dimensions are thus required. A current solution has been to use video imaging for the x–y components of the particle displacement and to analyse the diffraction rings around the particles to obtain the z component corresponding to the height of the particle. The diffraction ring method requires careful preparation of well parallelized illumination optics (Gosse and Croquette 2002). Off-focus imaging with fluorescent particles could equally have been used in this application (Speidel *et al* 2003).

Feedback control of the applied magnetic forces is also required to create a direct analogue of optical tweezers, i.e. to hold a particle with a constant force as it is moved across the field of view. The feedback control has been achieved electronically by on-line monitoring of the magnetic field and fluctuation analysis of the displacement of the probe particles (Gosse and Croquette 2002).

The frictional force on a particle in a Newtonian fluid can be used to calibrate magnetic tweezers if the field strength and dipole moment are both unknown. For example, in the driven motion of a superparamagnetic sphere, the probe quickly reaches its terminal velocity once the magnetic driving force is balanced by the frictional force (equation (8)). Dipole moments on the magnetic spheres can be separately quantified using a SQUID or a vibrating sample magnetometer.

A series of different arrangements have been designed to manipulate magnetic particles with a varying number of pole pieces (1, 2, 4, 6 and 8). There is as yet no consensus as to the most effective set up for rheological measurements.

Single pole piece magnetic microrheology was examined in the context of stress relaxation (section 3.1, figure 19 (Bausch *et al* 1999)). This is the simplest magnetic rheological apparatus to build, since it does not require measurement of the phase angle of the complex moduli, equation (22). Such a device with a single needle-shaped pole piece was used in a biological study of the formation of intracellular neurites (Fass and Odde 2003).

A different strategy has been used (Haber and Wirtz 2000) for the micromanipulation of DNA attached to a magnetic particle using *two pole* pieces (figure 30). For force extension

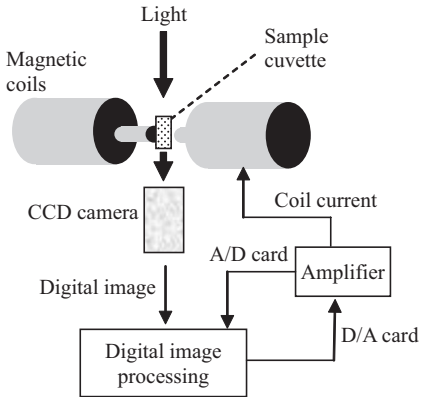

Figure 31. Magnetic microrheometer for studying the linear viscoelasticity of complex fluids (Keller *et al* 2001). The digital video images and the coil current are acquired simultaneously and stored on the hard disk of the personal computer.

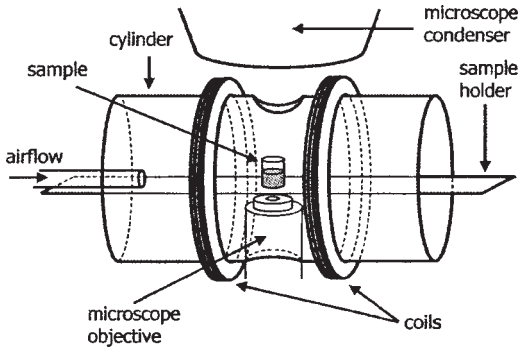

Figure 32. Magnetic tweezers designed for use with ferromagnetic particles (Trepot *et al* 2003). The air flow is used to control the sample and electromagnet temperature.

studies a constant magnetic field was applied to saturate the magnetic dipole moment of the paramagnetic probe particles and the particle was subsequently moved using a second gradient magnetic field, equation (41). The arrangement of combined constant and varying magnetic field cannot be used for oscillatory experiments, since the constant field would also need to oscillate, defeating its purpose in saturating the magnetization of the paramagnetic probes.

An oscillatory *two pole* piece magnetic bead microrheometer has been built which has a high frequency response (50 Hz, figure 31) (Keller *et al* 2001). This frequency response is made possible by using a fast CCD attached to a frame grabber card synchronized with an additional A–D card connected to the magnetic pole pieces to measure the phase shift. The apparatus was used to analyse the scaling behaviour of the linear rheology of actin, with results in agreement with the theory for semi-flexible polymer dynamics. Heterodyne detection could increase the frequency response by an order of magnitude in such studies (Fabry *et al* 2001).

Ferromagnetic tweezers with *two pole* pieces have been demonstrated (Trepot *et al* 2003). They were designed with large coaxial coils (280 mT, gradient $2T/m$, figure 32). These tweezers allowed for reasonable forces ($\sim$2 pN) and homogeneous fields (this type of design is used in NMR apparatus), but there is the complication of torques rotating the particles and the remnent hysteretic magnetic moment of the ferromagnetic particles. The arrangement

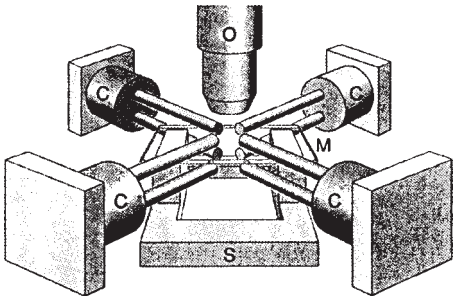

Figure 33. Four pole piece magnetic tweezers for particle manipulation in two dimensions (Amblard *et al* 1996). The split pole pieces are arranged above and below the microscope slide used to contain the sample material.

also suffered from the probe particles being too dense and experiencing problems with sedimentation. The sedimentation phenomena would preclude them being used for anything but the most viscoelastic specimens (to decrease v_{sed} in equation (5)). A possible adaptation developed in our laboratory is to place an additional single electromagnetic coil around the microscope objective to counteract the buoyancy force.

