

Microrheology

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Abstract

Several complementary techniques have been developed in recent years that make it possible to measure viscoelastic properties of soft materials on micrometer scales. Such methods provide new prospects for the characterization of local inhomogeneities in materials, for studies of material properties in small samples, including living cells, and for high frequency rheology. The main techniques discussed in this review are magnetic bead manipulation, observation of refractive beads with laser interferometry or multiply scattered light, and atomic force microscopy. Materials studied include synthetic polymers, thin polymer films, biological polymers in vitro and in vivo. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Rheology; Viscoelasticity; Biopolymers; Atomic force microscopy (AFM); Cell mechanics

1. Introduction and background

Many of the diverse material properties of such soft materials as polymer solutions, gels, and even filamentous protein analogues of these in cells stem from their complex structures and dynamics with multiple characteristic length and time scales. One of the most important and frequently studied material properties of such systems is the shear modulus. In contrast with ordinary solids, the shear modulus of polymeric materials can exhibit significant time or frequency dependence in the range of milliseconds to seconds or even minutes. In fact, such materials are *viscoelastic* — exhibiting both a viscous and an elastic response. Rheology, or the experimental and theoretical study of viscoelasticity in such systems, is of both fundamental and immense practical significance. The mea-

surement of bulk viscoelastic properties is usually done with mechanical rheometers that probe macroscopic (milliliter) samples at frequencies up to tens of hertz.

Recently, a number of techniques have been developed to probe the material properties of systems ranging from polymer solutions to the interior of living cells on microscopic scales. These techniques have come to be called *microrheology*, as they can be used to locally measure viscoelastic parameters. There have been several motivations for such developments. In many cases, and especially in biological systems, samples only come in small sizes. Another strong motivation for biological applications has been the prospect of being able to study inhomogeneities, for instance inside of cells. Furthermore, such techniques have provided the possibility to study viscoelasticity at frequencies above 1 kHz. Finally, the ability to study materials such as polymer solutions with probes spanning some of the characteristic microscopic length scales (e.g. approaching the inter-chain separation or

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mesh size of gels) will lead to new insights into the microscopic basis of viscoelasticity in these systems.

2. Rheology using small embedded probe particles

The general principle behind microrheology is to minimize the mechanical probe that is deforming the medium. The use of micrometer sized spheres as probes, in conjunction with modern high-resolution microscopy, has permitted the measurement of rheological material properties at the micrometer scale. Microrheology techniques using small particles or inclusions fall into two classes: those involving *active* manipulation of probe particles within the sample [1–4], and those employing *passive* observation of thermal fluctuations of such probe particles [5,6•,7•,8••,9,10•,11]. In either case, the probes used are typically chemically inert spherical beads of between a fraction of a micrometer to several micrometers in diameter.

The active manipulation of micron-size particles by magnetic fields was pioneered in the 1920s, when Freundlich and Seifriz studied the properties of gelatin using small magnetic particles [12]. Later modifications allowed the use of this technique in living cells [13], and in studies of spatial heterogeneities in mucus [14]. Recent advances in high-resolution and rapid microscopy have led to increased interest in similar micromanipulation techniques. Ziemann et al. [2], Schmidt et al. [3], and Amblard et al. [4] have used magnetic field gradients for direct manipulation of micron-sized magnetic beads to measure the viscoelastic response of biopolymer solutions and gels. These groups measured the particle displacement by video microscopy. In Ziemann et al. [2] the authors reported measurements of both the shear elastic *storage modulus* $G'(\omega)$ and *loss modulus* $G''(\omega)$ as a function of frequency up to approximately 20 Hz, while Schmidt et al. [3] describe an attempt to map the strain field by analysis of the motions of non-magnetic beads in the vicinity of a magnetic probe bead. The forces applied are calibrated by measuring the velocity of the same kind of bead exposed to the same field gradient in a purely viscous fluid of known viscosity. Both geometric considerations and the finite mesh size and possible inhomogeneities of the network complicate the determination of the strain. Initial studies assumed the application of point forces within a continuous, incompressible elastic medium [2], which is a problematic and unphysical boundary condition. Magnetic bead techniques have been extended to cells, as described later [15,16••].