Four pole piece tweezers have been constructed (Amblard *et al* 1996), in which the fields (and hence forces) were controlled using Hall probes glued to the bottom of the pole pieces (figure 33). Electronic feedback control of the current through the pole pieces was used to maintain a constant magnetic force on the probe. Both translational ($\sim$1 pN) and rotational ($\sim 10^{-14}$ N m torques) manipulation of the motion of the probe was possible with the equipment and the time resolution was $\frac{1}{30}$ s.

A *six pole* piece magnetic tweezer set-up has been reported (Gosse and Croquette 2002). The arrangement of the apparatus is shown in figure 34. The pole pieces are placed above the sample cell and the vertical component of the magnetic field gradient (equation (41)) allows positioning of the particles in the z direction (figure 34(a)). A sophisticated feedback mechanism is used to move the particles both horizontally and vertically. A constant challenge with magnetic probe manipulation is to quantitatively understand the response of the particle, which depends on the magnetic field two fold both through the induced magnetization M, and directly on the magnetic field strength (B). Calibration was achieved with the six pole piece tweezers by image analysis of the fluctuation spectrum of the probe particles. This analysis is mathematically similar to the method used with optical tweezers, i.e. the Langevin equation (equation (36)) is analysed (Strick *et al* 2003).

Eight pole piece tweezers have been built (Huang *et al* 2002). The arrangement of the eight pole pieces appeared to provide superior magnetic force control than those of Gosse *et al* although quantitative analysis of the fluctuation spectrum of the tracked particles was not made.

A unique study of the viscoelasticity of polymeric materials was made using the dynamics of a falling magnetic needle (Chu and Wang 1992). The position of the needle ($\sim$millimetres in length) was measured to great accuracy using light scattered onto a position detector. The needles were repeatedly magnetically levitated, allowed to sink for a small distance ($\sim 100\ \mu$m), repositioned at the initial starting point and the positional data averaged. The falling needle provided measurement of the viscosity as a function of shear rate for polymer solutions over a wide range of concentrations. Perhaps the biggest advantage of this technique is that it provides an extremely large range of measurable viscosities ($0.001\ \text{Pa} < \eta < 10^7\ \text{Pa}$), although it does sacrifice the ability to probe heterogeneity by using such large probe particles.

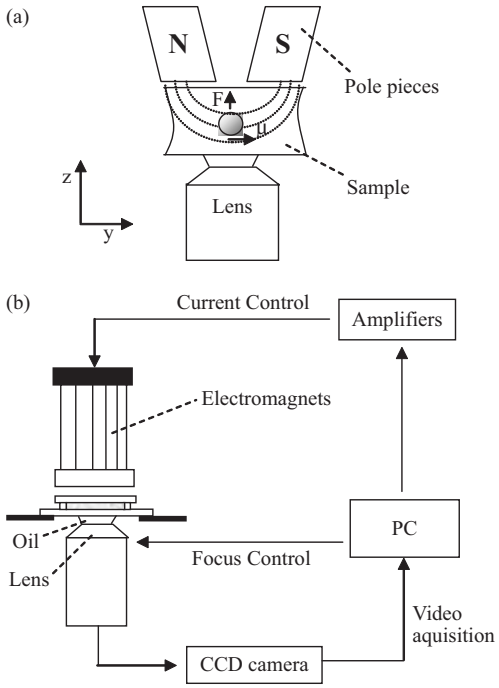

Figure 34. Three-dimensional magnetic tweezers for particle manipulation (Gosse and Croquette 2002). (*a*) A close up of the arrangement of the pole pieces around the sample, and (*b*) the global arrangement of the apparatus, with feedback control from the computer used to position the particles.

The study of the rotational motion of ferromagnetic probes has experienced a number of experimental studies (Laurent *et al* 2002, Wilhelm *et al* 2003). An impressive application of rotational magnetic microrheology (Fabry *et al* 2001) was made in which the shear modulus of cells was extracted from the complex torque and angular displacement, equation (47) at high frequencies.

Another method to study micro viscoelasticity using the rotational motion of probe particles has been made by means of the response of chains of magnetic colloids (Wilhelm *et al* 2003). It would require much further development for such a technique to become a standard characterization tool. The polydispersity of the chain lengths provides a complicated barrier to the analysis, although the 10 nm particles did have the strong advantage of avoiding the phagocytosis mechanism in living cells. This facilitates the microrheological analysis of intact biological organisms.

An interesting idea is to extend cross-correlation techniques (discussed in both the sections on particle tracking and optical tweezers) to magnetic microrheology. One possible method is to cross correlate the motion of 'driver' magnetic beads with that of passive marker particles, potentially providing quantitative agreement with the bulk rheology of high modulus heterogeneous materials (Evans 2004).

3.2.5. Atomic force microscopy. Dynamic AFM experiments are faced with the challenge of decoupling the viscoelasticity of the sample from the hydrodynamic interaction of the cantilever with the surface. This is a partially solved problem, but research continues driven by the wide range of possible applications.

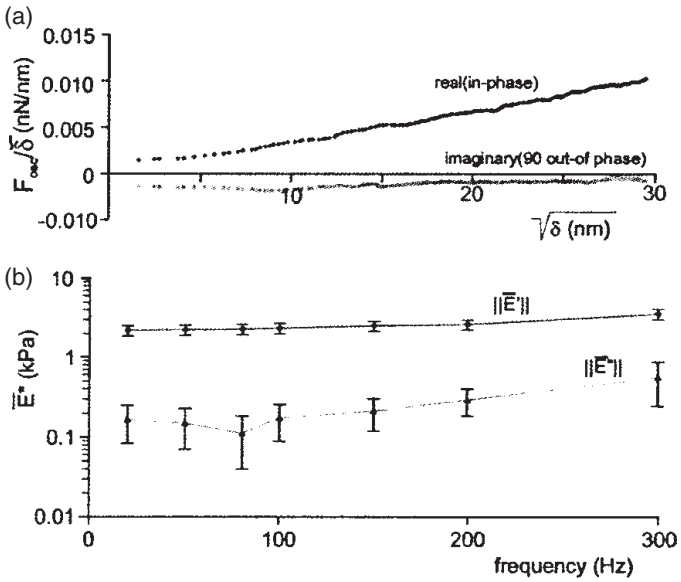

Figure 35. (*a*) The rescaled indentation force plotted against the square root of the amplitude of oscillation, and (*b*) the storage (E') and loss (E'') longitudinal modulus measured with AFM (Mahaffy *et al* 2000).

The question of dynamic AFM was approached by attachment of a polystyrene bead to the cantilever (Mahaffy *et al* 2000). Oscillating the cantilever above a surface provided the complex longitudinal modulus as a function of frequency. The spherical Hertz model was used to analyse the viscoelasticity and it is valid for adhesive forces at small indentation depths. The analysis procedure was established in the range $\delta/R < 0.4$, where δ is the amplitude of the oscillations of the cantilever and R is the radius of the polystyrene bead. The complex longitudinal modulus (E^*) was then calculated using the relationship

$$E^* = \frac{f_{\mathrm{osc}}}{\delta\sqrt{\delta_0 R}}, \tag{42}$$

where δ_0 is the initial indentation depth, and f_{osc} is the applied force on the bead corrected for the hydrodynamic drag force. In figure 35 the complex longitudinal modulus measured with AFM is shown as a function of frequency from the surface of a polyacrylamide gel (Mahaffy *et al* 2000).

AFM can also be used as dynamic mechanical testing apparatus using the power spectrum of the cantilever fluctuations (Benmouna and Johannsmann 2004). With this method the viscoelasticity of gelatine surfaces was examined with a micron sized glass sphere attached to the cantilever.

Lubrication forces can be negligible with very soft materials (viscoelasticity dominates) (Benmouna and Johannsmann 2004). An alternative approach using an unmodified pyramidal tip has been performed with such materials (Alcaraz *et al* 2003). An expression is used for the applied force (F) on the indentation depth (δ) into a purely elastic surface.

$$E = \frac{3E\tan\theta}{4(1-\nu^2)}\delta^2, \tag{43}$$

where E is the Young's modulus, θ is the indenter angle, and ν is the Poisson ratio. The result was extended to produce an expression for the complex shear modulus (G^*) using the

correspondence principle. The resultant equation is:

$$G^*(\omega) = \frac{1-\nu}{3\delta_0 \tan\theta}\left[\frac{F(\omega)}{\delta(\omega)} - \mathrm{i}\omega b(0)\right], \tag{44}$$

where F is the Fourier transform of the applied force, ω is the frequency, δ is the Fourier transform of the indentation depth as a function of time, and $b(0)$ is the drag coefficient. There continue to be some open questions concerning the role of tip surface hydrodynamics and the role of adhesive forces in this approach. The indentation method was used to examine the surface viscoelasticity of lung cells, but should be considered a qualitative measure until it is calibrated against a number of well understood soft condensed matter systems.

A number of different AFM techniques are being examined to study the dynamics of single molecules. In a particularly interesting example, both the cantilever and surface were oscillated during force measurement (Okajima *et al* 2004) facilitating the separation of the hydrodynamic effects.

3.2.6. Miscellaneous. There is a wide range of other microrheology experiments which do not fall into the aforementioned categories.

A piezorheometer has been described in a study (Constantin *et al* 2002) to examine the linear rheology of surfactant micelles in the frequency range of 1 to 6×10^4 rad s^{-1} with a sample thickness of $\sim$60 μm. The very small motion of the piezoelectric crystals (1 nm) used to apply an oscillatory force to the micellar solutions, ensured that the measured viscoelasticity was in the linear regime.

SFA has not been covered in detail in this review (Meyer *et al* 1998). The extreme sensitivity to which SFA can probe surface forces (Israelachvili 1992), implies that the technique will play a major role in the development of quantitative measurements of surface shear moduli and reference should be made to the specialized literature.

An assortment of microrheology techniques use fluorescent tags attached to biological molecules to study their motion (Le Goff *et al* 2002). Typically the fluctuation spectrum of the motion of the tags needs to be examined with a well-defined theoretical model, for the dynamics of the fluid to be understood, e.g. that of semi-flexible filaments (Morse 1998).

An active microrheology technique using electric fields to move charged colloidal particles has been demonstrated (Mizuno *et al* 2001). This electrophoretic microrheology method is probably less promising for the measurement of a general complex fluid than magnetic tweezers, since it is difficult to isolate and characterize the effects of the electrostatic and electrodynamic interactions of the sample on the probe particle motions.

The measurement of microrheology with quasi-elastic light scattering is not confined to multiple scattering (DWS) measurements. The compliance (and thus the linear rheology) can be extracted from the opposite limit of single particle scattering with photon correlation spectroscopy (Sohn *et al* 2004). This microrheology DLS technique requires that the viscoelastic specimen is contrast matched with the solvent and there is a large difference in refractive index between the probe and the specimen/solvent matrix. The x-ray analogue of this technique provides the possibility of x-ray photon correlation spectroscopy (Dierker *et al* 1995) microrheology of dense optically opaque complex fluids with gold probe particles. It could have a larger range of applicability than the optical technique due to the larger range of samples providing good contrast for x-ray scattering. A further promising x-ray technique examines the dynamics of microcrystals attached to biological molecules, providing the measurement of milliradian rotational motion with millisecond time resolution (Sasaki *et al* 2001).

4. Specific experimental systems

It is informative to consider the impact of the new microrheology techniques on specific classes of complex fluids. The complex fluids studied are predominantly aqueous, and both sample opacity and the magnitude of the moduli that can be measured place restrictions on the materials which can be successfully examined. Crucially the agreement with bulk rheology is considered in this discussion, since in many cases both the theory and the measurements in microrheology experiments rest on shaky foundations in this constantly developing field.