Instead of using active probe manipulation, it is possible, and even advantageous in many cases, to determine the rheological properties by observation

of thermal fluctuations of embedded probes. This is because these fluctuations reflect the exact linear response parameters and their complete frequency dependence. In practice, such an approach is possible provided the embedding medium is soft and the detection sensitive enough to observe thermal fluctuations in the probe position. Viscoelastic parameters of a polymer solution or gel have been measured either by directly detecting the thermal motion of a single embedded probe [6•,7•,8••,9] or, alternatively, by observing the intensity fluctuations that result from the multiple scattering of light by an ensemble of embedded particles [5,10•,11,17]. Single particle tracking in a microscope is not limited to the temporal and spatial resolution of conventional video analysis. Gittes and co-workers [7•,8••] describe a high-resolution technique for measuring the power spectrum of the position fluctuations of dielectric particles over a frequency range from 0.1 Hz to over 10 kHz by laser interferometry. Not only can this technique be used to measure particle displacement over much shorter time scales than can be done by video tracking, but it is also sensitive to much smaller particle displacements (as small as 1 nm). As a result, the shear modulus can be measured over a wide frequency range in soft materials (e.g. for shear modulus less than 100 Pa). Mason et al. [6•] and Xu et al. [9] describe high-resolution particle tracking methods based on photodiode detection and enhanced video microscopy.

Similar to these single-particle passive methods is a light scattering technique pioneered by Mason and Weitz [5]. In this technique, the motions of an ensemble of many embedded micrometer size particles are determined from the temporal correlation of light that is multiply scattered by the sample. This diffusing-wave-spectroscopy (DWS) technique has been used by a number of groups in recent years [10•,11,17–19]. A recent review of this rheology method can be found in [20•]. While DWS and single-particle methods are quite similar in that they both infer viscoelastic parameters from the thermal fluctuations of probe particles, there are advantages and disadvantages of both, as well as differences in the methods of analysis used by the various groups. On the one hand, a basic advantage of the DWS technique over the various single-particle tracking methods is that an ensemble average over many particles is inherently performed. In contrast, using single-particle tracking, it may be necessary to average the results for many different particles in order to obtain a statistically meaningful measurement of the bulk shear modulus. On the other hand, observation of the motion of single particles permits the study of inhomogeneities within the sample. Furthermore, it is possible to obtain the shear modulus by a quantitative

and direct transformation of the measured fluctuations of individual particles [7•,8••]. In Mason and Weitz [5] and subsequent DWS experiments [6•,10•,11] a less direct approach was used, which was shown empirically to agree well with conventional measurements in [5,6•]. This latter method of analysis has also been extended to relate the probe motion to the creep compliance [9].

Fundamental to any kind of rheology using probe particles is a quantitative modeling of the interaction of the probe with its surroundings. Theoretical discussions of some of the theoretical issues and limitations concerning the response of a sphere in a viscoelastic network can be found in [7•,8••,21•]. For both active and passive techniques, the calculation of a shear modulus assumes a continuous viscoelastic medium surrounding the probe particle. For this to be valid, one must at least have a large bead radius compared with the characteristic dimension of, for instance, a polymer network. The motion of a single spherical inclusion or *bead* in a viscoelastic medium can in certain limits be viewed as a generalization of the diffusion of such a particle in a purely viscous fluid. However, the complete interaction of such probe particles in a solution of solvent plus polymer is somewhat more complicated, and depends on the time-scale of interest. At least three qualitatively distinct behaviors are possible. (i) In a crosslinked polymer gel, at long times or for low frequencies the response of the surrounding medium can be regarded as that of a compressible elastic solid. (ii) Over shorter times, or for higher frequencies, the response of the surrounding medium becomes increasingly that of an incompressible viscoelastic fluid in which the complex shear modulus is frequency-dependent. The effective incompressibility is due to the increasing hydrodynamic coupling of polymer and solvent. The viscoelastic response of polymer solutions to bead motion involves contributions from both solvent and polymer. Theoretical treatments of this aspect of solution behavior include so-called *two-fluid* models, in which a phenomenological, viscous coupling is assumed to couple the network and solvent [22–24]. At sufficiently high frequencies, this viscous coupling effectively forces the two components to move as a single viscoelastic fluid, i.e. there is no more ‘draining’ of the network. This means that a treatment of the solution as a continuous, *incompressible* viscoelastic medium is valid. (iii) Finally, at very high frequencies, inertial effects may become important. For the motion of a micron-sized particle in a medium of density ρ and complex shear modulus G comparable to that of water, inertial effects of the medium can be neglected for frequencies ω up to approximately 1 MHz [8••]. This is because the viscoelastic penetration depth

$\sqrt{2\rho\omega^2 G}$, which characterizes the extent of the perturbation of the medium around the particle [25] is larger than the particle radius R .