4.1. Polymers

Theoretical models have been developed hand in hand with experiment in the field of polymer rheology over the last half century. Dynamic models for the low frequency motion of flexible, semi-flexible and rod-like polymer chains are now well developed. Such theory has been quantitatively used to understand the viscoelastic spectra (both linear and non-linear) from bulk rheological measurements. The reader is directed to the relevant books for theoretical introductions to the field (Edwards and Doi 1986, Rubinstein and Colby 2003).

For the experimentalist it is useful to develop an intuitive feeling for the result of a rheological measurement with reference to some standard examples. The Zimm (unscreened hydrodynamics), Rouse (screened hydrodynamics) and semi-flexible models provide predictions for the frequency dependence of shear moduli (G', G''), the magnitude of the shear moduli, the compliance and the viscosity of isolated chains (Ferry 1980). Furthermore these studies have been extended by dynamic scaling theory to semi-dilute unentangled/entangled and concentrated regimes. The viscoelasticity of polyelectrolytes (Dobrynin *et al* 1995), associating polymers (Semenov and Rubinstein 1998) and gels (Kavanagh and Ross-Murphy 1998) are less well understood and quantitative theories for their dynamics are still not available.

4.1.1. Neutral polymers. Flexible hydrophilic polymers in good solvents are a standard system for microrheology, since in principle their viscoelasticity is well understood. A prime example of such a material is PEO in water. The linear viscoelastic spectra from PEO show good quantitative agreement between DWS (Mason and Weitz 1995), laser tracking (Mason *et al* 1997), bulk and PTM results (van Zanten *et al* 2004). The laser tracking microrheology experiments demonstrate Rouse modes at high frequencies (G', $G'' \propto \omega^{1/2}$) for the overlapping chains (Schnurr *et al* 1997). The scaling law indicates that the hydrodynamics are screened at length scales above the mesh size and is in agreement with theory (Rubinstein and Colby 2003). Recently is has been claimed that DWS has found a new, as yet unexplained, dependence of the zero shear rate viscosity on the polymer concentration ($\eta \propto c^{4.7}$) (van Zanten *et al* 2004). In these studies, the high frequency compliance ($\langle \Delta r^2(t) \rangle$) is fitted by a single relaxation time Maxwell fluid

$$\langle \Delta r^2(t) \rangle = \frac{kT}{\pi a} \left(\frac{t}{\eta} + \frac{1}{G} \right), \tag{45}$$

where kT is the thermal energy, t is the time, η is the viscosity, G is the shear modulus and a is the particle radius. Such results on PEO need to be tested in detail, since these hydrophilic polymers are expected to provide model polymeric behaviour due to their tightly controlled polydispersity and well quantified polymer/solvent interaction.

DWS was used to study the associating polymer rheology of hydrophobically end-capped PEO (Lu and Solomon 2002). Good agreement was not found with the bulk rheology and there was a large effect of the probe size on the measurements. An in-depth study of the

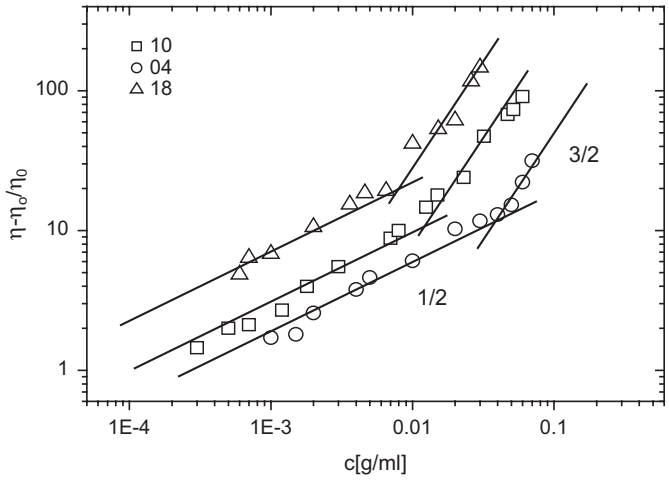

Figure 36. Zero shear rate viscosity data from particle-tracking microrheology experiments on flexible polyelectrolytes (maleic anhydride copolymers) at a series of different chain lengths (04, 10, 18, molecular weights are 82000, 196000, and 410000, respectively). The cross over between semi-dilute ($\sim c^{1/2}$) and entangled dynamics ($\sim c^{3/2}$) is shown and fair agreement is found with the degree of chain expansion measured with small angle neutron and x-ray scattering experiments (Di Cola *et al* 2004).

effect of sample heterogeneity on the DWS results gave six possible reasons for this lack of agreement of micro and macro results; failure of the assumption of continuum elasticity, the effect of probe particle inertia, the compressibility of the matrix network, chain adsorption on the surface of the probe particles, the entropic depletion of the polymer molecules, and structural heterogeneity of the associating polymer network. The authors argue convincingly that only the sixth point can account for the disagreement.

4.1.2. Charged polymers. Measurement of the rheology of non-associating linear flexible polyelectrolytes appears to be another success story of PTM. Figure 36 shows such PTM experiments with linear flexible polyelectrolytes (maleic anhydride copolymers) which had been previously examined with bulk rheological techniques (Di Cola *et al* 2004). Good quantitative agreement between the specific viscosity derived from micro and macro results was found. The scaling laws shown are in good agreement with the theoretical predictions for this class of charged polymers (Dobrynin *et al* 1995) and PTM has thus proven to be a rapid method for the analysis of the dynamic phase diagrams of flexible polyelectrolytes.

PTM experiments with branched flexible polyelectrolytes have also shown fair agreement between bulk and particle tracking data. Scaling theory (Dobrynin *et al* 1995) applied to x-ray and PTM data indicates that the chain backbones are almost completely extended (figure 37). Combs 16 and 13 have twice the backbone size of the other comb polymers shown in the figure, causing the large change in the entanglement concentration, and demarcating the transition between screened Zimm and reptative dynamics. The number and size of the side-chains causes a more minor change in the intrinsic viscosity with the trends expected from scaling theory.

The large depletion forces, which would be expected to act in polystyrene sulphonate comb/sulphonated sphere systems, do not present a barrier to micro/macro agreement. Further transmission DWS experiments have allowed measurement of the high frequency Maxwell-like terminal behaviour of dilute polyelectrolyte solutions up to 8×10^6 Hz (figure 38)

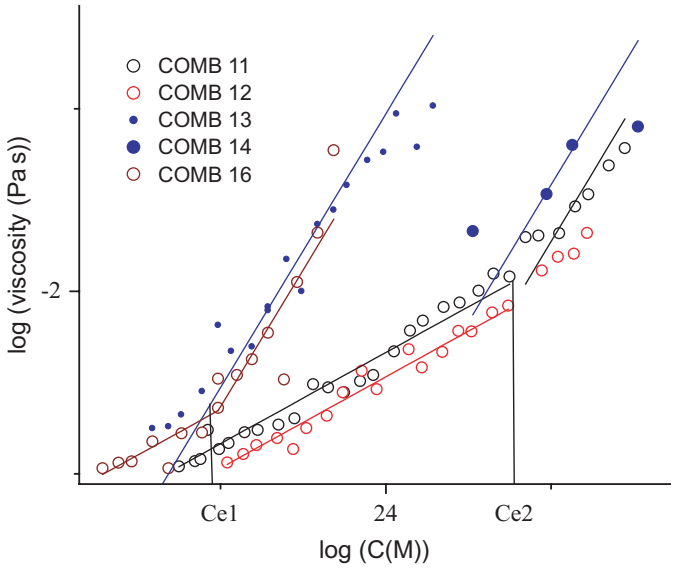

Figure 37. Zero shear rate viscosity data from particle-tracking microrheology experiments on well-defined anionically polymerized polystyrene sulphonate combs. The predictions from scaling theory for semi-dilute ($c^{1/2}$) and entangled ($c^{3/2}$) solutions are shown. The vertical straight lines (c_{e1} and c_{e2}) indicate the entanglement concentration marking the cross over between semi-dilute/entangled regimes.

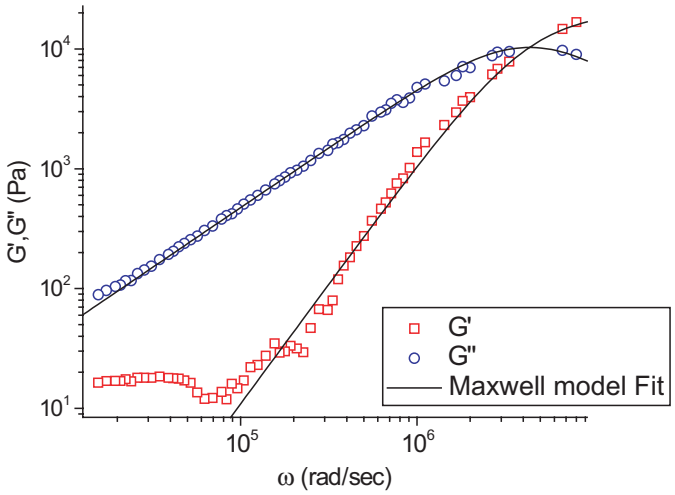

Figure 38. DWS data for the viscoelasticity of polystyrene sulphonate comb indicating the high frequency Maxwell type terminal behaviour of the charged flexible comb polyelectrolytes presented in figure 37. The contribution of the probe beads needs to be considered in very dilute solutions in which the sample viscoelasticity does no dominate.

(Papagiannopoulos *et al* 2005). This corresponds to the lowest Zimm rotational mode of the dynamics of the short polymeric chains.

Semi-flexible polyelectrolytes in high salt conditions have been comprehensively studied in the form of F-actin solutions (Morse 1998, Xu *et al* 1998). DWS indicates that semi-flexible

modes ($G', G'' \propto \omega^{3/4}$) are important for determining the viscoelasticity. The effects of network heterogeneity are cause for concern in microrheology experiments with semi-flexible polyelectrolytes and the agreement of single-particle experiments with bulk rheology is not guaranteed (Crocker *et al* 2000).

The area of *polyelectrolyte complexation* is of importance to the study of gene transfection. Oppositely charged chains (DNA) are absorbed to positively charged colloidal particles and the motion of the colloids can be studied with PTM as they traverse the cell membrane (Dawson *et al* 2004).

4.1.3. Biopolymers. *Actin* is one of the most often studied systems in microrheology experiments. The motivation in this is more for its importance in cell motility than actin being an ideal model complex fluid system. There are over 50 proteins known to associate with actin, many of which act as cross-linkers and will have a significant impact on the viscoelasticity of the specimens. This makes purification a veritable minefield (Bray 1992). Actin does offer large contour lengths and semi-flexibility, which are only available in a few other systems; DNA, viruses, F-sickle cell aggregates and self-assembled peptides (Aggeli *et al* 2001). Such samples therefore provide a significant elastic shear modulus at low polymer concentrations and thus PTM experiments can measure G' and G'' simultaneously. Actin occurs in a series of different roles in living organisms. Three principle categories are structural integrity, myosin-driven motility and self-assembling treadmilling motility. Cross-linker protein almost certainly existed in one of the early microrheology studies (MacKintosh *et al* 1995) and the theory includes this feature of fixed entanglements. Further bulk rheology experiments revisited the theory in detail (Gardel *et al* 2004) and found agreement using samples with carefully controlled densities of cross-linker proteins. Most strikingly, the elastic shear modulus scaled with polymer concentration (c) as $G' \propto c^{2.2}$ and not $c^{1.2}$ as with uncrosslinked materials. Fluid actin suspensions display better agreement with the theories of other authors (Farge and Maggs 1993, Gotter *et al* 1996, Morse 1998).