In general, the force f and displacement x of the bead are related by $x = \alpha f$, where α is the response function or compliance. In the low-frequency limit, in which the medium is characterized by a (real) shear modulus G and Poisson ratio ν , the response function is given by [7•,8••]

$$\alpha = \frac{1}{6\pi GR} \left[1 + \frac{\nu - 1/2}{2(\nu - 1)} \right].$$

For an incompressible viscoelastic medium, the Poisson ratio ν is $\frac{1}{2}$, resulting in a simple generalization of the Stokes–Einstein [26] drag on a moving sphere in a viscous liquid [7•,8••]: $\alpha(\omega) = 1/[6\pi G(\omega)R]$. Here, the shear modulus is complex and frequency-dependent and force and displacement are not necessarily in phase. The dynamic shear modulus $G(\omega) = G' + iG''$ can therefore be determined by simultaneous measurement of both a calibrated periodic force f_ω applied at frequency ω and the resulting displacement x_ω (including any phase shift between the two). This is the basis of the active methods described above. Alternatively, basic statistical mechanics shows that the same information can be extracted from the thermal fluctuations [27]. The fluctuation–dissipation theorem relates the imaginary part of the frequency-dependent response function or compliance $\alpha(\omega)$ to the power spectrum of fluctuations in position x :

$$\langle x_\omega^2 \rangle = \frac{4kT\alpha''(\omega)}{\omega}.$$

Provided that these quantities can be measured over a wide enough frequency range, dispersion relations [8••,27] of classical statistical mechanics allow one to determine the full complex response function $\alpha(\omega)$, and therefore the full complex shear modulus G .

The cytoskeletal protein F-actin has been a favorite model system in recent microrheology studies, as well as in numerous conventional rheology studies (for a recent review, see MacKintosh and Janmey [28]). F-actin is a semiflexible polymer: its persistence length is approximately 15 μm at room temperature, which can be deduced from thermal shape fluctuations [29–31]. Actin provides a convenient model system to study semiflexible polymer behavior, since its dynamics can be directly observed by fluorescence microscopy [32]. One spin-off of the recent studies of F-actin has been a significant reassessment of the theoretical situation concerning semiflexible polymers. For instance, microrheology has demonstrated frequency dependence of the shear modulus that differs substantially from that of conventional polymer

solutions and gels [4,7[•],8^{••},10[•],33,34], and which has been explained by recent theories [35[•],36[•]].

One issue that has yet to be adequately addressed concerns possible inhomogeneities caused by the probe particles. Apparent differences between conventional rheology and microrheology of F-actin solutions as obtained by the Munich group [2,3,37], as well as similar quantitative discrepancies between the theoretical predictions [35[•],36[•]] and the experimental shear moduli reported by Gittes and co-workers [7[•],8^{••}], may be explained by a depletion of filaments in the vicinity of the probe. Maggs [21[•]] has recently examined theoretically the limits of microrheology due to such effects. Although Maggs' model is not directly applicable to the range of frequencies studied by the experiments of other authors [5,6[•],7[•],8^{••},9,10[•],11,17], understanding such limits is important for quantitative application of microrheology.

3. Viscoelasticity measured by atomic force microscopy (AFM)

The atomic force microscope (AFM) has been applied in a variety of ways to study the dynamic properties of systems ranging from polymer networks over cells and membranes to single polymer filaments. In an AFM, a small tip is deflected when placed in contact with a surface. Sensitive measurement of the tip motion is made possible by the deflection of a laser. Because rather large forces can be applied with rather small tips, AFM is especially suited to studying stiff materials with elastic moduli of order 1 GPa [38–40[•],41]. Measurement and imaging of materials with moduli of order 1 kPa is also possible with techniques such as force modulation [42,43]. In general, however, there is a trade-off involving the tip size: in order to measure softer materials, a larger tip size is necessary. The interpretation of the measurements has usually been done within the Hertz model for the contact of elastic bodies [42,44]. This is limited to smooth and simply curved surfaces (say, a nearly spherical tip in contact with a flat surface), and for deformations that are small compared with the radius of curvature of the tip. However, uncertainties in the tip geometry make it difficult to be quantitative in practice. Furthermore, adhesive and wetting forces between the tip and sample make it difficult to determine the contact area between the tip and sample. This may also cause a jump to contact that makes it hard to determine the point of contact and thereby the subsequent indentation depth with respect to that point. Even without attractive interactions, the contact point is the harder to localize the softer the sample is.