A further novel feature of the phase behaviour of F-actin is that it can exhibit liquid crystalline phases. Experiments have been performed to measure the microrheology above and below the nematic/isotropic boundary, demonstrating the viability of particle tracking techniques in the oriented polymer phase. An elegant tracking fluorescence microscopy study of actin filaments measured the dynamics of the end to end distance and orientation of the ends of the self-assembled fibres on time (t). Power laws of $t^{3/4}$ and $t^{1/4}$ were found, respectively (Le Goff *et al* 2002). An interesting challenge with self-assembling biopolymer systems is to study the microrheology around the critical micellar concentration, since the dramatic change in sample morphology has a large effect on the viscoelasticity obtained.

Single actin aggregates immersed in a network (Goodsell 1992) are an important area of research, since cells are dense environments and the only study presented to date has been the forced reptation of actin filaments (Dichtl and Sackmann 2002). The gap between theory and experiment still exists in these systems.

Type I Collagen (table jelly) was studied (Velegol and Lanni 2001) in the form of denatured physically cross-linked hydrogels. The heterogeneity of the gel was mapped using a laser-trap microrheology technique. Examination of the shear modulus at 100 nm steps across the gel provided a range of different quantities with an order of magnitude spread in their values. Similarly, AFM has been used to map the viscoelasticity of gelatine surfaces (Benmouna and Johannsmann 2004). Collagen gels are deduced to be chemically heterogeneous and are not ideal systems to study the physics of gelation. A number of developments in their chemical analysis would be required, such as knowledge of the charge on the chains and the number of cross-links, to enable the gap between theory and experiment to be bridged (Dobrynin

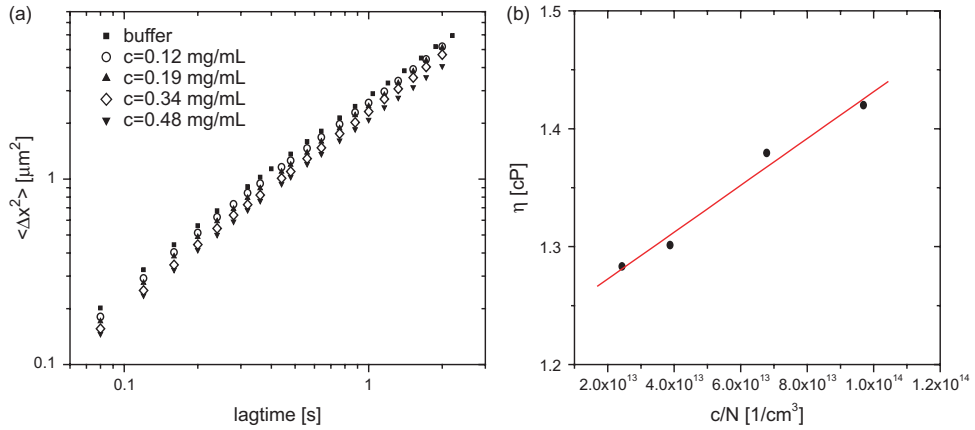

Figure 39. Particle tracking microrheology from the giant muscle protein titin. (*a*) Average MSDs at a series of titin concentrations, and (*b*) corresponding viscosity as a function of titin concentration showing the fit of the Flory/Fox equation (Di Cola 2004).

et al 2004). Collagens are however of large importance to the food industry and they play a number of roles in the human body, with their malfunction of interest to medical science in osteoarthritis.

Gliadins are the proteins found in wheat that are responsible for bread dough elasticity. The proteins were the subject of a particle tracking study (Xu *et al* 2002). Progress was made in quantifying the sample heterogeneity using the histogram method.

Xanthan linear viscoelasticity was examined with DWS (Pashkovski *et al* 2003). Xanthan was found to have rubber elasticity in agreement with the Mooney–Rivlin model (Ferry 1980). In the same study the shear moduli of carboxymethyl cellulose were found to demonstrate the signature of semi-flexibility at high frequencies G', $G'' \propto \omega^{3/4}$ (Pashkovski *et al* 2003).

Guar was examined with two point microrheology to demonstrate the technique with a challenging polydisperse heterogeneous system (Crocker *et al* 2000). Agreement was found with bulk rheology experiments using a newly developed theory (Levine and Lubensky 2000).

Starch dispersions with gelating agents were examined with DWS (Heinemann *et al* 2004). High frequency Rousse modes were observed in semi-dilute suspensions (G', $G'' \propto \omega^{1/2}$) and the evolution of the viscoelasticity during gelation was observed dynamically.

Aggrecan, which displays a comb comb morphology (a bottle brush of bottle brushes) (Papagiannopoulos *et al* 2005) was examined with PTM. Agreement was achieved between one- and two-particle results demonstrating that negligible sample heterogeneity occurs in these specimens. The zero shear rate viscosity scaled as $c^{1.5\pm0.1}$ in common with flexible linear and comb polyelectrolytes in the entangled regime, and Zimm modes in the shear moduli of dilute suspensions (G', $G'' \propto \omega^{2/3}$) were observed at high frequencies.

Titin is a giant muscle protein, which plays an important role in the passive elasticity of striated muscle. Although not technically a polymer (it is a single molecule with no unique repeating unit) it is included for continuity.

The viscosity of titin solutions was examined with PTM (figure 39) (Di Cola *et al* 2004). The radius of gyration calculated using the Flory–Fox equation was in agreement with the value measured with DLS experiments (figure 39(*b*)) (Higuchi *et al* 1993). The contribution of the semi-flexible internal modes was not observed in the viscoelastic measurements, since the contour length ($\sim$1 μm) was much larger than the persistence length ($\sim$10 nm). In contrast small angle neutron scattering was able to provide a clear signature of the persistence length (Di Cola *et al* 2004).

DNA PTM has been used to establish the size of super-coiled λ-DNA using intrinsic viscosity plots (Goodman *et al* 2002). There is currently a substantial use of intrinsic viscosity measurements as a function of polymer concentration to calculate molecular sizes using bulk techniques (Kulicke and Clasen 2004) and therefore PTM experiments could have a large impact in this field.