We will describe, in the following, recent AFM

experiments that explore the viscoelastic properties of polymer solutions and networks. Applications to biological cells will be described later. In most cases references to earlier work can be found in the cited articles. Two distinct modes of AFM have been widely used to locally test viscoelastic properties of biological and synthetic materials. In the 'force mapping method' [45] the tip of the AFM is parked over a point of the sample and then slowly brought in contact to indent the material and retracted again. The sample stiffness is mapped from point to point in this way. The method is quasi-static in the sense that the scan is performed slowly enough for viscous forces to stay negligible. In the 'force modulation method' [42] the sample is sinusoidally vibrated normal to the surface and the amplitude and phase of the tip response is monitored while the sample is laterally scanned. In both cases the indentation of the elastic material by the AFM tip is modeled with the Hertz model [42,44], which is valid for smoothly curved, homogeneous elastic materials and for small deflections.

Aimé et al. [38] report force-mapping experiments with relatively rigid (Young's modulus 700 MPa) films of polyacetylene and with softer films of diblock-copolymers of polystyrene–polyacetylene in air. They include the surface energy between tip and polymer in the calculation, but because of large uncertainties in the experimental geometry, cantilever stiffness and piezo hysteresis, results are only qualitative. Nakajima et al. [39] studied thin films of various mixtures of polystyrene and polyvinylmethylether, ranging in stiffness from approximately 1 MPa to 3.3 GPa by force mapping in air. They observed a hardening of the films with increasing probing velocity, qualitatively demonstrating a viscoelastic response. Domke and Radmacher [43] experimentally explored the validity of the Hertz model for thin films of gelatin of varying softness (20 kPa to 1 MPa) under liquid. As might be expected, application of the Hertz model leads to erroneous results for soft films when the indentation depth becomes significant compared with the film thickness.

Radmacher et al. [42] used force modulation in air on dried Langmuir–Blodgett films. Lock-in detection of the cantilever motion provides amplitude and phase of response. They used the Hertz model with a frequency dependent complex Young's modulus to interpret the data. The situation is, however, complicated by resonances of the cantilever and unknown tip geometry. Results are therefore not quantitative, but have proven to be useful for contrast generation in imaging. Kajiyama et al. [46] used force modulation to study the viscoelasticity of polyethylene single crystals in air. Without extracting viscoelastic parameters quantitatively, they used amplitude and phase of response for contrast generation in imaging. Details of

approximately 100 nm size could be resolved in this mode.

Using a variation of the force modulation technique, DeVecchio and Bhushan [40•] have demonstrated a technique for local viscoelastic measurement, in which good quantitative agreement was found with standard techniques for materials with shear moduli as large as approximately 1 GPa. Overney et al. [41] used both dc approaches and approaches with superimposed oscillations to probe the viscoelasticity of polymer brushes. Data were interpreted within a Maxwell model assuming a sample confined between parallel plates. It was found that deviations from the expected behavior are evident since the tip is not flat and can penetrate the film and laterally displace material. Friedenbergh and Mate [47] used a glass sphere instead of a conical tip to probe the viscosity of a thin layer of a low-molecular-weight polymer liquid, polydimethylsiloxane, by force modulation. The increase in viscous damping in close vicinity to the surface was found to follow predictions. Even with a purely viscous sample the phase shift of the cantilever response was small, since the oscillation frequency was far below resonance, which in turn was determined by the mass of the cantilever. This demonstrates that it is not always possible to directly interpret in-phase and out-of-phase response as elastic and viscous response of the sample. Haga et al. [48•] qualitatively imaged elastic properties of relatively soft agar gels (30–80 kPa) under water with a force modulation method and also used force mapping to obtain values for the Young's modulus of the gels. Under water, the phase shift of the response is difficult to interpret since the viscous damping of the whole cantilever in water contributes strongly. Using the Hertz model, their fits of the data gave results in reasonable agreement with those measured by conventional rheology. Several other problems contribute to the difficulties in interpreting force-mapping curves. On soft samples, the contact point can not easily be determined, leading to a large uncertainty in the indentation depth.