4.1.4. Gels. The gel state of a complex fluid occurs in a number of different guises (Kavanagh and Ross-Murphy 1998). Principally the division exists between chemical gels in which covalent bonds link the sub-units and physical gels in which weaker ionic and hydrophobic interactions are important for the association of sub-units. Gels like molecular glasses, their solid state analogues, present a challenging area of study due to the existence of non-ergodic disorder. An exact definition of the gelled state is problematic due to the complex interplay of synerisis, heterogeneity and non-ergodicity.

DWS has examined the rheological features of gels using mechanical averaging of correlation functions at different points across a polyacrylic acid gel (Nisato *et al* 2000). The local shear modulus was obtained as a function of the cross-linking fraction. DWS also has allowed the shear modulus to be extracted from the maximum MSD of probe particles (δ) in chemically cross-linked polyvinyl alcohol (PVA) gels at short times using equation (46) (Narita *et al* 2001).

$$\mu = \frac{kT}{6\pi R\delta^2}, \tag{46}$$

where R is the radius of the probe spheres. The equation is motivated by assuming the work necessary to move a particle of radius R a distance δ in a gel $(6\pi R\delta^2)\mu$ is equal to kT. These studies show that the high frequency viscoelasticity is the same for solutions and highly cross-linked PVA gels, whereas gels with low cross-linking exhibit lower storage moduli. Strongly associating polymers, such as hydrophobically modified PEO which form physical gels, have been studied using DWS (Lu and Solomon *et al* 2002). The single particle measure of the bulk rheology has been shown to break down in this case as discussed in section 2.2.

4.1.5. Single molecules. Single molecule experiments of polymers with well-defined hydrodynamic environments (i.e. distant from surfaces, with accurately controlled chemistries and well-defined ionic equilibria) are still relatively few in number. The majority have been restricted to giant biopolymers such as titin, DNA and actin, since they allow for imaging using optical microscopy and fluorescent probes (Dichtl and Sackmann 2002, LeDuc *et al* 1999). Small oligomers of dextran and IG domains (sections of titin) have been examined with dynamic AFM near surfaces, but robust data analysis has still not been established with these methods (section 3.2.5).

Individual titin molecules have been the subject of a number of single molecule experiments. Optical tweezer studies have tended to focus on equilibrium elastic properties as individual domains along the protein are unfolded followed by subsequent stress relaxation (Tskhovrebova *et al* 1997). A novel molecular combing experiment (Tskhovrebova and Trinick 2001) examined the dynamics of the titin molecules and the viscoelasticity.

There are a series of other single molecule techniques, which can be used for dynamic, and consequently rheological, measurements. These include single molecules in liquid droplets, near field scanning optical microscopy, wide field epi illumination and far field confocal microscopy. A good review has been written (Nie and Zare 1997).

4.2. Colloids

Organic hard sphere colloids at high frequencies were examined using PMMA particles mixed with probe silica spheres in DWS experiments (Sohn *et al* 2004). The high frequency viscoelasticity was extracted from the correlation functions and allowed the GSE equation (15) to be tested successfully. Charged polystyrene colloids have also been studied and their gelation was induced by controlling the ionic strength (Romer *et al* 2001). The buoyancy of the particles was tuned using a mixture of H_2O and D_2O. The time evolution of the viscoelasticity was examined with DWS with 1 min resolution of the time steps.

Surfactants were examined with transmission and backscattering CCD DWS to examine the motion of worm-like micelles (Cardinaux *et al* 2002). Although an accomplished technical feat, the data was unsatisfactory with incomplete agreement between bulk/micro viscoelastic spectra. A factor of 1.5–2 was needed to vertically rescale the curves of shear modulus against frequency. A single Maxwell relaxation mode was used to fit the data indicating that a single relaxation process controls the dynamics of these materials.

The membrane structures formed from surfactants can exhibit rich rheological behaviour (Helfer *et al* 2001). The in-plane and out-of-plane fluctuations of naturally occurring membrane lipids have been related to the structure of the underlying actin cytoskeleton (Helfer *et al* 2001). Microelasticity experiments of membrane structures are not covered in this review, but they have had an important impact in the field of membranes, e.g. micropipette aspiration (Zhelev *et al* 1994).

Hexane/water emulsions of sodium dodecyl sulfate were examined with echo DWS and the yielding of droplets was measured as a function of strain amplitude (Hebraud *et al* 1997). The solid/liquid shear transition of Gillette shaving cream was examined (Cohen Addad *et al* 1998) and the process of foam coarsening during the ageing process were studied.

Inorganic silica particles have been used as probes in optical tweezer studies (Verma *et al* 2000), so there is no practical barrier preventing further development of this field. Such inorganic colloidal materials are known to exhibit a range of phase behaviour. This includes solid, liquid, gas, liquid-crystal, glass and gel phases, and with each there is an individual rheological question raised (Larson *et al* 1999). Granular matter has been the subject of a number of particle tracking studies using direct optical imaging. Here evidence has been found for superdiffusive driven motion and geometry dependent caging phenomena (Choi *et al* 2004). However, the length scales considered with current measurements on granular matter (spheres of $\sim$millimetres diameter) are typically three orders of magnitude larger than encountered in microrheology experiments, although there is no pratical limit to a reduction in size towards the powder (micrometre-sized) state with the concomitant technological implications (pharmaceutical tablets, detergents, etc). There are a number of beautiful studies in the literature on the effects of confinement on granular particle dynamics (compare section 2.5) (Clement 1999).

4.3. Biological assemblies

Biophysics requires an understanding of the dynamics of living constructs of multi-component complex fluids such as the motility of cells, the division of chromosomes, the adaptation of the extracellular matrix, and the chemotaxis of bacteria (Heidemann and Wirtz 2004). These offer a whole series of challenges in both non-equilibrium thermodynamics and microrheological instrumentation.

Active actin networks (Keller *et al* 2003) formed from mixtures of actin, myosin and ATP are now being studied both theoretically and experimentally. Detailed predictions for

the rheological functions have been made and single actively transported actin filaments have been imaged (Humphrey *et al* 2002).

The viscoelasticity of the cellular cytoplasm has been approached (Bausch *et al* 1999). Magnetic beads are attached to the cell membrane and the viscoelasticity has been quantified as a function of position. The coupling of the membrane to the elasticity of the cytoskeleton can be examined (Bausch *et al* 1999).

The microrheology of living cells subject to the rotational motion of ferromagnetic probes has been studied (Fabry *et al* 2001). The complex modulus (G^*) was calculated from the applied complex torque as a function of frequency ($T^*(f)$) and the resultant complex angular displacement $d^*(f)$

$$G^* = \frac{T^*(f)}{d^*(\mathrm{f})} \tag{47}$$

by rotating ferromagnetic particles in a magnetic field as a function of the heterodyne frequency. The samples exhibited soft glassy rheology and the shear modulus could be fitted with a model of structural hysteretic damping.

The flagellar motor proteins that propel bacterial cells have been subject to a large number of experiments (Berg 1993, 2003). Here the tracked particle is a living object (the bacterium), that experiences driven biased diffusion as it searches for food molecules. Poisson statistics are observed for the probability distribution of the displacement of the particles with time and not the Gaussian statistics found for inert probes (Crocker and Grier 1996).

A barrier to the use of probe particles in living microorganisms is the phenomenon of phagocytosis. A number of single-cell organisms will ingest probe particles as if they were food packages. The tracking microrheology experiments in these cases are probing the complicated processes of phagoytic cup formation and not the viscoelasticity of the unperturbed cytoplasm (Feneberg *et al* 2001, Ishikawa *et al* 2003). Such phenomena will frustrate naïve studies of the microrheology of the components of living cells.

Fortunately, the questions concerning phagocytosis have been overcome with a number of cell lines. Particles in these cases have been engineered to experience a less complex process of endocytosis. They are compartmentalized within a lipid membrane, but these packages are allowed to move freely within the cell and are subjected to the representative thermal fluctuations due to the internal viscoelasticity of the cell. For example the microrheology of living cells has been considered using two-particle cross-correlation techniques (Lau *et al* 2003). In another experiment, 8 nm magnetic particles were used, which formed linear aggregates in the cell allowing their rotational motion to be studied. These probes followed a well-defined process of endocytosis, facilitating the analysis (Wilhelm *et al* 2003).

The questions on cell/particle interaction are intimately connected with the field of gene therapy (Suh *et al* 2004). Here the probe particles are carriers of DNA and the question is how to move the particles across the cell wall to deliver the replacement genetic information to the cell nucleus.

AFM has been used to probe the rheology of the surfaces of a number of viscoelastic cellular materials such as lungs (Alcaraz *et al* 2003) and fibroblast cells (Mahaffy *et al* 2000). More work is still needed to be certain that the measurements are characteristic of the specimens, and not the hydrodynamics of the cantilever or the adhesive forces with the tip.

5. Future avenues of research

Microrheological techniques need to be rigorously established with heterogeneous materials to establish themselves alongside bulk rheometers as a range of standard characterization

techniques. Once this is accomplished, further minimization of sample volumes should be possible using AFM, single-fibre DWS (Popescu *et al* 2002) and magnetic/optical tweezers. However, the technical questions relating to these measurements are not just confined to the instrumentation; further theoretical developments are needed to enable the experimentalist to understand what they have measured. The statistical analysis of single molecular rheological events requires thorough investigation to guide the research as the length scale of microrheological measurements is reduced. The study of the viscoelasticity of the surfaces of complex fluids is still in it infancy and big breakthroughs are expected in this area.

The lion's share of experiments reported to date consider the microrheology of aqueous solutions, which are facilitated by the easy availability of electrostatically stabilized neutrally buoyant colloidal probes. Only a small subset of the possible aqueous complex fluid systems has been examined and a wide range of unexplored areas of soft condensed matter still exist. New probe chemistries in a series of non-aqueous solvents (preferably sterically stabilized) would greatly enhance the range of complex fluids that can be studied.

Magnetic tweezers and video particle tracking apparatus, which are both relatively cheap to construct, have as yet not been commercialized. This would cause a rapid expansion in the usage of microrheology methods.

6. Conclusions

The microrheological techniques discussed, in all their various guises, offer a series of unique probes of the viscoelastic behaviour of complex fluids. Their new contributions have been reviewed to both the measurement of the high frequency viscoelasticity and the linear rheology of minute specimens of complex fluids in a range of confined geometries.

The future for sub-micrometre rheology is very promising. The techniques described here neatly complement the development of a range of single molecule experiments and would help extend these methods to provide a description of the time response of single molecules. There is an extensive overlap of microrheology methods with biophysical studies; in particular, questions such as how molecular motors dissipate energy (Howard 2001), how DNA can be transported into a cell for gene therapy (Dawson *et al* 2004), and the dissipative properties of blood clots (Ryan *et al* 1999), would all profit from a consideration of the unified principles of microrheology.

There are many medical and industrial applications for the rapid microanalysis of viscoelastic samples. These require developments in colloidal synthesis to be made hand in hand with experimental techniques.

Video PTM, magnetic/optical tweezers, DWS, SFA and AFM are all now well-established techniques for the measurement of how a selection of complex fluids store and dissipate energy. Currently, microrheology techniques can provide accurate measurements of the linear viscoelasticity of a number of complex fluids over a wide range of frequencies, provided the static structure of the sample is well characterized and the chemistry of the probe/sample interaction is well understood (Valentine *et al* 2004). Extension of these methods to the routine quantification of the viscoelasticity of heterogeneous materials will be an important advance. Recent cross-correlation methods, although mathematically very sophisticated, look to be a promising solution.

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