4. Microrheology and applications to cells

One strong motivation to develop microrheology techniques has been a desire to locally measure the mechanical properties of cells. For this, microrheology is necessary first, because cells are typically of the order of tens to hundreds of microns in size, and second, because the interior of cells is highly non-homogeneous, so that mechanical material constants are only meaningful if measured locally. A large fraction of cellular materials such as the cytoskeleton or the cell membrane have structural functions. The physical

properties of these materials as well as their regulation are crucial for cell integrity, a multitude of transport processes, cell division, cell locomotion, as well as the response of cells to mechanical stress and external mechanical stimuli. In particular, cell motility and mechanical integrity are strongly influenced by the *actin cortex*, a network of filamentous polymeric proteins [49]. This part of the mechanical framework of cells can be approximated as a homogeneous elastic medium. A number of methods have been developed to probe the rheological properties of the cytoskeleton, including active manipulation — pulling and twisting — of magnetic particles by magnetic fields [1,15,16••,50,51]. In a recent attempt to measure stresses in moving cells, Guilford et al. [52] used micron-sized magnetic particles that had been ingested by macrophages. Bausch and coworkers [15,16••] in Munich have applied magnetic tweezers [2,3] for the measurement of stress and viscoelastic properties of living cells. They applied forces up to 10^4 pN on approximately $5\mu\text{m}$ particles, in order to measure shear moduli and viscosities in cells. They analyzed the deflection of the beads using video microscopy and image processing methods. They report local measurements of viscoelastic moduli within the cytoplasm of living macrophages using a creep response of magnetic particles subject to constant force [16••]. They also attempt to characterize the displacement field of the cytoplasm surrounding probe particles subject to magnetic forces. The latter directly demonstrated the highly non-homogeneous cellular environment, which calls into question the interpretation of such results in terms of bulk shear moduli. Most of the experiments reported so far have not attempted to take into account inhomogeneities in the cells. There are usually rigid elements of some kind in the vicinity of the probe, actin stress fibers, microtubuli or microtubule bundles, the cell membrane or the substrate of the sample chamber. Slow elastic deformations (video frequency or below) have necessarily a long spatial range and will probe inhomogeneities, apart from the fact that they overlap with active dynamics of the cell. A further problem is the pronounced non-linearity of the elastic behavior of most biopolymer networks due to the semiflexible nature of the molecules. The elastic response is amplitude-dependent and strong strain hardening occurs. These difficulties represent a major challenge for future applications of microrheology in living cells.

Cells can also be mechanically probed from the outside. Recently atomic force microscopy has been used to improve overall spatial resolution over earlier cell-poking with glass needles. A variety of tissues, cells and sub-cellular structures in varying conditions have been tested, including bone cross-sections [53], human platelets [54], secretory granules from

mast cells [55], cholinergic synaptic vesicles [56], fibroblast-like cells [57], and mouse F9 embryonic carcinoma cells [58]. Curves of force versus indentation depth, which are taken spot by spot in a raster-scan mode (force mapping, see above), are usually modeled using the Hertz model. The assumption is always that the tested medium is a homogeneous semi-infinite viscoelastic continuum with a smooth boundary. This is an oversimplification in most cases and resulting numbers for Young's moduli have to be considered as estimates. However, AFM makes it possible to follow the change of properties on living cells in physiological conditions and has been used, for example, to study lamellipodia of mobile cells [59•]. Most experiments are done at low frequencies to allow for viscous relaxation. This avoids the problem of having to model the viscous effects in a difficult geometry, with a relatively large probe very close to a solid surface, interacting with an unknown complex structure. The elastic deformations that are probed can be long range, and are perturbed by active conformational changes in the cells. By varying the frequency of the probe motion or by using tapping mode [60], however, the depth of mechanical probing for imaging purposes can be adjusted. In the extreme, a cell surface looks rather hard when probed at approximately 15 kHz.

5. Conclusions

It remains a challenge to make both the microrheology approaches using imbedded probe particles and the AFM techniques described above more quantitative. This is especially true of the possible applications to biology, where these techniques have so far been only qualitative tools to study relative changes or variations in viscoelasticity, e.g. within cells. However, the ability to probe the properties of materials at a small scale has many potential applications, which have only begun to be explored.

Acknowledgements

The authors wish to thank Fred Gittes, Josef Käse, Rachel Mahaffy, and Dave Weitz for helpful conversations. FCM and CFS were both supported in part by the Whitaker Foundation and by NSF Grant Nos. DMR 92-57544 (FCM) and BIR 95-12699 (CFS).

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- of special interest
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